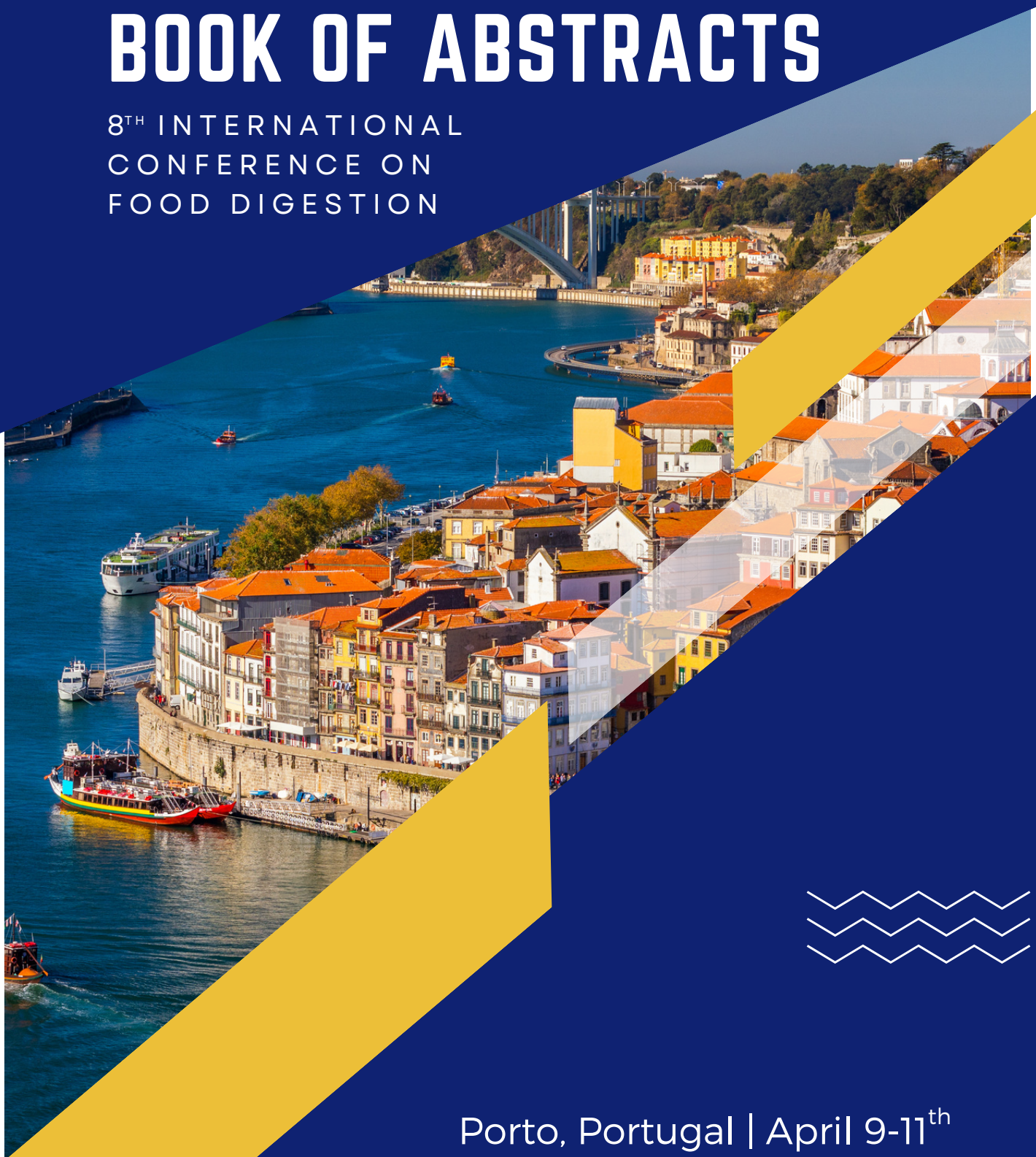
ICFD 2024

BOOK OF ABSTRACTS

8TH INTERNATIONAL CONFERENCE ON FOOD DIGESTION



Porto, Portugal | April 9-11th 2024

INFOGEST

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ICFD 2024

ICFD 2024

WELCOME ADDRESS

Dear Colleagues & Friends,

On behalf of the Organizing and Scientific committees, we are delighted to welcome all of you to the city of Porto, Portugal, for the **8th International Conference on Food Digestion (ICFD2024)**. This conference is organized within the framework of INFOGEST, a global network created in 2011 that now gathers approximately 800 researchers (academics and food companies) from 300 institutions in 60 countries. An INFOGEST branch has been established in Latin America, facilitating collaboration between researchers from Europe and other continents, who will be connected during this conference.

As a cornerstone event in the field of Food, Nutrition, and Health, our goal is to enhance the health properties of food through the exchange of knowledge on the digestive process. **Over 400 delegates will engage in discussions covering the key themes of this conference**:

- Oral processing and sensory properties of foods
- Food structures, digestion and imaging technologies
- Bioaccessibility/absorption of beneficial and harmful compounds
- *In vitro*, *in vivo* and *in silico* models of digestion and absorption
- Impact of diet on gut microbiota

Thank you for joining us this week to experience the renowned warmth, hospitality, and rich culture of Portugal. Enjoy the science, the local food, the Porto Wine at its birthplace and our city. Porto, with its 800 years of history stands as a testemony of resilience and vitality. Designated as a UNESCO World Heritage site since 1996 and awarded several times as the World's Leading Touristic City Destination, it exudes charm and character at every turn.

The cultural night and conference dinner will be held at the historic heart of Vila Nova de Gaia, just a short distance from the center of Porto, offering an amazing view to the Porto city and Douro River. It is the perfect landscape to an unforgetable sunset, exquisite dinner and the enchanting melodies of our music.

The **FOODinteract Research Team** from the **University of Porto** and **LAQV (Associated Laboratory for Green Chemistry)** is committed to ensuring that ICFD2024 will be an unforgetable experience for all of you.

Professor Isabel M.P.L.V.O. Ferreira & Dr Miguel A. Faria
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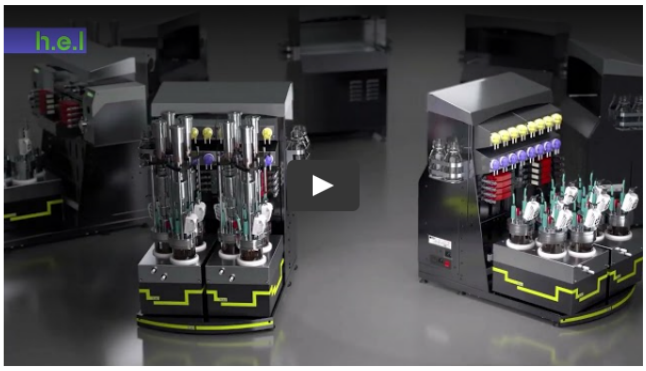

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CONFERENCE AND EVENTS

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CONFERENCE PROGRAMME

Tuesday, 9th April 2024

13:00	**Registration Open**
14:00 - 14:20	**Opening session**
First Session Day 1	**Oral processing and sensory properties of foods** *Chairs: Isabel Ferreira and Didier Dupont*
14:20 - 15:00	**Invited Speaker Anwesha Sarkar, UK,** **"3D tongue-like surface: A quantitative tribology tool to probe oral processing"**
15:00 - 15:50	Oral communication | Session - Day 1
15:50 - 16:30	Coffee Break + Posters Session Day 1
Second Session Day 1	**Food structures, digestion and imaging technologies** *Chairs: Susana Casal and Frédéric Carrière*
16:30 - 17:45	Oral communication | Session Day 1
17:45 - 18:25	**Invited Speaker Harjinder Singh, New Zealand** **"Gastric structuring of food materials to optimise nutrient absorption: challenges and opportunities"**
18:25 - 18:30	Introduce **INFOGEST BR** - Latin American Branch
19:00 - 20:00	**Welcome reception at Sheraton Hotel**

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CONFERENCE PROGRAMME

Wednesday, 10th April 2024

First Session Day 2	**Bioaccessibility/absorption of beneficial and harmful compounds** *Chairs: Isidra Recio and Miguel Faria*
08:30 - 09:50	Oral communication	Session Day 2 - Morning
09:50 - 10:30	**Invited Speaker Manuela Pintado, Portugal** **"*In vitro* food digestion as tool for assessing bioactivity and bioaccessibility"**
10:30 - 11:10	Coffee Break + Posters session Day 2
First Session Day 2	**Bioaccessibility/absorption of beneficial and harmful compounds** (continuation) *Chairs: Beatriz Miralles and Pasquale Ferranti*
11:10 - 12:30	Oral communication	Session Day 2 - Morning
12:30 - 14:00	Lunch time (poster session change)
Second Session Day 2	***In vitro, in vivo* and *in silico* models of digestion and absorption** *Chairs: Joana Costa and André Brodkorb*
14:00 - 15:20	Oral communication	Session Day 2 - Afternoon
15:20 - 16:00	Invited Speaker **John Van Camp, Belgium** **"*In vitro* bioavailability and impact of food-derived amyloid-like protein fibrils on health-related markers"**
16:00 - 16:40	Coffee Break + Posters session Day 2
Second Session Day 2	***In vitro, in vivo* and *in silico* models of digestion and absorption** (continuation) *Chairs: Isabel Mafra and Alfonso Clemente*
16:40 - 18:00	Oral communication	Session Day 2 - Afternoon
18:30 - 19:00	Buses leave Hotel for Cultural Dinner venue
20:00	**Cultural Dinner**

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CONFERENCE PROGRAMME

Thursday, 11th April 2024

Day 3 Part 1	**Impact of diet on gut microbiota** *Chairs: Linda Giblin and Sara Cunha*
09:00 - 10:05	Oral communications | Topic 5
10:05 - 10:45	Invited Speaker **Andrea Gianotti, Italy** **"Role of food formulation and processing on the gut microbiome mediated health and diseases: how in vitro approaches can explain causalities and mechanisms "**
10:45 - 11:30	Coffee Break + Posters session | Topic 4
Day 3 Part 1 continuation	**Impact of diet on gut microbiota** (continuation) *Chairs: Isabel Ferreira and Miguel Faria*
11:30 - 12:20	Oral communications | Topic 5
12:20 - 12:40	**INFOGEST Update Didier Dupont**
12:40 - 13:00	**Awards and closing session**
13:00 - 15:00	**Lunch time**
15:00 - 18:00	**WG meetings**

FULL PROGRAMME

PDF ORAL COMMUNICATIONS

PDF POSTER COMMUNICATIONS

ICFD 2024

KEYNOTE LECTURES

Portugal | April 9-11th, 2024

Topic 1: Oral processing and sensory properties of foods | Invited Speaker

(23210) - 3D TONGUE-LIKE SURFACE: A QUANTITATIVE TRIBOLOGY TOOL TO PROBE ORAL PROCESSING

Sarkar, Anwesha (Portugal)[1]

1 - Food Colloids and Biorocessing Group, School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, United Kingdom

Abstract

Oral tribology at multiple length scales1-3 is emerging as a new frontier in food science to quantify friction and lubrication of food-saliva mixtures in the oral surfaces. Tribological assessment is providing fundamental insights into the physics of oral processing and sensory (textural) perception of food. Although gustatory function of tongue papillae is well investigated, the uniqueness of papillae within and across individuals remains elusive. First, I will discuss the novel machine learning framework on 3D microscopic scans of human papillae, uncovering the uniqueness of geometric and topological features of papillae. Models trained on these features with small volumes of tongue papillae from 15 healthy participants predict the type of papillae with an accuracy of 85%. Remarkably, the papillae are found to be distinctive across individuals and an individual can be identified with an accuracy of 48% among the 15 participants from a single papillae. Understanding the importance of tongue papillae, I will then talk about how we have fabricated unique 3D soft biomimetic surface that simulates the topography of papillae, wettability and deformability of a real human tongue. We demonstrate the unprecedented capability of these surfaces to replicate the theoretically defined and simulated collision probability of papillae representing mechanoreception and to closely resemble the tribological performances of real human tongue masks. These novel 3D tongue simulator can offer plethora of opportunities acting as an in vitro screening tool thereby accelerating the product development cycle of food, and also particularly helpful for testing formulations targeted for specific vulnerable paediatric and geriatric population where sensory tastings remain challenging.

Acknowledgments

The European Research Council is acknowledged for its financial support (Funding scheme, ERC Starting Grant 2017, Project number 757993) for this work.

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Keywords : Oral tribology, friction, lubrication, machine learning, papillae

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Invited Speaker

(24487) - IN VITRO FOOD DIGESTION AS TOOL FOR ASSESSING BIOACTIVITY AND BIOACCESSIBILITY

P, Maria Manuela (Portugal)[1]

1 - CBQF—Centro de Biotecnologia e Química Fina—Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

Abstract

The beneficial effects of bioactive molecules on consumer health are intricately linked to their absorption and interaction within the gastrointestinal tract (GIT), including their impact on gut microbiota. Understanding the concepts of bioaccessibility and bioavailability is crucial, as they determine the effectiveness of these molecules. Bioaccessibility refers to the amount of a compound available for absorption in the GIT, while bioavailability denotes the fraction of these compounds that reaches systemic circulation. Exploring the potential of bioactive molecules found in extracts or fractions obtained from natural resources requires characterization of their behavior throughout the GIT. This characterization helps assess their potential biological properties and toxicity post-passage, understand their interaction with gut microbiota, and design suitable protection strategies or select appropriate matrices for incorporation.

In vitro digestion models serve as valuable tools due to their speed, cost-effectiveness, reproducibility, and ability to correlate well with *in vivo* results. This presentation will highlight the progress of the use of GIT *in vitro* models in our research group (Bioproducts and Bioactives) to assess different bioactive extracts, ingredients, or products, with a recent focus on the use of the INFOGEST *in vitro* digestion model. Our group has obtained several bioactive molecules, extracts, and ingredients from agrofood byproducts and natural unexploited resources, and screened different biological properties to envision their potential applications in food and nutraceuticals. Thus, the bioaccessibility and bioavailability of present key bioactive molecules, such as phenolic compounds, antioxidant fiber, carotenoids, peptides, polyunsaturated fatty acids, and probiotic bacteria, have been tested using GIT *in vitro* models to fully understand their effective bioactive potential. During the presentation, key examples of bioactives from different sources will be presented and discussed, with emphasis on composition affected by GIT conditions, related biological properties post-passage, and impact on gut microbiota. Additionally, we will illustrate how food matrix effects significantly influence the bioaccessibility of key bioactives and consequently determine overall bioavailability. Ultimately, our findings underscore the importance of understanding gastrointestinal behavior for optimizing bioactive ingredient delivery and efficacy in functional food and nutraceutical applications.

Acknowledgments

The author would like to thank the CBQF under the FCT - Fundação para a Ciência e Tecnologia project UIDB/Multi/50016/2020

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https://doi.org/10.1016/j.tifs.2022.02.028

Keywords : Bioactive molecules, In vitro Gastrointestinal tract models, Bioaccessibility, Biological properties, Gut microbiota

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Invited Speaker

(23332) - IN VITRO BIOAVAILABILITY AND IMPACT OF FOOD-DERIVED AMYLOID-LIKE PROTEIN FIBRILS ON HEALTH-RELATED MARKERS

Van Camp, John (Belgium)[1]; Luyckx, Trui (Belgium)[1]; Grootaert, Charlotte (Belgium)[1]; Monge-Morera, Margarita (Belgium)[2]; Delcour, Jan (Belgium)[2]; Rousseau, Frederic (Belgium)[3]; Schymkowitz, Joost (Belgium)[3]; Carpentier, Sebastien (Belgium)[4]

1 - Laboratory of Food Chemistry and Human Nutrition, Faculty of Bioscience Engineering, Ghent University, Ghent Belgium; 2 - Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), KU Leuven, Leuven, Belgium; 3 - Switch Laboratory, VIB-KU Leuven Center for Brain & Disease Research, Leuven, Belgium and Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium; 4 - Laboratory of Tropical Crop Improvement, Faculty of Bio-Science Engineering, KU Leuven, Leuven, Belgium

Abstract

In this presentation, we demonstrate how appropriate processing conditions are able to induce amyloid-like protein fibrils (ALFs) in food proteins. These fibrils are stabilized by cross β-sheet structures and can be used to improve the techno-functional properties of food formulations. ALFs show structural similarity to disease-related endogenous amyloids, and therefore also need to be evaluated in the context of food safety. We give an overview on the gastrointestinal digestion, intestinal absorption, and systemic dissemination of ALFs, and discuss assessments of potential ALF cross-seeding of endogenous precursor proteins linked to (non)neurodegenerative amyloidosis. We highlight these processes in both healthy and predisposed individuals. We conclude that the health impact of ALF consumption merits additional research efforts to determine the exact extent to which ALF ingestion may influence the general health status. We further illustrate these findings with more recent literature as well as with own research results obtained using *in vitro* digestion, absorption and bio-activity models.

*for more information see Luyckx et al., Mol. Nutr. Food Res. 2022, 2101032. This work was part of the PhD of Trui Luyckx performed within the Strategic Basic Research project ProFibFun, funded by the Research Foundation-Flanders (FWO, SBO grant S003918N, Brussels, Belgium)

References

Keywords : protein, amyloid, bioavailability, digestion, absorption

Topic 4: Food structures, digestion and imaging technologies | Invited Speaker

(24490) - GASTRIC STRUCTURING OF FOOD MATERIALS TO OPTIMISE NUTRIENT ABSORPTION: CHALLENGES AND OPPORTUNITIES

Singh, Harjinder (New Zealand)[1]

1 - Riddet Institute, Massey University, Private Bag 11 222, Palmerston North, New Zealand

Abstract

With the growing crisis in diet-related diseases (e.g. type 2 diabetes, cardiovascular diseases, obesity), there is increasing recognition within research community the need for better understanding of the behaviour of foods within the human digestive tract (GIT). Human foods are derived from a diverse range of animal and plant sources and contain diverse phases, structures, and matrices. The human digestive tract must physically and chemically break down these structures/matrices to release nutrients, which can then be absorbed and metabolised in the body. Digestion of food in the human digestive system is very complex, with a combination of physical and biochemical processes involved in food disintegration, transport and absorption of nutrients, and elimination the undigested food material.

Stomach is the most crucial unit of the GIT. This is where food material structures are extensively modified due to the mechanical action and the presence of digestive juices (containing minerals, enzymes, and highly acidic pH). The dynamics of food structure breakdown and rearrangement play a key role in gastric emptying and consequently the delivery of nutrients in the duodenum. Different foods have been shown to undergo major re-structuring and phase changes during gastric digestion, which consequently influences post-prandial physiological and metabolic responses. However, the fundamental mechanisms involved in gastric emptying, breakdown and mixing kinetics of different food materials in the stomach, particularly in-vivo models, need to be better understood. This presentation focuses on recent research on interactions in liquid food systems (milk and emulsions) within the environment of the GIT, especially in the stomach, using both *in vitro* and *in vivo* digestion models. Selected examples of recent work carried out at the Riddet Institute on the gastric digestion behaviour of colloidal dispersions (milks of different species, plant-based milks etc), demonstrating the importance of colloidal interactions and gastric re-structuring, will be discussed.

Topic 5: Impact of diet on gut microbiota | Invited Speaker

(24491) - ROLE OF FOOD FORMULATION AND PROCESSING ON THE GUT MICROBIOME MEDIATED HEALTH AND DISEASES: HOW IN VITRO APPROACHES CAN EXPLAIN CAUSALITIES AND MECHANISMS

Gianotti, Andrea (Italy)[1,2,3]; Nissen, Lorenzo (Italy)[1,2,3]; Casciano, Flavia (Italy)[1,3]

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Abstract

The last decade research demonstrated that gut microbiome (GM), present across the entire digestive tract but predominantly abundant in the colon, is an important determinant of physiological effects that are derived from the gastrointestinal processes affecting solubility, stability and bioavailability of dietary substrates including chemical constituents and microorganisms (beneficial or harmful). Moreover many endogenous or exogenous (dietary of environmental) factors were recognized as direct determinants of the structure and function of the intestinal microbiota community. Specifically, dietary components may contribute to benefit not only to benefit (eubiosis) or alterate (dysbiosis) gut microbiome composition but also to its functionality with important effects on systemic disorders including both human intestinal diseases, systemic inflammation, metabolic syndromes etc.

The restriction to study *in vivo* the behavior of GM during a diet intervention has led researcher to develop *in vitro* gut fermentation models. This approach permits to explore over the influence on GM of a vast variety of factors such as dietary compounds, microbial pathogens, bioactives, pharmaceuticals and toxic substances. The setting of *in vitro* models provides to cultivate the human GM under regulated environmental conditions and study the microbiota shifts and their related metabolites over time. After a brief overview on the main fermentation models used in food science, some experimental case studies carried out by MICODE model will be considered to show the opportunity of understanding the mechanisms of prebiotic potential of foods as effect of reformulation, cooking processes and missuses of targeted foods by general healthy population. Finally, a brief example of gut microbiota effects coming from dietary xenobiotics will be provided to open the discussion on the potential use of gut models as risk assessment strategy to evaluate the alteration of human or animal microbiome by food additives or dietary contaminants.

As scientific community, we are asked to fill the gap on standardized and validated protocols to share results and provide predictive tools specifically designed to better understand the role of gut microbiota in food digestion process.

ICFD 2024

ORAL PRESENTATIONS

Portugal | April 9-11th, 2024

ICFD 2024

TOPIC 1

ORAL PROCESSING AND SENSORY PROPERTIES OF FOODS

Portugal | April 9-11th, 2024

Topic 1: Oral processing and sensory properties of foods | Oral Communication

(22787) - INFLUENCE OF ADDITION OF ALGINATE HYDROGELS ON ORAL PROCESSING BEHAVIOUR AND SENSORY PERCEPTION OF DIFFERENT FOOD MATRICES

Garcia-Fuentes, Alvaro R. (Netherlands)[1,2]; Genova, Gergana (Netherlands)[3]; Aguayo-Mendoza, Monica G. (Netherlands)[1]; Troost, Freddy J. (Netherlands)[1,4]

1 - Food Innovation and Health, Centre for Healthy Eating and Food Innovation (HEFI), Maastricht University, Venlo, The Netherlands; 2 - University College Venlo, Faculty of Science and Engineering, Maastricht University, Venlo, The Netherlands; 3 - MSc. Health Food Innovation Management, Faculty of Health, Medicine and Life Sciences, Maastricht University, Venlo, The Netherlands; 4 - Department of Human Biology, NUTRIM Institute of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands

Abstract

Background: Alginate hydrogel beads are an effective mean to deliver bioactive food ingredients to target locations in the gastrointestinal tract. Hydrogels in foods inherently modify the product's sensory characteristics and consumer acceptability. Food matrices varying in texture (i.e. solid, semi-solid, or liquid) require different oral processing and this can in turn influence foods' sensory perception. This study aimed to investigate the influence of adding hydrogel beads to food matrices varying in textural properties on oral processing behaviour and sensory perception.

Methods: 3 products, chocolate slab (solid), semolina (semi-solid), and tomato soup (semi-solid), either containing 60g of beads or without beads (control), were produced. 33 participants evaluated all samples in a randomized order. To characterize oral processing, bite size, consumption time, and eating rate were determined from 3 regular bites of each sample. Sensory characteristics were evaluated using the Ideal Profile Method. Participants scored perceived and ideal intensity of 22 attributes on a 100mm VAS scale. Liking of samples was determined using likert scales from 1 to 10. Data were analysed using ANOVA, when significant ($p<0.05$) Bonferroni post-hoc was done. Multivariate analyses were performed on sensory data to assess similarities of perceived and ideal product spaces.

Results: Bite size was significantly larger from chocolate slab with beads (4.3 ± 0.3 g) compared to chocolate without beads (2.5 ± 0.2 g). Consumption time was significantly different between products, independent of beads content, with chocolate having the largest consumption time (12.8 ± 0.3 s), followed by semolina (8.1 ± 1.2 s), and tomato soup (3.5 ± 2.8 s). Eating rate was significantly slower for tomato soup with beads (2.3 ± 0.2 g/s) compared to the product without beads (3.3 ± 0.3 g/s). Addition of beads had a significant effect on the overall sensory perception of the different samples, mainly affecting attributes of appearance and texture. The ideal profile for foods with beads was between the perceived profiles of products with and without beads. Liking was significantly decreased in all foods when beads where added.

Conclusion: Addition of hydrogel beads increased bite size of solid samples, while in liquid samples it slowed down eating rate. Sensory evaluation showed that addition of beads modified overall foods' sensory profiles, independently of their food matrix. Ideal sensory profiles of food with beads were different from the perceived sensory profiles of foods with as well as without beads. This shows that the ideal sensory profile for a food product containing beads does not consist of a homogeneous food matrix. Future research will optimize food products to incorporate alginate beads and to achieve the consumers' ideal profile to become more acceptable for consumption.

References

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Santagiuliana M et al. 2018;80:254–63.

Keywords : Food oral processing, Sensory perception, Composite foods, Food matrix carriers, Alginate hydrogels

Topic 1: Oral processing and sensory properties of foods | Oral Communication

(21430) - MECHANISMS BEHIND FAT RELATED PERCEPTION IN PLANT-BASED BURGERS VARYING IN FAT TYPE

Corrà, Lucia (Switzerland)[1]; Tecuanhuey, Maria (Switzerland)[1]; Girardi, Alicia (Switzerland)[1]; Devezeaux De Lavergne, Marine (Switzerland)[1]

1 - Nestlé Research Center

Abstract

Fat related perception of plant-based burgers is a key driver of consumer liking. To understand the mechanisms behind the perception of different oils typically used in plant-based burgers, we propose an integrated approach considering sensorial, mechanical, and physiological aspects of the consumption of pure oils and burgers.

Four edible oils and corresponding plant-based burgers, containing 8.5 wt% oil, were selected: Cocoa, Coconut, Shea Stearin and a Canola/Cocoa mix. Burgers were comminuted *in vitro* via a grinding step followed by a mixing step with artificial saliva, to mimic oral processing. Juices were extracted from the stimulated boli via filtration, structural images of the Juices were obtained with a fat and a protein die using Confocal Scanning Microscopy (CLSM) and moisture and fat content was measured in the Juices. Friction properties of the oils and Juices extracted *in vitro* from burger were measured using a ball-on-three pins configuration in an Anton Par Rheometer. Oils and burgers containing oils that were previously died with curcumin were orally processed by subjects (N=10) and the residual oil coating on the tongue was measured after spitting out using a spectrophotometer. Finally, all samples were analyzed by a sensory panel using RATA (N=10), including the “fatty” and “oily-mouthcoating” attributes.

Results of sensory, friction and oil deposition discriminated between pure oils, but did not predict results in application. Juices obtained from burgers containing different fats had the structure of an oil in water emulsions as shown by CLSM, and had similar compositions in term of total fat content and moisture. Friction properties of the *in vitro* juices showed that the Shea sample elicited the least friction. Oil deposition on the tongue was highest for the burger containing Coconut oil. Sensory results showed that small but significant differences in “fatty” and “oily-mouthcoating” existed between the burgers. Oil deposition on the tongue did not explain fat related sensory attributes, whereas friction of the extracted Juices inversely correlated with the two fat related attributes.

These results suggest that fat perception in complex food matrices is not predicted by the sensory perception of the fats alone. In addition, the mechanism of fat sensing from simple oils and plant-burgers seemed related to friction properties and not to oil deposition properties on the tongue.

Acknowledgments

Keywords : Mouthfeel, Fat perception, Tribology, Plant-Based

Topic 1: Oral processing and sensory properties of foods | Oral Communication

(21450) - THE INFLUENCE OF ORAL PROCESSING BEHAVIOUR ON NUTRIENTS DIGESTION

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Abstract

The effect of food properties on oral processing behaviour, bolus properties and *in vitro* digestion of macronutrients was determined in various food matrices to better understand the role of oral processing behaviour in nutrients digestion. Oral processing behaviour impacts bioavailability of macronutrients. For chicken and soy-based vegetarian chicken, natural chewing time differed considerably (5x fold) between healthy consumers. For both foods, longer chewing times resulted in the formation of more and smaller bolus fragments and consequently led to higher *in vitro* protein hydrolysis. Oral processing behaviour may contribute to individual differences in glycaemic responses to foods, especially in plant tissue where chewing behaviour can modulate the release of starch from the cellular food matrix. In a cross-over trial, participants consumed two carbohydrates-identical test meals (brown rice; chickpeas) with long or short chewing time. Longer chewing resulted in more and smaller bolus particles, higher saliva uptake in the bolus and higher *in vitro* starch hydrolysis for both meals. No significant effect of chewing time on glycaemic response (iAUC) was found for both meals, whereas brown rice showed significantly and considerably higher *in vitro* starch hydrolysis and glycaemic response (iAUC) than chickpeas regardless of chewing time. This suggests that differences in the innate structure of starch based foods have a larger impact on postprandial glucose responses than differences in mastication behaviour although oral processing behaviour showed consistent effects on bolus properties and *in vitro* starch digestion. Oral processing increases the accessibility of lipids to digestive enzymes of cellular plant-based foods such as nuts and almonds which are often consumed with accompanying foods. At similar bite size, the addition of chocolate and iceberg lettuce to almonds decreased chewing time and increased eating rate. Almond bolus particle sizes were similar for almonds consumed alone and with chocolate, while consuming almonds with lettuce generated fewer and larger almond bolus particles. Predicted lipid bioaccessibility of almonds consumed with iceberg lettuce was lower than for almonds consumed alone and almonds consumed with chocolate. Eating rate correlated significantly and positively with the mean area of bolus particles and significantly and negatively with predicted lipid release. We conclude that differences in food properties and oral processing behaviour translate into differences in food bolus properties and nutrients digestion and bioavailability highlighting the impact of the food matrix and consumption context on digestion.

Keywords : Food matrix, oral behaviour, digestion, plant protein, food structure

Topic 1: Oral processing and sensory properties of foods | Oral Communication

(22611) - QUANTITATIVE ULTRASOUND TO EXPLORE TACTILE PERCEPTIONS OF FOOD ELICITED BY TONGUE-PALATE FRICTION: A BIOMIMETIC APPROACH

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Abstract

The tongue has a central role in the sensory experience of texture perception of food. Tongue mechanoreceptors have varied ranges of sensitivity, both in terms of amplitude and frequency of the mechanical stimuli to which they are exposed to. Understanding the behavior of these sensory receptors requires the development of innovative methods, with experimental approaches that integrate both the characteristics of the food and the complex properties of the tongue.

In this work, a biomimetic device was composed with artificial tongues (polyvinyl alcohol cryogels) actuated by two translation stages to generate custom sequences of compressional and shearing motions against a rigid plate playing the role of the hard palate. The roughness of the tongues was designed to be reminiscent of the diversity of human tongue papillae height (20–140 µm). Newtonian aqueous solutions of glycerol (1–1400 mPa.s) were deposited on their surface and their frictional behavior was investigated during sequences of shearing motions against the palate.

A multi-axes strain gauge sensor and a piezoelectric accelerometer were used to measure forces and vibrations between the tongue and the palate. In addition, non-destructive ultrasound (US) waves were propagated within the tongue with a pulse recurrence frequency of around 1 kHz, using a single-element sensor (5 MHz) positioned under the tongue. Signal processing methods were developed to characterize the evolution of the time of flight (ToF) of the echo corresponding to the reflections of US at tongue-palate interface.

The low frequency component of ToF evolution (0–40Hz) was found to correlate both with the normal pressure exerted by the tongue on the palate, and with the thickness of the lubricating film at tongue-palate interface. At higher frequencies (>40 Hz), ToF fluctuations were correlated with vibrations induced by stick-slip phenomena between the tongue and palate, characteristic of the mixed lubrication regime. The study paves the way for the use of US methods (non-invasive and non-destructive) for the continuous monitoring of friction phenomena between the tongue and the palate. Implemented on such a biomimetic system, they make it possible to study the respective contributions of the properties of foods and of the physiological specificities of individuals on the biomechanical phenomena at the origin of texture perceptions.

References

Glumac et al., *Biotribology,* **35-36** (2023) 100257

Keywords : Texture, Tongue, Tribology, Ultrasound, Biomimicry

ICFD 2024

TOPIC 2

FOOD STRUCTURES, DIGESTION AND IMAGING TECHNOLOGIES

Portugal | April 9-11th, 2024

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Oral Communication

(22583) - QUANTIFYING THE IMPACT OF GUT MICROBIAL METABOLISM ON THE BIOAVAILABILITY AND PHARMACOKINETICS OF STEVIOSIDE

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Abstract

The gut microbiome is metabolically active and is expected to influence the bioavailability and systemic disposition of food ingredients. This may occur directly via gut microbiome-mediated metabolism, or indirectly through alterations of intestinal absorption kinetics by microbial metabolites; however, there are limited examples where the effect of microbial metabolism on the pharmacokinetics of food ingredients has been quantified. Here we demonstrate the investigation of the effect of the gut microbiome on the biotransformation and systemic bioavailability of the natural sweetener stevioside. Orally ingested stevioside is not absorbable, but instead is deconjugated by the gut microbiota to liberate steviol which is bioavailable. In the liver, steviol is converted to steviol glucuronide, which is then secreted by the kidneys into urine for excretion. Using this framework, microbial hydrolysis data from stool incubations with stevioside, hepatic glucuronidation of steviol and renal transporter secretion of steviol glucuronide were incorporated in a physiologically-based pharmacokinetic model to predict the stool, plasma and urinary concentration-time profiles of stevioside and its metabolites steviol and steviol glucuronide. The simulated time-courses were successfully validated against published human *in vivo* clinical data for low and high stevioside dose scenarios. Interestingly, our simulations revealed that stevioside exhibits a microbially-controlled, pre-systemic metabolism of stevioside to steviol occurring in the colon. This rate-limiting step directly determines the bioavailability and the systemic pharmacokinetics of steviol. In addition, we demonstrate that there is saturable, dose-dependent intestinal absorption of steviol, resulting in non-linear systemic pharmacokinetics. This occurs at a higher consumption (40 mg/kg) of stevioside. Our finding implies that increased bioassessibility does not automatically translate to a proportional increase in bioavailability. Finally, we demonstrate that stevioside systemic kinetics can be modulated by adjusting the degree of microbial metabolism, which can be accomplished by strengthening the glycosidic bond to resist microbial hydrolysis, or by increasing the degree of conjugation (as found with other steviol glycosides e.g. Rebaudioside A). In summary, we demonstrate a workflow to quantify and test the influence of gut microbiota on pharmacokinetics of food ingredients. A mechanistic understanding of the key steps that control bioaccessibility and bioavailability allow rational design of the structure of food ingredients to achieve the desired kinetic behaviour.

Acknowledgments
This work was supported by grant numbers ASTAR-DP-2019-012 and SC34/22-109000 awarded to James Chan

Keywords : Pharmacokinetics, Bioavailability, Bioaccessibility, Steviol, Gut microbiome

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Oral Communication

(22803) - ASSESSING THE IMPACT OF IN VITRO GASTROINTESTINAL DIGESTION ON THE BIOACCESSIBILITY AND BIOACTIVITY OF POLYPHENOLS-RICH EXTRACTS FROM CITRUS BY-PRODUCTS

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Abstract

Citrus juice processing worldwide creates about 120 million tons of waste yearly due to discarded by-products, such as peels and pulps, leading to an unprecedented environmental burden. Orange (Citrus sinensis) and lemon (Citrus limon) peels, for instance, contain valuable bioactive compounds (e.g.: polyphenols, essential oils, fiber) which can be repurposed for novel food and health applications [1]. On the other hand, digestive conditions affect a significant portion of the global population. Diseases such as irritable bowel syndrome, inflammatory bowel diseases and digestive cancers impact millions [2]. Thus, phenolic compounds have been researched and found to exhibit antioxidant and anti-inflammatory properties, protecting the gastrointestinal system from oxidative stress and inflammation and promoting a balanced gut microbiota by allowing the growth of beneficial bacteria while inhibiting pathogenic ones, thereby enhancing digestive function and fortifying the intestinal barrier [3].

This study aimed to evaluate the impact of gastrointestinal digestion (GID) on polyphenols-rich extracts from both lemon and orange peels, assessing their bioaccessibility and antioxidant activity. The by-products were subjected to an enzymatic extraction followed by centrifugation, leading to the obtention of the oranges' (OPE) and lemons' (LPE) extracts. After, the bioaccessibility of both extracts was assessed through the GID using INFOGEST 2.0. Moreover, the polyphenolic content (HPLC-DAD) and *in vitro* antioxidant activity (ABTS and DPPH assays) of each GIG step (oral phase, gastric phase and intestinal phase) were assessed. The main polyphenolic compounds found on OPE fractions and their respective bioaccessibility index (w/w DE) after digestion were hesperidin (70 %), narirutin (75 %) and sinensetin (60 %) and nobiletin (76 %). Regarding the LPE, the main analyzed compounds were eriocitrin (40 %), hesperidin (89 %) and narirutin (75 %). Therefore, these flavonoids kept relatively stable over the tract. In addition, the chlorogenic acid present in OPE showed a bioaccessibility index of 56%. Yet, when analyzing the DPPH and ABTS assay results, all GID fractions from OPE and LPE extracts presented antioxidant activity. Moreover, both extracts showed an increasing tendency to have antioxidant activity over the GID, which might be related to some of the inherent hydrolysis throughout the digestion process [4].

Overall, this research intends to elucidate the usage of polyphenols-rich extracts from citrus side-streams as functional ingredients to improve digestive wellness, offering insights into their bioaccessibility and antioxidant activity patterns, through the INFOGEST 2.0, while emphasizing the relevance of waste's upcycling into valuable resources within the global food industry, applying a circular economy approach.

References

Vilas-Boas et al., 2022; Rose et al., 2022; Kumar et al., 2019; Cavia et al.,2023.

Acknowledgments

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Keywords : Citrus peels, Digestive Health, Polyphenolic extracts, Circular Economy, Hesperidin

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Oral Communication

(22626) - EFFECTS OF ENZYMATIC HYDROLYSIS, THE MAILLARD REACTION AND IN VITRO GASTRO-INTESTINAL DIGESTION ON ANTIOXIDANT PROPERTIES OF FEATHER KERATIN

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Abstract

Feather keratin is a cysteine-rich, underutilised protein, generated as waste in the poultry industry. This study aimed to investigate the antioxidant activity of soluble chicken feather keratin prepared by different processing methods. Firstly, the keratin isolate (KI) was obtained by extraction with L-cysteine and then hydrolysed with either pepsin, trypsin, chymotrypsin, subtilisin or papain. The most extensively hydrolysed preparation was obtained with subtilisin (KI-S, DH 36%). In an attempt to improve its bioactive and sensory properties (Fu et al., 2019), KI-S was then subjected to thermal treatment (90, 105 or 120°C for 1, 2 or 3h) with either glucose (G) or xylose (X) to induce Maillard reaction. The reaction's progress was quantified by measurement of spectral markers of intermediate (A_{294}) and advanced (A_{420}) Maillard reaction products. The molecular weight (MW) of resulting preparations was analysed by size exclusion chromatography and their antioxidant potential was determined using several assays (ABTS, Fe^{2}-chelating and FCR). For the next part of the study, KI, KI-S and KI-S-X-105°C-1h were subjected to *in vitro* static gastrointestinal digestion (SGID) using the INFOGEST protocol. Polarised Caco2-HT29MTX monolayers were treated with SGID-samples to monitor gut barrier health biomarkers and bioavailable free amino acids. Among the hydrolysates, KI-S exhibited the highest antioxidant activity regardless of assay employed. Post Maillard reaction, KI-S-X-105°C-1h showed the highest ABTS scavenging and Fe^{2+}-chelating properties, while the FCR assay indicated an increase in the content of aromatic products with rising temperature and reaction time. A_{294} and A_{420} indicated that temperature <105°C was insufficient to trigger the Maillard reaction and that X was more Maillard-reactive than G. Post-SGID, the MW <1 kDa peptide fraction increased from 7 to 67% for KI, from 63 to 77% for KI-S and from 65 to 78% for KI-S-X-105°C-1h. The keratin digests did not affect the epithelial barrier integrity as monolayer transepithelial electrical resistances were similar to controls ($p>0.05$). The keratin digests did not significantly change mRNA transcript levels of tight junctions (JAM-1, occludin, ZO-1) nor oxidative stress biomarkers (SOD, CAT, GPx2) compared to SGID control ($p>0.05$). The most abundant bioavailable amino acids were cysteine, valine, arginine, glycine, histidine and alanine. In conclusion, in the quest for a circular economy to utilise waste streams for dietary protein, we have produced keratin preparations that differ in antioxidant activity, MW and bioavailable amino acid profile, but do not harm the gut barrier. Further investigations are ongoing to explore other bioactive and structural properties of the keratin preparations to determine optimal processing conditions for potential food applications.

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Acknowledgments

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Keywords : antioxidant activity, digestion, feather keratin, Maillard reaction

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Oral Communication

(22657) - GASTROINTESTINAL LIPID HANDLING OF AN INFANT FORMULA WITH LARGE PHOSPHOLIPID COATED LIPID DROPLETS IS DIFFERENT FROM STANDARD INFANT FORMULA AND CLOSER TO HUMAN MILK

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Abstract

Objectives and study

Lipid globule structure varies in early life nutrition and can impact emulsion stability, lipid digestion, and (enteroendocrine mediated) gastric emptying. These effects may result in different rates at which lipids become accessible for intestinal absorption. Here, we compared the lipid bioaccessibility rate of human milk (HM) containing large lipid globules (mode diameter ~4 µm) which are coated with a 3-layered milk fat globule membrane (MFGM), Nuturis infant formula (IF) with large milk phospholipid coated lipid droplets (mode diameter 3-5 µm), a standard IF with small (mode diameter 0.4 µm), protein coated lipid droplets, and a standard IF with added MFGM.

Methods

Infantile dynamic gastrointestinal conditions were simulated using tinyTIM with an advanced gastric compartment. Gastric- and intestinal-content, and filtered (≤0.05 µm) intestinal-content, consisting of bioaccessible lipids, were collected in 30 min intervals. The lipid content in time was analyzed using gas-chromatography with flame-ionization-detection, and curve fitted using 4-parameter logistic regression. Enteroendocrine cholecystokinin secretion by Caco-2 cells following incubation with bioaccessible lipids, was measured

Results

HM and Nuturis IF showed lipid layering during gastric digestion, resulting in a greater gastric lipid emptying halftime for HM (ANOVA, Tukey $p=0.005$), but not for Nuturis IF ($p=0.084$) compared to standard IF. Both HM and Nuturis IF subsequently showed greater bioaccessible lipid halftimes than standard IF ($p\leq0.005$), suggesting that intestinal lipolysis rate was also affected. Standard IF bioaccessible lipids elicited increased cholecystokinin secretion compared to that of HM and Nuturis IF ($p\leq0.001$). Addition of MFGM phospholipids to the standard IF did not impact lipid behavior nor cholecystokinin secretion.

Conclusions

Nuturis IF lipid handling during gastrointestinal transit is different from standard IF, resulting in a slower lipid bioaccessibility rate, which is closer to that of HM. A slower lipid bioaccessibility rate is hypothesized to lead to lower postprandial plasma lipid levels, which can impact lipid partitioning, metabolism and growth.

Keywords : Lipid digestion, emulsion stability, cholecystokinin, bioaccessibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Oral Communication

(22712) - BIOACCESSIBILITY OF RICE MYCOTOXINS: A COMPARATIVE IN VITRO STUDY OF ADULT AND ELDERLY DIGESTION

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Abstract

Nowadays, older adults (age > 65 years) represent a significant portion of the global population, growing faster than any other age group worldwide due to the increase in average life expectancy. One of the most widely recognized factors to be addressed in favoring active and healthy aging is the improvement of elderly dietary patterns (1). Besides nutrients, foods can carry various contaminants, such as mycotoxins, to which humans are exposed. The elderly population, is one of the most vulnerable, reason why it is vital to understand the fate of these toxicants during digestion to better evaluate their impact on elderly health.

In this context, this work evaluated ochratoxin A (OTA), citrinin (CIT), and zearalenone (ZEN) bioaccessibility, isolated and combined, when ingested in the matrix rice. *Carolino* white rice was boiled, spiked with the compounds, and subsequently submitted to the INFOGEST harmonized *in vitro* digestion protocol for adults and the elderly. Mycotoxin bioaccessibility behavior was monitored after gastric and intestinal phases by LC-MS/MS. Data obtained under elderly gastrointestinal conditions were compared to those obtained using the INFOGEST protocol for adult digestion (1, 2).

Regarding the gastric phase, the ranking of bioaccessibility is displayed as ZEN>OTA>CIT, while the intestinal phase is CIT>OTA>ZEN. It should be noted that when the mycotoxins were isolated, the observed values were higher than when combined. Results clearly showed that mycotoxin bioaccessibility was deeply affected by the elderly gastrointestinal conditions. In the intestinal phase of the elderly simulating protocol, when mycotoxins are isolated and combined, CIT and OTA were more bioaccessible than with the adult protocol, contrary to the ZEN behavior.

The differences found in mycotoxins bioaccessibility between the adult and elderly digestion simulation are relevant and could be helpful for further accurate risk assessment studies.

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Keywords : Bioaccessibility, Digestion, Mycotoxins, Elderly

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Oral Communication

(21436) - RELEASE OF BIOACTIVE COMPOUNDS FROM MICROCAPSULES DURING IN VITRO DIGESTION

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Abstract

The addition of omega-3 polyunsaturated fatty acids (ω-3 PUFA), mainly eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3), to food has become increasing important in the last years, due to well-known beneficial effects of these fatty acids and the insufficient consumption of fish, seafood or algae to reach the recommended intake EPA plus DHA, which is around 0.25 g per person and day [1]. Among the evaluated strategies to enrich meat and meat products in ω-3 PUFA, the use of fish oil microcapsules seems to be the most appropriate one [2], and it has also been proved quite accurate to release the encapsulated material at targeted sites [3]. In this context, the selection of the coating wall materials of the microcapsules is decisive to achieve the stability of the bioactive compounds in the stomach and high release in the intestine.

Thus, this study aims to i) evaluate the release of EPA and DHA in different types of fish oil microcapsules, as a neat and delivered in different meat products, at the end of the oral, gastric and intestinal *in vitro* digestion phases and ii) calculate the bioaccessibility of these bioactive compounds.

For that, lecithin-maltodextrine (MO) and lecithin+chitosan-maltodextrine (MU) microcapsules of fish oil were produced by using spray-drying and added to two meat derivatives: cooked and dry-cured sausages (C-SAU and D-SAU, respectively).

Both types of microcapsules showed similar EPA (4–4.5 mg/g samples) and DHA (8–9 mg/g samples) quantities. The behaviour of releasing of EPA and DHA from MO and MU was similar, principally taking place at the intestine phase. However, the percentage of release of EPA and DHA during the whole in vitro digestion analysis was higher in MO than in MU, while their bioaccesibility was a bit higher in MU. This finding may indicate the major resistance of the multilayer structure of chitosan-maltodextrine to the gastric conditions than of the maltodextrine layer of MO.

The quantities of EPA and DHA in all meat samples (44–64 mg EPA + DHA/100 g sample) exceeded the level established by the European Union to label a food as "source of ω-3 fatty acids" (40 mg EPA + DHA/100 g product). The amount of EPA and DHA bioaccesible was higher in C-SAU-MU and D-SAU-MU batches (0.35 and 0.33 mg EPA + DHA per gram of sample digested), in contrast to C-SAU-MO and D-SAU-MO batches (0.25 and 0.24 mg EPA +DHA per gram of sample digested).

Therefore, the type of microcapsule of fish oil do not influence the EPA + DHA enrichment, but it did in their bioaccessibility, being better when using MU vehicles. This could point out the importance of analysing not only the quantity of EPA and DHA in the enriched food but also the bioaccesibility of these bioactive compounds in most products as possible.

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Keywords : microcapsules, omega-3 enrichment, meat products, release, bioaccessibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Oral Communication

(21484) - DIGESTION-RELEASED EGG PEPTIDES INDUCE GLP-1 SECRETION AND MODULATE POST-PRANDIAL GLYCEMIA

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Abstract

The intestine is considered the largest endocrine organ in the body due to the variety and the relevance of the hormones secreted at this level. Digestion products from nutrients promote the release of anorexigenic hormones and incretins, such as GLP-1, which is involved in glucose homeostasis regulation. Our previous studies showed that the peptide fraction from egg white digests exerts a potent GLP-1 secretagogue effect in the enteroendocrine cell line STC-1 (1).

The screening of different gastrointestinal-resistant peptides in STC-1 cells, showed the sequences from lysozyme (LZ), ^{109}VAWRNRCKGTD^{119} and ^{123}WIRGCRL^{129} to be potent GLP-1 and CCK inducers. To determine the key sequence, amino acid deletions and a peptide library based on ^{123}WIRGCRL^{129} was created. Our results showed a decrease in hormone release for the shorter form ^{125}RGCRL^{129}, and the relevance of ^{124}Ile and the two amino acids at the C-terminal end for cell activation. GLP-1 secretion induced by peptide ^{123}WIRGCRL^{129} and six alanine-substituted analogs was also assessed in mice jejunal organoids, along with an egg white gastrointestinal digest, and the amino acid Phe, as positive control. GLP-1 response induced by Phe was faster than that exerted by the whole digest or the LZ-derived peptide, but the peptide elicited similar GLP-1 response although it was tested at a 20 times-lower concentration than Phe (peptide at 1 mM *vs* Phe at 20 mM). Moreover, the results in jejunal organoids confirmed the relevance of the amino acid ^{124}Ile in the LZ-derived peptide, the action of peptidases on ^{123}Trp, and the involvement of ERK- and AMPK-mediated pathways in cell activation and GLP-1 secretion. Oral glucose tolerance tests in Wistar rats after oral administration of ^{123}WIRGCRL^{129} at 0.1 mM showed a significantly reduction of the blood glucose levels compared to the glucose overload group, while no changes were found in the group receiving the corresponding amino acid mixture at the same concentration. Although more studies are needed to elucidate the mechanism of action of these and other peptides released during digestion, our finding suggest the potential therapeutic application of egg white peptides against type II diabetes.

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Acknowledgments

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Keywords : GLP-1, mice jejunal organoids, egg white peptides, type II diabetes, enteroendocrine cells

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Oral Communication

(22625) - IN VITRO GASTROINTESTINAL RELEASE KINETICS OF VARIOUS AMINO ACIDS ENCAPSULATED IN SOLID LIPID PARTICLES

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Abstract

Many food compounds exert beneficial bioactivity at specific sites of the gastrointestinal tract, either through physical interactions with other molecules, or through biochemical metabolism. To optimize bioactivity, encapsulation strategies are developed for the targeted delivery of various compounds, which are usually protected from the gastric environment. The colon might be targeted, in which case the compounds are also protected in the small intestine.

In this study, our goal was to check whether encapsulated amino acids could pass through upper digestive tract with minimal release, thus potentially reaching the colon of piglet, which was the animal model in the research project. To do so, we investigated the release kinetics of various amino acids encapsulated in solid lipid particles through the mouth, stomach, and small intestine *in vitro*. To reflect the digestion of piglet, an Infogest static digestion method adapted to infant was used. Digestion experiments in the absence or presence of enzymes were conducted to test the role of lipolysis. Solid lipid particles of various sizes loaded with either branched-chain amino acids (valine + isoleucine + leucine), lysine, or tryptophan, were fully characterized (size, structure, thermal properties, composition).

Results show that gastrointestinal release of amino acids depend on solid lipid particle size, structure, and amino acid type. Although gastric release was usually high, larger solid lipid particles released amino acids more slowly than smaller ones. When solid lipid particles presented an ordered smooth surface, amino acid release was also slower than when their surface was disordered and coarse. Although lipolysis of solid lipid particles was high in the presence of enzymes, amino acid release was significantly different in the presence or absence of enzymes only in the case of tryptophan, the less hydrophilic compound. This means that the release of bioactive compounds in such encapsulation systems does not only depend on the hydrolysis processes, but most likely by their capacity to diffuse through the encapsulating structure into the aqueous medium.

References

Acknowledgments

Keywords : encapsulation, amino acids, lipid particles, gastrointestinal, release kinetics

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Oral Communication

(22795) - THE POTENTIAL HEALTH BENEFITS OF BETALAIN PIGMENTS – EVIDENCE FROM IN VITRO AND IN VIVO STUDIES

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Abstract

The consumption of beetroot and other betalain-containing foods has been associated with a number of health benefits such as lowering of blood pressure and benefits to diabetes and cardiovascular health, although the evidence underpinning some of these effects is very limited. In particular, the contribution of betalains, a group of red and yellow pigments, is unclear, with gaps in knowledge regarding their specific effects, their bioavailability, transformation and excretion. We have purified some of the main betalain pigments; and whilst cellular uptake was very low, all betalains were efficient to markedly alleviate cellular inflammatory response. However, only the red pigment group demonstrated marked radical scavenging capacity, indicating differing potential for oxidative stress alleviation. We have piloted the transformation of betalains during the gastrointestinal passage confirming their low systemic bioavailability and fast elimination in vivo. Urinary metabolome analysis following beetroot juice intake in humans showed dominance of betanin, the main red pigment, whereas stool analysis revealed severe pigment decomposition and higher inter-individual variation in fecal pigment composition compared to urine. Transient increases in *Akkermansia muciniphila* and reductions in *Bacteroides fragilis* species were observed after two-week beetroot juice intake, as well as rises in fecal butyric acid levels. Our research demonstrates the potential of betalain-rich food to modulate molecular mechanisms and to affect gut microbiota and metabolite profiles, with likely impact on human and animal health.

References

Keywords : beetroot, betalains, inflammation, bioavailability, microbiota

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Oral Communication

(21564) - ASSESSMENT OF THE PROTEIN QUALITY AND DIGESTIBILITY IN PLANT-BASED MEAT ANALOGUES

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Abstract

Nowadays, there is a high concern about food sustainability and animal welfare. In particular, there is an increasing number of consumers who approach plant-based diets for environmental or for ethical reasons. Furthermore, there is a general awareness about the need of producing more environmentally sustainable food. Therefore, the need to lean towards diets based on vegetable products is becoming evident and this lifestyle is already adopted by a good segment of the population. From this point of view, plant-based meat analogues, which have been developed in order to replicate its sensory qualities such as appearance, bite, mouthfeel, and taste , are becoming more popular on the market and they have become part of the diet of different groups of consumers. This study was performed on selected commercial products to evaluate the similarity level of the plant-based products examined – in terms of macronutrient composition, protein quality, and protein digestibility – with the meat products they intend to resemble. Moreover, new formulations of plant-based were tested in order to assess how the treatment carried out on protein and the addition of ingredients can affect the protein quality and digestibility of the final products. A complete molecular characterization, at a protein level, was assessed by using chromatographic and mass spectrometry technique. Digestibility studies were approached by applying INFOGEST static protocol. The study leads to conclude that some plant-based meat analogues can substitute meat, at least in terms of protein quality and digestibility. Anyway, being the supply on the market wide and vast and the composition of these products very heterogeneous, there is the need for careful choice by the consumer in order to select products with high quality. Furthermore, there is the need of more awareness that not all of these products have enough protein quality to meet the needs of all population groups. The consumption of some of these products to vulnerable segments of the population (such as children) could lead to replacing meat with a product of lower protein quality.

Keywords : meat analogues, protein digestibility, protein quality, amino acids, plant proteins

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Oral Communication

(21411) - VITAMIN BIOACCESSIBILITY OF FOODS FERMENTED WITH RIBOFLAVIN-OVERPRODUCING BIFIDOBACTERIA

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Abstract

Certain populations worldwide have vitamin deficiencies and thus, manufacturing fermented food matrices with probiotic cultures capable to enrich the content of vitamin, such as riboflavin, in the final product is an appealing alternative to prevent the issues associated with this vitamin deficiency. In our group, several riboflavin-overproducing derivative strains from two parental *Bifidobacterium longum* subsp. *infantis* after roseoflavina exposition were generated. The quantification of this vitamin by a HPLC, using a fluorescence detector, showed that the overproducing strains were able to release up to 1,000-fold more amount than the parental ones in culture medium. Two of the riboflavin producing strains, IPLA60012 and IPLA60015, and their corresponding parental ones, IPLA60011 and CECT4551^T respectivelly, were used to elaborate fermented drinks based on dairy or almond drink matrices. Overall, dairy matrix showed higher riboflavin concentrations and demonstrated better preservation of the bifidobacterial strains survival upon one month of refrigerated storage than the almond fermented product. IPLA60002 was the strain producing and maintaining the highest vitamin concentration under cold storage (fermented milk: 1.4 ±0.10 mg/L; fermented almond-beverage 0.34±0.02 mg/L). Besides, following simulated *in vitro* static digestion (Infogest protocol) of the biofortified products, between 59.8-84.6 % of the riboflavin present in the fermented foods were recovered, the highest occurring in the dairy matrix fermented with IPLA60012. Additionally, fermented milks also allowed a higher survival rate of the bifidobacteria in the three phases of static digestion than the vegetable-based product. The final percentage of survival was highly dependent on the strain, but IPLA60012 having the best performance. These results set the ground to facilitate the incorporation of riboflavin producing bifidobacterial cultures into fermented matrices. In one hand, biofortified foods can be obtained naturally enriched in riboflavin which is stable and bioaccesible under cold storage. On the other hand, the fermented foods protected bifidobacteria along the gastrointestinal passage which might also allow the *in situ* (colon) production of riboflavin. Finally, it is worth noting that the biofortified products were manufactured at 37°C in aerobic conditions, which is a key trait for the application of oxygen-sensitive bifidobacteria.

References

Acknowledgments

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Keywords : food digestion, biofortification, riboflavin, Bifidobacterium, probiotic

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Oral Communication

(21494) - MICROBIAL ENZYMES ENHANCE MACRONUTRIENT DIGESTIBILITY IN A DYNAMIC DIGESTION SIMULATION

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1 - BIO-CAT, Inc.; 2 - The TIM Company

Abstract

Background: Several clinical observational studies show that digestive function declines naturally with age beginning in the fifth decade of life. Oral enzyme supplementation is a candidate strategy to support digestion and nutrition in older adults. Dose-ranging experiments in a static in vitro simulation of gastrointestinal (GI) digestion helped define a mixture of six microbial enzyme preparations ("Macro Digest") that enhance protein, fat, and carbohydrate digestion from several mixed macronutrient substrates [1]. The objective of this study was to test Macro Digest's effects on digestibility and bioaccessibility of proteins and carbohydrates in a dynamic simulation of GI digestion. **Methods:** The tiny-TIMsg dynamic simulation system was used to model typical dynamics of postprandial gastric emptying, gastric pH, peristalsis, pepsin output, intestinal transit time, intestinal pH, and pancreatin output [2]. Aging was modeled by reducing pepsin and pancreatin output to 70% of standard fed-state conditions. A canned test meal (CTM) comprising canned chicken, peas, and instant potatoes was used as the substrate [1]. Macro Digest and a maltodextrin control were each investigated at a dose of 95.6 mg per 150 g CTM. Simulations proceeded for 6 hours to model the average upper GI tract transit time of a meal. Luminal samples were withdrawn from the gastric and small intestinal compartments every 15 minutes. Digested and soluble compounds were continuously removed from the intestinal compartment via a semi-permeable membrane (5–7 kDa pore size). Analytical testing of luminal and dialysate samples included spectroscopy to measure free amino nitrogen (FAN) and HPLC to measure amino acid (AA) and glucose concentrations. Given the exploratory nature of the study, only one experiment was performed per group. **Results:** Across the first 120 minutes of simulated CTM digestion, Macro Digest supplementation increased FAN, total AA, and glucose concentrations of gastric luminal samples by 2.3- to 5.3-fold, 2.5- to 3.4-fold, and 15.3- to 21.1-fold, compared to control. Notably, at 30 minutes into the simulation, gastric luminal essential AA and leucine concentrations were 4.6- and 9.5-fold higher with Macro Digest. Across the full 6 hours, Macro Digest increased intestinal luminal FAN, total AA, and glucose concentrations up to 2.7-fold, 33%, and 3.1-fold greater than control. Macro Digest also increased dialysate glucose concentrations across the entire simulation, as well as FAN dialysate concentrations over the first 2.5 hours, compared to control. **Conclusions:** A supplemental mixture of microbial enzymes enhanced protein and carbohydrate digestibility and bioaccessibility from a mixed meal under aging-adapted conditions of reduced pepsin and pancreatin output. These preliminary in vitro observations warrant replication and further study under standard digestion simulation conditions.

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Keywords : digestion, enzyme, protease, lipase, amylase

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TOPIC 3

BIOACCESSIBILITY/ABSORPTION OF BENEFICIAL AND HARMFUL COMPOUNDS

Portugal | April 9-11th, 2024

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Oral Communication

(21425) - ASSESSING RAT INTESTINE EXTRACT FOR POLYPHENOL DEGLYCOSYLATION IN APPLE TISSUE VIAINFOGEST

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Abstract

The study of *in vitro* polyphenol bioaccesibility using commercially available isolated and pure standards has allowed to unveil the interactions of polyphenols with the gastrointestinal tract and its implications on metabolic response. However, only polyphenol aglycones are commercially available at affordable cost to conduct digestion experiments. In edible plant tissues, polyphenols are predominantly glycosylated, and the attached sugar must be removed following consumption before absorption can take place. Lactase-phlorizin hydrolase (LPH), and maltase-glucoamylase (MGA) enzymes in the brush border of the small intestine epithelial cells are responsible for removing the attached sugar from polyphenols, which has been shown to have major implications on polyphenol absorption and binding to digestive enzymes and epithelial transporters. Despite the relevance of glycosylation on polyphenol absorption and bioactivity, most *in vitro* digestion protocols, including the standardized INFOGEST 2.0, omit the presence of brush-border enzymes. In this work, we investigated the bioaccesibility and hydrolysis of apple polyphenols during *in vitro* digestion with and without Rat Small Intestine Extract (RSIE) treatment. LPH, MGA and sucrase-isomaltase (SI) activities in RSIE were calculated via HPAEC-PAD. Whole apple, apple pomace and apple juice were selected as model systems representing materials containing hydroxybenzoic acids (HBAs), hydroxycinnamic acids (HCAs), flavanols, flavonols, and dihydrochalcones, and with different matrix effects. The semi-quantification of 36 polyphenol glycosides and their respective aglycones via UHPLC-ESI-QTOF-MS/MS analysis in the three apple fractions revealed changes in polyphenol profiles explained by polyphenol degradation and release from the plant cell wall matrix. Results showed a slight but significant deglycosylation of HCAs by pancreatin. Importantly, a notable deglycosylation of HCA and flavonoids was only observed when RSIE was present. The degree of HCA deglycosylation was ameliorated when the plant cell wall matrix was present. Importantly, the presence of RSIE significantly decreased the bioaccessibility of all polyphenol groups, explained by their physical entrapment and/or binding to the RSIE tissue. These results underline the importance of incorporating brush border digestion in polyphenol research. Nevertheless, the decrease in polyphenol bioaccessibility with RSIE suggests the need to produce affordable MGA and LPH in pure form for conducting *in vitro* digestion assays.

Acknowledgments

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Keywords : Brush Border Enzymes, Plant Cell Wall, Polyphenols, Lactase Phloridzin Hydrolase, Mass Spectroscopy

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Oral Communication

(21445) - AMYLASE ACTIVITY ASSAY EVALUATION AND OPTIMIZATION: AN INFOGEST INTERNATIONAL RING TRIAL

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Abstract

Background: The accurate characterization of digestive enzymes is a pre-requisite for conducting *in vitro* digestions that are physiologically relevant and comparable across different studies. α-amylases (of salivary and pancreatic origin) are key enzymes for starch digestion. At present, α-amylase activity is determined using a protocol based on a single-point measurement obtained at 20 °C (Bernfeld, 1955). This assay has long been used, however, no inter-laboratory comparison trials have yet been carried out, and previous work within INFOGEST Working Group 5 – Starch digestion revealed that there can be wide variations between the results obtained by different labs.

Aim: This inter-laboratory study aimed at testing and evaluating the repeatability and reproducibility of a newly-developed version of the protocol used to quantify α-amylase activity prior to in-vitro digestions.

Method: Human saliva pooled from 10 healthy adults (from Medix Biochemica) and three porcine enzyme preparations (pancreatic α-amylase and pancreatin from Sigma, and pancreatic α-amylase from Megazyme) were tested in 13 laboratories, from 12 different countries, across 3 continents. Amylase activity assays were performed according to a newly developed version of Bernfeld's protocol which is based on multiple time-point measurements. Other differences include the duration and temperature (37 °C) of incubations. A subgroup of 5 laboratories has also analysed all products at 20 °C using the new version of the protocol.

Results: Mean intra-laboratory coefficients of variation (CVs) between 7% and 13% were observed for the four products tested, at both incubation temperatures. Inter-laboratory CVs ranged between 9% and 21%. Similar results were obtained regardless of the type of instrument used for the incubations (thermoshaker with shaking *vs.* static incubation in a water bath). The amylolytic activity of each product at 20 °C and 37 °C differed by 3.5 fold (± 0.2).

Discussion and Conclusion: Previous tests within the working group, using the classic version of the protocol, resulted in high inter-laboratory CVs (ranging from 65% up to 85%). In the present study, inter-laboratory CVs were reduced up to four times, reflecting a significant improvement in the reproducibility of the assay. The newly-developed protocol is therefore recommended to ensure accurate determinations of amylase activity levels in future studies.

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Keywords : Amylase, Starch, Digestion, Saliva, Pancreatin

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Oral Communication

(21504) - DEVELOPMENT OF IN SILICO PREDICTION MODEL FOR TRUE ILEAL PROTEIN DIGESTIBILITY

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Abstract

Proteins are essential for optimal body development and physiological maintenance. Proteins are broken down into amino acids during digestion and only available after the small intestine absorbs the amino acids. In 2013, the Food and Agriculture Organization of the United Nations proposed a new scoring system, called the digestible indispensable amino acid score (DIAAS), to evaluate protein quality. Protein quality is considered important to understand its effects on human health. For instance, whey intake promotes muscle synthesis in older people. To calculate DIAAS, amino acid content and true ileal digestibility of the tested ingredient need to be determined. However, the digestibility assessment requires invasive animal experiments, which is costly and raises ethical issues. Furthermore, digestibility is considerably influenced by the types of food ingredients and processing or cooking methods.

This study developed an *in silico* digestibility prediction model for DIAAS evaluation considering the types of ingredients and processing or cooking methods. A random forest model was developed using existing data on protein digestibility and nutrient components of various ingredients. The explanatory variables were amino acid content, ingredient types, cooking or processing information, and nutrient composition, while the objective variable was the true ileal digestibility. Good prediction accuracy was verified through cross-validation. The feature importance analysis revealed that amino acid content and processing methods were the major parameters for predicting digestibility. To further understand the characteristics of each method, digestibility data were compared between *in silico, in vitro,* and *in vivo* models. *In silico* and *in vitro* models showed good predictivity for *in vivo* digestibility of animal proteins; however, differences were observed in the types of amino acids and ingredients with high predictivity in plant proteins. DIAAS values were calculated using *in silico* and *in vivo* digestibility models, and a good correlation was found between *in silico* simulation and *in vivo*-derived DIAAS.

This study demonstrates that protein digestibility can be predicted, and DIAAS can be evaluated conveniently and accurately using the *in silico* model, further useful in assessing the nutritional values of protein and amino acids in various foods, especially when combined with the *in vitro* model.

Keywords : protein digestibility, DIAAS, machine learning, in silico, database development

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Oral Communication

(21534) - GASTRIC DIGESTION OF SKIMMED MILK USING A NEAR REAL DIGESTIVE TRACT (NERDT)

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Abstract

The biomechanics of the gastrointestinal tract influence food disintegration and digestion in vivo. Most currently available dynamic digestion models lack, however, the ability of mimicking the oro-gastrointestinal morphology and contractions, possibly overlooking the effect of mechanical forces in the digestive process. Recently the NEar Real Digestive Tract (NERDT), a biomechanically-relevant digestion simulator equipped with a silicon real-size model of the human stomach has been developed. The simulator mimics the oro-gastrointestinal morphology and anatomical structures, biochemical environments, peristaltic contractions and dynamic aspects present in vivo in adult humans through a set of rollers that can be controlled precisely. In the present study, the NERDT was used to investigate the gastric digestion of skimmed milk in the presence and absence of pepsin. The objective was two-fold: to determine the suitability of the NERDT to reproduce the in vivo gastric digestion behavior of skimmed milk, and to evaluate the respective influences of pepsin and the gastric biomechanics on the overall gastric digestion kinetics. The trends observed in gastric emptying, pH and the final mass of milk curd remaining in the stomach were similar with and without pepsin. However, in the presence of pepsin, the gastric coagulation of milk tended to take place sooner, the particles appeared more fragmented, and more peptides were produced towards the end of the experiments. These results confirm the key role of pepsin on milk protein coagulation and further hydrolysis of the particles, but also illustrate that gastric emptying kinetics are predominantly governed by the gastric biomechanics. The experimental results were close to the expected results calculated based on in vivo data, hence demonstrating that the NERDT can be set to achieve desired gastric emptying curve and pH evolution curve.

Keywords : In vitro gastric model; Gastric digestion; Skimmed milk; Milk coagulation; Pepsin

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Oral Communication

(22585) - MIGUT - A SCALABLE TRIPLE-STAGE GUT MODEL SYSTEM

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Abstract

The triple-stage gut model (TSGM), developed by Gibson et al. [1], has been a fundamental tool for studying the gut microbiome *in vitro*, shaping our understanding of the critical role the microbiota plays in regulating host metabolism and physiology. This model allows for close control of experimental conditions, facilitates sampling of different regions of the colon and has a track record of predicting *in vivo* clinical outcomes. Despite its historical significance, the TSGM's design has remained largely unchanged since its inception in the 1990's, posing practical limitations on its scalability.

In an effort to address the shortcomings of the TSGM, we present MiGut, a system consisting of four miniaturised gut models [2]. We have re-engineered the TSGM to improve reliability, automation, and scalability, while preserving key features such as number of vessels, pH control, and retention time. We demonstrated equivalence between MiGut and the TSGM by performing a side-by-side study with all models running under identical conditions. Here, we demonstrated that the microbial ecologies (measured via qPCR) were highly similar under steady state conditions and had comparable dynamics when exposed to perturbations (in this case a series of antibiotics). Moreover, we have shown that replicate MiGut models are highly repeatable and recapture the microbial compositions of different faecal inocula (measured via 16S rRNA sequencing). MiGut is now used across multiple laboratories at the University of Leeds for cutting-edge research into how nutrition, disease, and novel therapeutics affect the gut microbiome.

With MiGut, dozens of triple-stage bioreactors can be run simultaneously, leading to enhanced insights through biological replicates and complex studies. Furthermore, the reduced working volume means that a single-donor faecal inocula can be used compared with larger models that require a pooled sample. This will allow for inter-individual microbiota differences to be studied *in vitro*, leading the way to more targeted healthcare and nutrition. By expanding our ability to generate clinically reflective *in vitro* data, this technology will help us understand how various factors – such as nutrition, antibiotics, infection, therapeutics, and supplements – impact the microbiota. Ultimately, MiGut is a key tool in this exploration that will lead to a more comprehensive view of the gut microbiome.

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Keywords : chemostat, gut model, engineering design, bioreactor, microbiome

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Oral Communication

(22619) - IN SILICO EXPLORATION OF GASTRIC CHEMO-FLUID DYNAMICS: A FOCUS ON PROTEIN HYDROLYSIS

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Abstract

Protein plays a vital role as a necessary nutrient in our diet, and its digestion initiates within the stomach. The primary digestive enzyme responsible for protein breakdown in the stomach is pepsin, which is secreted by gastric chief cells. Utilizing pepsin, food proteins are enzymatically cleaved into smaller fragments, a process known as protein hydrolysis. Gaining a comprehensive understanding of the biomechanical processes involved in gastric mixing and hydrolysis, including the inherent variability in gastric motility among individuals, holds great potential in the field of nutrition and the design of future foods.

Studying gastric chemo-fluid dynamics through experimental methods presents numerous challenges. In vitro models fail to accurately replicate the complex wall motion and chemical response to food, whilst in vivo approaches are usually impractical and constrained by ethical considerations. Nevertheless, recent advancements in computational power and non-invasive imaging techniques have opened up novel possibilities for studying gastric chemo-fluid dynamics. In silico modeling of several processes in the stomach have been conducted previously to assess various aspects of gastric motility. Existing Computational Fluid Dynamics (CFD) models have not evaluated the effect of variations in stomach anatomy and contractions among the individuals.

Subject-specific CFD models of gastric motility were developed in this study utilizing stomach geometry and motility data obtained from Magnetic Resonance Imaging. These models were also used to simulate protein hydrolysis. The mixing, breakdown and emptying of a liquid meal, representing a protein shake made with water, was simulated over a span of 90 minutes. Pepsin was secreted from the stomach walls and its pH dependent activity was predicted by this model. Gastric acid was also secreted from the stomach walls which leads to a spatially and temporally varying pH within the stomach. Subsequently, the rate of protein hydrolysis was compared between the two different subjects.

Keywords : Computational models, Proteins, Computational fluid dynamics

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Oral Communication

(22636) - AN INFOGEST INTERNATIONAL CONSENSUS STATIC IN VITRO DIGESTION MODEL ADAPTED TO THE GENERAL OLDER ADULT POPULATION AND ITS APPLICATION TO DAIRY PRODUCTS

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Abstract

Understanding the mechanisms of food digestion is of paramount importance to determine the effect foods have on human health. Significant knowledge on the fate of food during digestion has been generated in healthy adults due to the development of physiologically-relevant *in vitro* digestion models. However, it appears that the performance of the oro-gastrointestinal tract is affected by ageing and that a model simulating the digestive conditions found in a younger adult (<65 y) is not relevant for an older adult (>65 y). The objectives of this work were: (1) to conduct an exhaustive literature search to find data on the physiological parameters of the older adult oro-gastrointestinal tract, (2) to define the parameters of an *in vitro* digestion model adapted to the older adult, (3) to apply it to the digestion of dairy products. International experts have discussed all the parameters during a dedicated workshop organized within the INFOGEST network. Data on food bolus properties collected in the older adult were gathered, including food particle size found in older adult boluses. In the stomach and small intestine, data suggest that significant physiological changes are observed between young and older adults. In the latter, the rate of gastric emptying is slowed down, the pH of the stomach content is higher, the amount of secretions and thus the hydrolytic activities of gastric and intestinal digestive enzymes are reduced and the concentration of bile salts lower. The consensus *in vitro* digestion model of the older adult was applied to the digestion of two fermented dairy products formulated with a ratio of whey proteins to caseins of 80/20 and 20/80. Results showed that the digestion conditions used (young vs. older adult) influenced significantly the kinetics and extent of proteolysis in the gastric phase but not in the intestinal phase.

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Keywords : elderly, static digestion, INFOGEST, dairy

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Oral Communication

(22664) - GETTING THE BILIARY SURFACTANTS RIGHT FOR PHYSIOLOGICAL RELEVANCE OF IN VITRO DIGESTION

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Abstract

A series of extensive studies undertaken over the recent years by our research group has provided a comprehensive analysis of the role of major biliary surfactants, specifically bile salts (BSs) and phospholipids (PLs), in the intestinal digestion processes. Our meticulous *in vitro* experimentation has revealed how the interplay between these surfactants at various BS/PL ratios crucially impacts both the intestinal lipolysis and proteolysis.

Our findings demonstrate that the presence of BSs and PLs synergistically enhances the lipolysis process, with a significant increase in efficiency observed at specific BS/PL ratios. Notably, a ratio of over two-fold excess of BSs to PLs was identified as most physiologically relevant and particularly effective in replicating the physiological conditions of the human small intestine, leading to a significant reduction in triglyceride oil-water interfacial tension and enhanced lipolysis rates. In the *in vitro* proteolysis study, the magnitude of protein digestion by intestinal proteases was also highly dependent on the presence of both BSs and PLs in the simulated digestion environment.

In exploring the role of these surfactants in intestinal digestion processes, we have shown that the total concentration of BSs is more crucial than their individual composition in both lipolysis and proteolysis. This discovery holds substantial implications for the design of physiologically relevant *in vitro* models. A separate study, which involved the use of real human bile, has established that simple mixtures of individual BSs and PLs can accurately simulate the complex behavior of human bile, thus providing a practical and effective approach for replicating human digestion conditions *in vitro*. This is particularly relevant for *in vitro* models, where the use of difficult-to-obtain human bile is often very problematic.

Our studies have been crucial in revealing the complex dynamics of BS and PL interactions at the molecular level during digestion. Overall, the findings highlight the critical importance of accurately mimicking the physiological conditions of the human gastrointestinal tract in *in vitro* digestion studies. Our results indicate distinct roles of BSs and PLs in lipolysis and proteolysis, strongly suggesting that any future *in vitro* studies aiming to simulate human digestion should take into account the impact of biliary PLs – not just BSs – to accurately mimic the physiological role of bile in intestinal lipolysis and proteolysis.

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Acknowledgments
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Keywords : Biliary surfactants, Intestinal lipolysis, Proteolysis, Bile salt/phospholipid ratio, In vitro digestion models

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Oral Communication

(22672) - THE IN VITRO DYNAMIC DIGESTION : A SUITABLE MODEL TO MIMIC THE IN VIVO DIGESTION OF INFANT FOODS IN TERMS OF FOOD DECONSTRUCTION AND PROTEIN HYDROLYSIS

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Abstract

Human infants are preferably fed human milk (HM), but a majority still receive infant formula (IF) as a HM substitute. Optimization of IF is still required to improve HM biomimetics, including digestion behavior biomimetics. While this can be studied *in vivo*, such experiments have to be reduced and appropriate *in vitro* models are needed. The present study aimed to compare food deconstruction and protein digestion of HM vs. IF using two infant digestion models, the mini-piglet and the *in vitro* dynamic gastrointestinal system (DIDGI®). Mini-piglets (Yucatan) were fed either a mature HM (n=9) or a standard IF (n=9) during 6 days. Piglet digesta were collected along the digestive tract 30 min after the last meal. The same foods were digested in triplicate using a term infant *in vitro* dynamic model with regular digesta sampling along time. Microstructure (confocal microscopy and laser diffraction) and protein digestion (SDS-PAGE, hydrolysis degree, peptidomics) were investigated in both digestion models. Data were statistically analyzed thanks to ANOVA and multidimensional analyses (hierarchical classification and multiple factor analyses). The microstructure of the digesta differed between HM and IF in a similar manner *in vitro* and *in vivo* along digestion. The meal dilution and emptying were similar between both digestion models, with a faster emptying for HM. Proteolysis, as investigated by SDS-PAGE, were similar between digestion models, with a lower hydrolysis level for HM caseins. Peptide mapping along the sequence of the major proteins was well correlated between models, particularly in the stomach and the proximal jejunum ($r > 0.6$). Similar result was found for bioactive peptide release. The ratio between bioaccessibility (in vitro) and bioavailability (in vivo) of amino acids was high (50-80%) at the cleavage sites of the pancreatic enzymes, more precisely for Arg, Tyr, Lys, Phe and Leu, but was much lower for the other amino acids (<30%). In overall, the *in vitro* dynamic gastrointestinal digestion model well predicted the *in vivo* digestion of HM and IF, particularly for protein hydrolysis, peptidomics and food deconstruction, while further improvement is needed to better correlate bioaccessibility and bioavailability.

References

Acknowledgments

Keywords : human milk, infant formula, digestion model, digestion kinetics, proteolysis, bioactive peptides

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Oral Communication

(22706) - INTERNATIONAL STANDARDIZATION PROCESS OF THE IN VITRO DIGESTIBILITY METHOD BASED ON THE STATIC INFOGEST METHOD

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Abstract

The FAO recommends the digestible indispensable amino acid score (DIAAS) as a measure of protein quality, which is determined in *in vivo* experiments on human or pig. However, these are costly and raise ethical concerns. In addition, a rapid and reproducible *in vitro* method for measuring protein quality is needed for product development.

In the previous COST Action INFOGEST, a standardized static *in vitro* digestion protocol was established, validated in inter-laboratory experiments and tested for its physiological relevance for human and pig digestion. Based on this static INFOGEST method, an analytical workflow was developed to quantify *in vitro* protein digestibility and to calculate DIAAS and proxy DIAAS, and the results were highly comparable to human and pig *in vivo* data. The standardization process within the International Dairy Federation (IDF) and the International Standardization Organization (ISO) was initiated with the aim of establishing a harmonized and validated *in vitro* digestibility method that allows the calculation of *in vitro* DIAAS. An initial international collaborative study with five dairy products and a technical school enabled the protocol to be further improved.

Thanks to these measures, a second collaborative study (N= 15) with seven substrates (five dairy products and two plant sources) achieved a significant reduction in variability (CV < 10 % for total digestibility) and provided data on the repeatability and reproducibility of the method. The IDF/ISO standard method for *in vitro* digestibility will help manufacturers to evaluate protein quality during product development and will also be a useful tool to compare the quality of different protein sources.

Acknowledgments
The authors would like to thank all the participants for their support in improving the protocol and their participation in the two collaborative studies.

Keywords : in vitro Digestion, Protein digestibility, Protein quality, in vitro DIAAS

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Oral Communication

(22729) - PIONEERING IN VITRO MODELS OF THE IMMATURE GUT BARRIER FOR ADVANCING INFANT FORMULA

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Abstract

Newborns have a leaky gut barrier, a key feature for nutrients and immune modulators absorption in the first few days of life. Yet, prolonged permeability poses health risks. Breast milk contributes to maturing the infant gut barrier, reducing permeability. To advance next generation infant formula that can mimic breast milk functionality, this project aims at improving *in vitro* leaky gut barrier models (1) to assess infant formulas for their ability to reduce permeability.

Day 25 polarised Caco-2/ HT29-MTX monolayers were treated with different concentrations (0.5, 0.8, 1 mM) of the bile salt sodium glycodeoxycholate (GDC). Treatment with GDC notably reduced Trans-Epithelial Electrical Resistance (TEER) compared to media alone ($P<0.01$), without compromising cell viability. Moreover, GDC-treated monolayers (0.8, 1 mM) exhibited higher permeability to lactulose and mannitol ($P<0.05$), similar to newborn babies. Alcian Blue staining indicated higher acidic mucins in 0.8 and 1 mM GDC monolayers compared to control ($P<0.01$). At 1 mM GDC treatment, mRNA transcripts of tight junction proteins Zonulin-1 and Occludin were significantly increased compared to controls ($P<0.05$). However, intriguingly, at 0.8 mM GDC, Occludin immunofluorescence microscopy revealed a lower intensity ($P<0.01$) compared to control monolayers, together with a lower colocalization with actin, indicating an altered cell structure. Effect of GDC was reversible, as monolayers recovered 8 hours after removal of GDC (ie TEER values increased). 0.8 mM GDC was selected for the subsequent analysis.

To investigate the ability of infant foods to improve the barrier function of 0.8 mM GDC treated monolayers, 3 commercial infant formulae (milk, soya and amino acid based) and breast milk (pooled, 6 donors) were subjected to static *in vitro* gastrointestinal digestion, using the INFOGEST protocol adapted to the infant gut (2). BCA (protein content), OPA (free amino groups), SDS-PAGE and the analysis of free and total amino acids in the bioavailable fraction of the digesta showed that the dairy and soya infant formulae had comparable protein digestibility to breast milk. The exposure of GDC treated and untreated monolayers to infant formulae digesta did not modify TEER, but results were similar to breast milk digesta ($P>0.05$).

Ongoing work involves a full assessment of the GDC monolayers exposed to Infant formula and breast milk digesta, including Occludin immunofluorescence microscopy and colocalization with actin after digesta exposure, real-time TEER measurements and profiling of amino acids /peptides absorbed through the monolayer.

Establishing robust *in vitro* models for this life stage represents an important step in the development of infant formulae that promote gut barrier maturation. GDC-treated Caco-2/HT29-MTX monolayers offers an *in vitro* gut barrier with increased permeability that is reversible.

References

1(Kondrashina et al., 2021)

[2](Ménard et al., 2018)

Acknowledgments

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Keywords : sodium glycodeoxycholate, Caco-2/ HT29 MTX, in vitro newborn gut barrier, breast milk and infant formula

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Oral Communication

(22781) - EVIDENCE OF INCREASED GLUTEN-INDUCED PERTURBATIONS IN THE NUCLEOPHILIC TONE AND DETOXIFYING DEFENCES OF INTESTINAL EPITHELIAL CELLS IMPAIRED BY GASTRIC DISFUNCTION

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Abstract

The food digestive process is very complex, as each digestive phase have different enzymes involved and all this is dependent of an efficient symbiotic interaction of the gastrointestinal tract, the mucosa and the gut microbiota. Modifications in these parameters can lead to differences in the digestive process, which in turn can contribute to variations in the food matrix - peptide sequence and size - promoting variations in terms of immunogenicity and toxicity to the intestinal epithelium [1]. It has been increasingly demonstrated over the past few years that some proteolytically resistant gluten peptides may directly affect intestinal cell structure and functions by modulating pro-inflammatory gene expression and oxidative stress . The relationship between oxidative cell damage and Celiac Disease (CD) is supported by several studies on human intestinal epithelial cell lines, such as the Caco-2 cell model, already shown to be particularly sensitive to the pro-oxidative and pro-apoptotic properties of gluten protein digests. Through providing valuable evidence concerning some of the pathophysiological mechanisms that may be at play in gluten-related disorders, most of these *in vitro* studies have been employing simplified peptic-tryptic/chymotryptic digestion schemes and intestinal cell systems that do not fully resemble mature enterocytes in terms of their characteristic tight junctions, microvilli and membrane transporters. Herein the peptide profile and pro-oxidative effect of two different gastrointestinal gliadin digestions was thoroughly characterized and comprehensively compared: one following the complete INFOGEST workflow and a second one by-passing gastric processing, to assess the dependence of gliadin-triggered downstream cell effects on pepsin activity. In both matrices, gluten-derived immunogenic peptide sequences were identified by non-targeted LC-MS/MS. The present study provides important first-hand data concerning the still unexplored peptide composition, gastric-dependence, and immunogenicity of physiologically representative gliadin protein digests, revealing the generation of many different gliadin peptides, some of which potentially immunoreactive *in vivo*. Moreover, this study highlights the necessity of employing more complex and integrated *in vitro* cell systems when modelling and exploiting gluten-induced perturbations in the nucleophilic tone and inflammatory status of intestinal epithelial cells. [2]

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[1] DOI:10.3390/nu10091129;

[2] DOI:10.1016/j.foodres.2023.113317

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Keywords : Celiac disease, Oxidative stress, Gluten peptides, Gluten Digestion

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TOPIC 4

IN VITRO, IN VIVO AND IN SILICO MODELS OF DIGESTION AND ABSORPTION

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Topic 4: Food structures, digestion and imaging technologies | Oral Communication

(21508) - FIBRE , REGARDLESS OF ITS STRUCTURE, INCREASES PYY RELEASE

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Abstract

Ileum is an important player in appetite regulation. It contains a high density of L-cells that secrete appetite supressing hormones Glucagon like Peptide-1 (GLP-1) and Peptide YY (PYY) in response to luminal contents including nutrients and bacterial fermentation products short chain fatty acids (SCFAs)[1]. Food structure can impact the digestion of foods and therefore molecules arriving to ileum which can impact bacterial fermentation and appetite hormone release[2]. For example, plant foods with intact cellular structures can act like barriers to digestive enzymes and increase intracellular nutrient delivery to ileum which can increase appetite hormone release. However, access to human ileum is difficult and little is known about the interplay between food structures, ileal environment and appetite hormone release.

To understand the impact of food structures on ileal environment and appetite hormone release, a randomised crossover trial was conducted. Healthy human volunteers (n=10) were admitted as inpatients for 4 days and randomly assigned to one of the three intervention diets: High fibre, intact structures (I-HF); High fibre, disrupted structures (D-HF) or Low fibre, disrupted structures (LF). A nasoenteric tube was inserted into the distal ileum for sample collection from intact human ileum. On day four, ileal and blood samples were collected (fasted and every 60min for 480min postprandially).

We demonstrate for the first time the highly dynamic, wide ranging molecular environment of the intact human ileum over time. The numbers of bacteria and the concentrations of SCFAs in the ileum dramatically dropped from fasted to postprandial states in all groups which was replaced by dietary metabolites. Ileal microscopy pictures demonstrated that only I-HF diet delivered intact cells with intracellular starch to ileum. Despite this, both I-HF and D-HF, regardless of their structure, increased PYY release compared to a low fibre diet during 0-240min (vs D-HF P=0.005 and vs I-HF P=0.012). This was associated with increased fullness in D-HF (p=0.028) and reduced composite appetite score in I-HF group (p=0.024) compared to LF at 120min. High fibre diets also increased ileal stachyose and disrupted high fibre diet increased certain ileal amino acids compared to the low fibre diet. Treatment of human ileal organoids with ileal fluids or amino acid and stachyose mixture stimulated PYY expression in a similar trend to blood PYY levels, confirming the role of ileal metabolites in PYY release.

These data demonstrate that dietary fibre, independent of its structure, induces metabolite profile changes in the ileum that relate to the release of PYY. . This can lead to the design of diets and food products that can target ileal metabolic pool to promote satiety and act as a weight management strategy.

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Keywords : Ileum, Food Structure, Appetite Regulation, Nasoenteric Tube, fibre

Topic 4: Food structures, digestion and imaging technologies | Oral Communication

(21552) - INTRAGASTRIC BEHAVIOR OF AN EXPERIMENTAL INFANT FORMULA MAY BETTER MIMIC HUMAN MILK THAN A CONTROL FORMULA

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Abstract

Background: During breastfeeding the macronutrient composition of breastmilk changes gradually from relatively low-fat (foremilk) to relatively high-fat (hindmilk), initially exposing the gastrointestinal tract to a relatively low fat concentration. In contrast, infant formulae (IF) are consumed homogenous. Mild processing and addition of milk fat globule membrane (MFGM) may impact gastric emulsion instability, potentially impacting the phased release of nutrients into the intestine as observed with breastfeeding.

Objective: This study compared gastric emulsion stability, gastric emptying, and the postprandial plasma metabolome of an experimental minimally processed IF (EF) with an altered fat-globule interface with that of a control IF (CF).

Methods: Twenty healthy males participated in this double-blind randomized crossover trial. Gastric MRI scans and blood samples were obtained before and after consumption of 600 ml CF or EF over a 2-h period. Outcomes included gastric top layer formation, total gastric volume, and blood parameters (FFA, insulin, glucose, and NMR-metabolomics).

Results: The EF showed an earlier onset (13.4 min, p=0.017), smaller maximum volume (49.0 ml, p= 0.033), and a shorter time to maximum top layer volume (13.9 min, p=0.022), but similar top layer volume over time AUC (p=0.915) compared to CF. Total gastric volume did not show a treatment*time effect. Insulin concentrations were lower after EF. FFA and glucose did not differ. The EF yielded higher serum concentrations of phospholipid- and cholesterol-related metabolites likely affected by the addition of MFGM.

Conclusion: A mildly processed, experimental infant formula with the addition of MFGM displayed faster gastric creaming compared to a control formula, thereby potentially better mimicking the behavior of breastmilk which leads to phased release of nutrients into the intestine. Overall physiological benefits of this difference in gastric behavior remain to be studied further in infants.

Keywords : MRI, Emulsion stability, Fat layer, Gastric emptying, Infant formula

Topic 4: Food structures, digestion and imaging technologies | Oral Communication

(21528) - QUANTIFYING INTESTINAL LIPOLYSIS WITH MAGNETIC RESONANCE IMAGING USING FRESH CREAM

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Abstract

Understanding lipid digestion is crucial for health strategy development in managing energy intake and nutrient bioavailability. Lipolysis kinetics can be studied by assessing the chemical composition, which requires sample purification and lipid extraction. For online monitoring of lipolysis, non-invasive methods like MRI remain to be developed.

A recent study suggested that the MRI water-fat separation method enables quantifying undigested lipids, but not lipolytic products (Musse et al., 2023). To find out the mechanism behind this and to assess the feasibility of monitoring lipolysis using this approach, the lipid quantification by MRI during *in vitro* intestinal digestion of a commercial fresh cream was supplemented by thin-layer chromatography (TLC) for quantifying lipolysis and Time Domain (TD)-NMR for characterization in details of transverse (T_2) relaxation times.

The TLC analysis showed that around 96% of the triacylglycerols (TAG) was hydrolysed into lipolytic products (including free fatty acids (FFA), diglycerides (DAG), and monoglycerides (MAG)) after the *in vitro* intestinal digestion. The MRI results demonstrated that the loss of lipid signal correlated with the degree of lipolysis of the samples. The TD-NMR results showed a remarkable difference in the T_2 of undigested lipids (~120 ms; in this study, mostly TAG) and that of lipolytic products (~2 ms; FFA, DAG, and MAG). The very short T_2 of lipolytic products is likely due to the semi-crystalline structures they formed with bile salts (micelles, vesicles, liposomes, etc.) and they were suspended in the aqueous phase. Notably, The MRI water-fat separation method is not able to capture the signal from such fast-relaxing protons (short T2), which explains why the signal of lipolysis products cannot be detect. Nonetheless, the MRI method proves effective in quantifying lipolysis by monitoring the decreasing amount of undigested lipids during *in vitro* digestion. Moreover, the MRI imaging parameters could even be optimized to acquire rapid mapping on lipolysis in 13 seconds (within a breath hold), opening up many opportunities for future *in vivo* applications.

In conclusion, magnetic resonance techniques are potential methods for investigating digestion processes. MRI water-fat separation method can quantify the lipolysis of cream during *in vitro* digestion and may serve as a promising method in studying real-time lipolysis of other foods in the gastrointestinal digestive tract, both *in vitro* and *in vivo*.

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Keywords : Lipolysis, Magnetic Resonance Imaging (MRI), Time-domain Nuclear Magnetic Resonance (TD-NMR), Water-fat separation method, Transverse relaxation time (T2)

Topic 4: Food structures, digestion and imaging technologies | Oral Communication

(22777) - IMPACT OF THE MICROSTRUCTURE OF TWO BEEF CO-PRODUCTS ON POSTPRANDIAL PLASMA AMINO ACID KINETICS

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Abstract

The increasing demand for proteins is driving the search for alternative food sources. From this point of view, the valorization of co-products, such as meat co-products that are little valorized in human nutrition, is a promising way, in line with the principle of the circular economy. The present study focused on two protein ingredients of bovine origin, co-products of the fat rendering process, namely Greasy Greaves Recovered Proteins (GGRP) and Water Recovered Proteins (WRP), previously shown to display valuable but different functional properties. The aim of the present study was to evaluate the nutritional quality of these proteins and the relationship between the structure of these two meals and the postprandial plasma amino acid (AA) kinetics. The structure adopted by these two meals during the gastric digestion was investigated using an *in vitro* semi-dynamic model (INFOGEST). Gastric digesta were collected at 0, 40, 80 and 120 min; their microstructure was analyzed by confocal microscopy, particle size distribution by laser diffraction, and their viscosity was measured by oscillation test. The nutritional quality and postprandial AA kinetics were determined *in vivo* on ten growing pigs, cannulated at the ileal level and catheterized in the jugular vein, and receiving over a 2.5-day period one of the two experimental meals according to a cross-over design. Ileal digesta and blood samples were collected during the 9 postprandial hours and AA contents were analyzed by ion-exchange chromatography. Data were statistically analyzed using linear models.

WRP and GGRP showed similar, moderate true ileal protein digestibility (81-84%, $p>0.05$). However, due to their different amino acid profiles, the DIAAS was much lower for WRP than for GGRP with values of 18 vs. 74%, respectively, with Trp being the first limiting AA for both protein sources. The plasma AA concentration reached its maximal value between 3 h and 5 h postprandial for WRP and GGRP, thus qualifying both protein ingredients as slowly digested sources. GGRP tended to have a slower appearance rate of plasma AA. This could be explained by the higher viscosity of this meal (25 to 35 times higher) than that of WRP meal ($p < 0.05$). Nevertheless, the viscosity of both meals sharply decreased during digestion by an equivalent factor (around 34), from 1.3 to 0.04 Pa.s at 10 s^{-1} for WRP, and from 35 to 1 Pa.s for GGRP. In addition, the GGRP digesta contained a higher proportion of very large particles than the WRP ones. The higher viscosity and larger particle size for GGRP digesta than for WRP ones could explain the trend for the slower rates of digestion and absorption of GGRP. Overall, the GGRP bovine co-product appears to be an interesting source of dietary protein for human, while WRP has mainly valuable functional properties, but few nutritional properties.

References

Acknowledgments

Keywords : beef co-product; protein; in vivo; in vitro; digestion; structure

Topic 4: Food structures, digestion and imaging technologies | Oral Communication

(21444) - CONTROLLING WHITE RICE GLYCAEMIC INDEX BY STRUCTURING WITH GELLAN GUM: MRI STUDY IN VIVO

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Abstract

Background: White rice is a staple food in many parts of the world and its consumption has been increasing. However, white rice has a high glycaemic index and consumption of white rice has been linked to increased risk for obesity and type-2 diabetes mellitus. In recent *in-vitro* studies we have shown that addition of food hydrocolloids such as low acyl gellan gum (LAGG) to the cooking process of white rice has potential to control starch digestion kinetics [1]. Mechanism may involve formation of a protective layer on the rice grains that reduces rice bolus break down and enzymatic hydrolysis but effectiveness *in-vivo* remains to be investigated.

Objectives: to determine the effect of adding LAGG to jasmine white rice on postprandial glycaemic, gastrointestinal and appetitive responses in humans.

Methods: 12 healthy adults, mean age 26±2 years and BMI of 23±1 kg/m^2, participated in a randomised, controlled, crossover study. They consumed isoenergetic meals of jasmine rice (232 kcal) cooked with and without 3% (weight over dry rice weight) LAGG. Blood glucose level was measured using fingerpick method. Intragastric meal appearance and volume were assessed using magnetic resonance imaging (MRI) and appetite questionnaires were collected at fasting baseline and postprandially for 120 min.

Results: all 12 participants completed the study. The incremental area under the curve (iAUC 2 hours, iAUC2h) for blood glucose for the white rice + LAGG meal (93 ± 16 mmol/L · min) was significantly lower than that for the white rice control meal (160 ± 18 mmol/L· min), P=0.0007. Blood glucose rose postprandially to a peak at T=30 minutes, with the rice control meal peak (7.3 ± 0.2 mmol/L) significantly higher than that for the rice + LAGG meal (6.5 ± 0.2 mmol/L), P < 0.01. The MRI images showed that when rice was cooked with LAGG there were multiple rice boluses persisting throughout the digestion time and that postprandial gastric volumes were lower compared to those of rice control, though the difference was not significant. There were no significant differences in composite appetite score AUC2h between meals.

Conclusions: Modifying the cooking process of jasmine white rice with LAGG was effective in reducing blood glucose responses in healthy humans. The modification of the cooking process is simple and relatively inexpensive. If confirmed, this could potentially provide an intervention to help reduce the post prandial glycaemic response to white rice, potentially impacting on the rising levels of obesity and type 2 diabetes seen in populations consuming white rice as a staple food.

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Acknowledgments

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Keywords : Imaging, blood glucose, stomach, jasmine rice, low acyl gellan gum

Topic 4: Food structures, digestion and imaging technologies | Oral Communication

(21413) - SCATTERING TECHNIQUES TO STUDY THE NANOSTRUCTURE AND DIGESTION MECHANISM OF NOVEL FOODS

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Abstract

The relationship between structure, functionality and bioavailability is extremely important for a rational design of novel food products with improved nutritional and techno-functional properties. Although the digestibility and bioaccessibility of food products can be estimated through *in vitro* models, the nanostructural changes taking place during the gastrointestinal digestion process are still not completely understood. For instance, the nanostructural assembly of the digestion products through intermolecular associations or by interaction with components present in the physiological medium have not been studied to date. In this context, small angle scattering techniques represent an extremely powerful tool, since they allow investigating the nanostructure of food systems and their digestion products in their native hydrated state.

In this work, some examples will be shown to demonstrate how small angle X-ray scattering (SAXS), combined with complementary techniques such as microscopy, spectroscopy and rheology, can provide very useful information on the nanostructures formed upon gastrointestinal digestion of different types of food systems, such as hybrid protein-polysaccharide hydrogels and different seaweed-based products. Our results evidence that the composition and multi-scale structure of the food products, which can be tuned by processing, have a strong effect on the digestion mechanism. Interestingly, the digestion products can interact with the bile salts present in the intestinal phase, leading to the formation of different types of structures, such as lamellae, micelles and vesicles. Furthermore, the presence of dietary fibres has a major impact not only on the protein digestibility, but also on the nanostructural assembly of the released peptides. This is expected to be key to understand the intestinal transport of the digestion products and determine the nutritional quality of novel protein sources and ingredients.

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Acknowledgments

This research was funded by the European Union (ERC, PRODIGEST, 101086483).

Keywords : nanostructure, hydrogels, seaweeds, scattering, proteins

ICFD 2024

TOPIC 5

IMPACT OF DIET ON GUT MICROBIOTA

Portugal | April 9-11th, 2024

Topic 5: Impact of diet on gut microbiota | Oral Communication

(22744) - PROTEIN INGREDIENT QUALITY OF INFANT FORMULAS IMPACTS GUT PHYSIOLOGY AND MICROBIOTA IN MINI-PIGLETS USED AS A HUMAN INFANT MODEL

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Abstract

Infant formulas (IFs), the only adequate substitute to human milk, are complex matrices that require numerous ingredients and processing steps. Previously, we showed that the quality of the dairy protein ingredients within IFs modulated protein microstructure and *in vitro* and *in vivo* digestive kinetics (protein digestion and amino acids plasma concentration). Therefore, the aim was to assess the consequences on gut maturation and microbiota, an important actor within the microbiota-gut-brain axis.

Three isonitrogenous IFs were formulated with whey proteins from different origins (cheese whey: IF-A, vs. ideal whey: IFs-B/C) and casein with different organizations (micellar: IFs-A/B, vs. non-micellar: IF-C). Twenty-four Yucatan mini-piglets (2- to 21-day-old), used as an infant model, received one of the three IFs. Digestive contents, faeces, and tissues were analysed using metagenomic, histological, ex vivo permeability and gene expression approaches and a metabolomic analysis was done on serum. Univariate and multivariate statistical analyses were performed.

Piglets fed with IF-C had a significantly higher colonic paracellular permeability than those fed with IF-A, which was also associated with a slight immune boost, potentially as the result of increased antigens passage through the gut barrier stimulating the mucosal immune system. Colonic transcellular amino acid transporters were less expressed in piglets fed with IF-C than with IF-A which could be the result of the increased paracellular permeability with IF-C, favouring paracellular transport. These results suggested a combined effect of whey origin and casein supramolecular organization on intestinal physiology in favour of IF-C, whose parameters were closer to those recently reported for human milk-fed piglets (Charton *et al*., 2022). Even though gut microbiota composition was moderately changed between diets, faecal short-chain fatty acid composition differed according to the whey protein origin, with higher butyrate concentration for ideal whey than for cheese whey. Differences in microbiota fermentative activity may result from differences in digestive kinetics previously observed *in vitro* between cheese whey-based IF (A) and ideal whey-based IFs (B and C), which could modulate the colonic substrate available for the microbiota. Serum metabolomic analysis showed that Trp metabolic pathway was different between IF-A-fed piglets and IF-C-fed piglets with higher serum concentrations of Trp, kynurenine and 3-indole acetic acid in IF-C-fed piglets than IF-A, IF-B being intermediate. Seric polyamines, bacterial metabolites from protein digestion, were also more concentrated in piglets fed with IF-C than IF-A.

This study suggests that the use of ideal whey and the modulation of casein supramolecular organization are possible avenues to keep improving IFs towards more human milk biomimetics.

Keywords : Infant formula, Protein ingredient quality, Gut physiology, Microbiota, Animal model

Topic 5: Impact of diet on gut microbiota | Oral Communication

(21527) - MODULATORY EFFECTS ON GUT MICROBIOTA OF SIMULATED GASTROINTESTINAL DIGESTS FROM MICROALGAE

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Abstract

As a result of the continued increase in the world's population, the current dietary pattern established by international health organizations and consumers is changing towards the consumption of foods that improve health and have a positive impact on the environment. The relationship between the gut microbiota and human health is being more clinically and scientifically recognized, making it a key target in the study and treatment of many different pathological conditions [1]. Among the promising sources of sustainable functional foods, microalgae have attracted attention due to their exceptional nutritional and biological value and minimal environmental impact [2]. Microalgae can be used to produce valuable molecules such as proteins, polyunsaturated fatty acids, essential amino acids, antioxidants, vitamins, minerals and fiber. Despite the health benefits attributed to microalgae, the evidence on the effects that these organisms and the compounds released during their transit through the gastrointestinal tract can exert on gut microbiota is still limited. The aim of this work was to evaluate the impact of simulated gastrointestinal digestion of four microalgae species, *Arthrospira platensis, Chlorella vulgaris, Tetraselmis chuii* and *Nannochloropsis gaditana* on intestinal microbiota as analyzed through colonic fermentation. Intact and pre-treated (combination of freeze-thaw cycles and ultrasounds) microalgae biomasses were subjected to gastrointestinal digestion simulating physiological conditions. The non-absorbable fraction (NAF) of the gastrointestinal digests was separated through centrifugation and subjected to simulated static colonic fermentation, collecting samples at 0, 24, 48, and 72 h. The effects on microbial populations, protein content, short-chain fatty acids (SCFAs) and ammonium production was evaluated. Additionally, a metataxonomic analysis was performed at 0 and 48 h, identifying different bacterial taxa through massive sequencing of the 16S rRNA gene. The NAF showed a time-dependent reduction of protein content and consequent increase of the ammonium production, thus indicating that microalgae protein was used as nitrogen source for the microbiota growth. The NAF also exerted potent modulating effects on the microbial population and SCFAs production. Precisely, NAF from *N. gaditana* digest significantly stimulated the production of butyric acid, the recognized intestinal health biomarker. The metataxonomic analysis revealed modulatory effects of microalgae digests on the composition and distribution of the bacterial gut microbiota, mainly over the growth of phyla associated with chronic inflammatory processes. Overall, the prebiotic effects and the generation of postbiotic products of these species reinforce the potential of microalgae as promising food ingredients with gut microbiota modulatory properties.

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Acknowledgments

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Keywords : in vitro digestion, microalgae biomass, colonic fermentation, gut microbiota

Topic 5: Impact of diet on gut microbiota | Oral Communication

(21410) - BRAZILIAN PROPOLIS MICROENCAPSULATION: EFFECTS ON BIOLOGICAL ACTIVITIES AND GUT MODULATION

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Abstract

Brazil is a major producer and world exporter of propolis from *Apis mellifera*. Propolis is a resinous substance produced by bees, which is associated with a myriad of biological properties, such as antimicrobial, anti-inflammatory, and especially antioxidant. Although propolis is legally considered a food in several countries, its consumption is restricted to the form of aqueous or hydroalcoholic extracts due to its characteristic bitter taste, which makes it challenging to apply it as a functional ingredient in foods. Thus, the objective of this work was to microencapsulate an organic propolis extract using arabic gum and vegetal fat as carriers using a spray-dryer equipment to preserve the health benefits associated with propolis intake and minimize its undesirable sensory characteristics. The developed particles were evaluated by an *in vitro* simulation of gastrointestinal digestion, based on the INFOGEST protocol, and a cell model for transepithelial transport. The scavenging activity of reactive oxygen species (ROS) in Caco-2 cells, as well as the chemical composition determined by HPLC-PDA, were evaluated in the ethanolic extract of propolis, whether encapsulated or not during the digestion and cell transport assays. The prebiotic potential effect was evaluated *in vivo* for 14 days in isogenic mice fed every day with both the particles and non-encapsulated propolis extracts and then the genomic analysis of their faecal microbiota was performed. As results, the HPLC-PDA analyses showed that the release of phenolic compounds in the propolis particles occurred gradually during the gastrointestinal digestion. The cellular antioxidant potential of the intestinal fraction for the propolis particles was higher ($p < 0.05$) than for the non-encapsulated propolis indicating that the process of encapsulation protects the compounds against the digestion conditions. Regarding the microbiota assay, a modulation effect of the microbiota in animals treated with the propolis particles (10 mg/kg) and the non-encapsulated extract (10 mg/kg), when compared with to the control animals (phosphate buffer) was observed, including a decrease in the abundance of Gammaproteobacteria class. That gram-negative bacteria are commonly related as immunostimulant which can make the organism more susceptible to infections. The results of both treatments showed a significant increase in gram-positive bacteria of the genus *Ligilactobacillus*, with a probiotic potential by its anti-inflammatory effects. In conclusion, there is evidence that propolis has beneficial potential to a positive modulation of microbiota. The antioxidant activity was better preserved with the encapsulation process, which represents a promising technology to include propolis in foods as a functional ingredient.

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Keywords : caco-2, bioactivity, phenolic compounds, natural products, prebiotic

Topic 5: Impact of diet on gut microbiota | Oral Communication

(22770) - UNRAVELLING THE FULL THERAPEUTICAL POTENTIAL OF POLYUNSATURATED FATTY ACIDS: A COMPLETE STUDY OF OMEGA-3 AND CONJUGATED FATTY ACIDS BIOACCESSIBILITY, BIOAVAILABILITY PREDICTION AND IMPACT IN GUT MICROBIOTA.

Salsinha, Ana Sofia (Portugal)[1,2]; Araújo-Rodrigues, Helena (Portugal)[1,2]; Cima, André (Portugal)[1]; Machado, Manuela (Portugal)[1]; Rodríguez-Alcalá, Luis Miguel (Portugal)[1]; Relvas, João B. (Portugal)[2,3]; Pintado, Manuela (Portugal)[1]

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Abstract

Omega 3 EPA and DHA are polyunsaturated fatty acids with relevant health benefits. Conjugated linoleic and linolenic acids are known for their anti-carcinogenic effect, anti-inflammatory properties, and body weight reduction [1]. To achieve therapeutical doses, high amounts of these fatty acids' food sources must be consumed. Thus, the intake of enriched oils with a high concentration of these fatty acids is often used. But several factors influence their bioavailability.

The **first step** of this study consisted of using the INFOGEST *in vitro* protocol of gastrointestinal tract digestion. It was assessed the bioaccessibility of these fatty acids in different matrixes: Pomegranate and Fish oil and Omega-3, CLA, and CLNA soft-gel enriched capsules. After digestion, the recovery index for the major fatty acids is very low: Pomegranate oil is 2%, Fish oil 11-13%, CLNA 17%, CLA 6%, and Omega 3 capsules 3%. Higher initial concentrations of these PUFAs seem to be related to higher degrees of oxidation. Importantly, bioaccessibility studies of similar matrixes are very scarce and absorption studies are absent in most of the works. Consequently, in the **second step** of this work intestinal permeability studies were performed using a size exclusion method, resorting to 3.5 kDa dialysis membranes, and a model of Caco-2/HT29-MTX co-culture. In the co-culture model, it was observed that a significant incorporation of bioactive fatty acids into the intestinal cells, which may affect their permeability performance. Interestingly, in both models, most fatty acids remain in the non-bioaccessible fraction (colon-available), which may be relevant in gut microbiota modulation.

Gut microbiota has been demonstrated to play an important role in the maintenance of a general good health condition [2] and there is a great potential for diet to modulate it. Polyunsaturated fatty acids' impact on gut microbiota is still poorly defined. Thus, the **third step** of this study consisted of using an *in vitro* human fermentation model to determine the role of omega 3, CLA isomers, and punicic acid on microbiota modulation in healthy human donors. Fish oil, Omega 3, and CLA samples presented a positive impact on *Akkermansia* spp. and *Bifidobacterium* spp. growth. Moreover, all the samples supported *Roseburia* spp. growth after 24 h of fermentation and, importantly, they were able to maintain the Firmicutes: Bacteroidetes ratio near 1. All the bioactive fatty acids samples, except Pomegranate oil, were able to significantly increase butyrate levels compared to those found in the positive control (FOS) sample. Moreover, Fish oil and Omega 3 samples were able to increase the concentration of GABA, alanine, tyrosine, phenylalanine, isoleucine, and leucine between 12 and 24 h of fermentation, which may be relevant in gut-brain axis modulation.

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Acknowledgments

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Keywords : omega-3, conjugated linoleic acid, conjugated linolenic acid, bioaccessibility, gut microbiota

Topic 5: Impact of diet on gut microbiota | Oral Communication

(22666) - PARTICLE SIZE OF BARLEY HUSK INFLUENCES PHENOLIC ACID RELEASE, AS WELL AS MICROBIOTA SUCCESSION AND SHORT CHAIN FATTY ACID PRODUCTION IN VITRO

Ntouranidi, Sofia (Netherlands)[1,2]; Fryganas, Christos (Netherlands)[1]; Zoetendal, Erwin G. (Netherlands)[2]; Smidt, Hauke (Netherlands)[2]; Fogliano, Vincenzo (Netherlands)[1]; Capuano, Edoardo (Netherlands)[1]

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Abstract

Barley husk is a cereal by-product rich in dietary fiber and phenolic acids, and its use as ingredient in food formulation have potential benefits for human health. The physical structure of fibre-rich ingredients is often neglected as a potential factor able to affect fibre behavior in the large intestine. In this study, we evaluated the effect of milling, as a simple way for manipulating physical structure, on fibre fermentability, release of phenolic acids as well as in the modulation of gut microbiota composition. Barley husk was milled in four fractions of different particle sizes ranging from 50 to 1000μm. The fractions were pre-digested with the INFOGEST protocol and fermented *in vitro* with pooled human fecal inoculums from five healthy donors. Microbial activity was determined for up to 48h by measuring short chain fatty acids and gas production. Total bacterial load and microbiota composition were determined with 16S rRNA gene-targeted qPCR and 16S rRNA gene amplicon sequencing, respectively. Twelve hydroxybenzoic and hydroxycinnamic acids and several metabolic products of phenolic acids metabolism were quantified by LC-MS/MS. Apart from butyrate, which was abundant after fermentation of the coarsest particles at 48h, compared to the other particle sizes, no significant effect of milling barley husk was observed on SCFA and gas production. Reduction of particle size increased the level of bound phenolics and their bioaccessibility, especially for ferulic and coumaric acid during *in vitro* digestion. Ferulic acid, amongst others, was released during fermentation and converted to phenylpropionic and phenylacetic acid derivatives. Preliminary analysis of the microbiota composition showed distinct differences in succession among the different particle sizes. The results indicate that physical structure manipulation of barley husk could potentially stimulate different microbial populations and affect metabolite production.

Topic 5: Impact of diet on gut microbiota | Oral Communication

(21492) - TRYPTOPHAN CATABOLISM BY GUT MICROBIOTA IS MODULATED BY THE FOOD MATRIX AND THE DIET

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Abstract

Gut microbiota has been widely acknowledged for its impact on human and animal health. In recent years, microbiota-derived tryptophan (Trp) catabolites have gained increasing attention because they can activate the aryl hydrocarbon receptor (AhR), indirectly enhancing intestinal epithelial barrier function, and contributing to intestinal homeostasis. In this work, we aimed to gain a better understanding of the effects of dietary factors on the modulation of Trp catabolism by gut microbiota and the effect of such modulation on the activation of the AhR. Through a combination of in vitro models of colonic fermentations, pig models, chromatography coupled with mass spectroscopy and cell assays, we demonstrated that the level and the pattern of microbial Trp metabolites: 1) depends on the accessibility of tryptophan to microbial cells (i.e. how Trp is presented in the large intestine, e.g. whether free, locked in proteins or within intact plant particles); 2) differs along the length of the gastrointestinal tract (i.e. among small intestine, proximal and distal colon) and 3) it is modulated by the composition of the diet, particularly by the presence of dietary fibre. Activation of the AhR cannot be easily predicted based on the level and pattern of Trp metabolites because of synergistic/antagonistic effects in mixtures of metabolites as well as interference from other dietary components. The results of this investigation help understanding the relationship between diet and microbial metabolites in general, and of tryptophan catabolites in particular, and the modulating effect of the food and matrix and the diet on gut health.

Keywords : tryptophan catabolites, food matrix, dietary fibre, aryl hydrocarbon receptor

Topic 5: Impact of diet on gut microbiota | Oral Communication

(22592) - IMPLICATIONS OF POLYPHENOL BINDING ON THE PREBIOTIC EFFECT OF PLANT CELL WALLS AND RESULTING CATABOLITES

Bechtner, Julia Doris (Denmark)[1]; Lopez-Rodulfo, Ivan Misael (Denmark)[1]; Gallego-Lobillo, Pablo (Denmark)[1]; Hosek, Jiri (Denmark)[2]; Schwab, Clarissa (Denmark)[2]; Martinez-Martinez, Mario (Denmark)[1]

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Abstract

Polyphenols (PPs) can interact with and bind to cellulose, pectin, and other components of the Plant Cell Wall (PCW), with most PPs in fruits and vegetables being bound to the PCW when it is ruptured either during consumption or processing. These interactions can be strong enough to alter PP profiles of the resulting fractions upon processing (e.g., cold pressing) and digestion, where 15-100 % of ingested dietary PPs may traverse the small intestine when bound to PCWs. Thus, PCWs can play an important role in transporting PPs to the proximal colon, where microorganisms have the potential to release and metabolize these compounds. Yet, it is still elusive whether PP-PCW interactions can cause sustained changes in microbiota community composition and activity, and if this could affect the prebiotic potential of dietary fibers. Moreover, due to the high diversity of PPs and PCWs, studies on the three-way interaction among PPs, PCW polysaccharides and the gut microbiota are often highly reduced in complexity. In this work, we synthesized multicomponent cellulose hydrogels using *Komagataeibacter xylinus* while incorporating pure pectin and different concentrations of purified tamarind xyloglucan to improve our understanding of PP-PCW interactions. Furthermore, the effect of xyloglucan, a major non-cellulosic polysaccharide of dicot PCWs and often disregarded in the generation of PCW models, on PP binding was studied. The dynamics and partitioning profiles of PP binding during soaking in a PP extract from apple was studied via UHPLC-ESI-QTOF-MS/MS, which identified 43 different PPs. Hydroxybenzoic- and hydroxycinnamic acid glucosides and flavonol glycosides were the most abundant PPs bound to the PCWs. *In vitro* batch fermentations conducted at physiologically relevant concentrations using fecal slurries as inoculum were performed on selected three-component PCWs, with and without bound PPs. Microbiota composition and fermentation activity were monitored by 16S rRNA gene sequencing and short chain fatty acid (SCFA) analysis, respectively. We detected a decrease in bound PPs during fermentation with a concomitant generation of compounds identified by untargeted mass-spectroscopy related to PP catabolism. Changes in microbiota composition and SCFA formation were mostly attributable to the addition of PCWs. This work thus not only deepens our understanding on the transport of PPs to the colon but also provides novel insights into the microbial response to PPs non-covalently bound to a food matrix.

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Acknowledgments

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Keywords : gut microbiota, food waste, apple, polyphenol bioaccessibility, dietary fiber

Topic 5: Impact of diet on gut microbiota | Oral Communication

(22695) - EFFECT OF PESTICIDE USE AND SPROUTING INHIBITOR ON POTATO CROPS ON GUT MICROBIOTA AND HEPATIC GENE EXPRESSION IN MICE

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1 - Université de Lille; 2 - Junia

Abstract

Purpose: The widespread consumption of potatoes is attributed to their easy availability and affordability. While potatoes provide numerous benefits, concerns arise due to their high carbohydrate content, often associated with health issues like obesity and diabetes. Nevertheless, they are rich in health-promoting compounds such as ascorbic acid, vitamins (C, B3, B6), proteins, minerals, and dietary fibers. Resistant starch, found in potatoes, acts as a beneficial nutrient for the gut microbiome. However, pesticides are commonly used during potato crop cultivation to combat diseases like mildew. Additionally, sprouting inhibitor treatments are employed for extended potato storage. Despite the known health hazards associated with pesticides, limited research has been examined on the combined effect of pesticide mixtures within food matrices. This study aims to explore the influence of pesticide treatments during crop cultivation and sprouting inhibitor treatments during storage, on the gut microbiota population and hepatic gene expression.

Methods: Lyophilized bio or conventional potato with or without sprouting treatment or water was administered through orogastric gavage in mice over a 3-week period. Then, mice were euthanized to collect organs like the liver. Feces were collected before the initial gavage and after 3 weeks of gavage. Nutrigenomic analysis was conducted on liver RNA, and metagenomic analysis was performed on feces.

Results: Findings from this study demonstrated notable modifications in gut microbiota and liver gene expression in mice following the consumption of potato bio or conventional, with or without storage (sprouting inhibitor treatment).

Conclusion: This study highlights the effect of pesticide treatment and storage of potato on both gut microbiota population and gene expression modulation in the liver.

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Keywords : Potato, Gut microbiota, Gene expression, Health, Pesticide mixtures

Topic 5: Impact of diet on gut microbiota | Oral Communication

(22647) - CAN PHLOROTANNINS NAVIGATE THE GASTROINTESTINAL TRACT? UNRAVELING GUT MICROBIOTA MODULATION AND STRUCTURAL TRANSFORMATIONS

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Abstract

Seaweeds have been a staple in human nutrition for centuries, serving as a rich source of bioactive compounds, particularly phlorotannins, an exclusive group of polyphenols found in brown algae with promising health benefits [1]. Despite their recognized potential, the fate of phlorotannins when crossing the gastrointestinal tract, their impact on gut microbiota, and the generation of potential metabolites remain poorly understood. This study aims to unveil novel insights into the modulatory effects of phlorotannin extracts on gut microbiota and explore their stability and transformations during gastrointestinal transit.

Following the INFOGEST protocol and subsequent fermentation with human colonic bacteria, a modest, yet positive influence on gut microbiota growth was observed, with Enterococcus spp. displaying the most significant response. Additionally, phlorotannin extracts exhibited the interesting ability to elevate propionate and butyrate levels.

The amount of phlorotannins reaching the colon was, however, substantially diminished compared to the initial content of non-digested extracts. The progressive loss of phlorotannins during each stage of gastrointestinal digestion (GID) suggests susceptibility to structural transformations that can consequently affect their bioactive properties. UHPLC-ESI-MSn analysis revealed notable differences in phlorotannin profiles between non-digested and digested extracts, particularly in fuhalol-type compounds, which were entirely absent after GID. Nevertheless, both non-digested and digested extracts exhibited potent inhibitory effects on cellular NO● production in LPS-stimulated Raw 264.7 cells. This implies that, despite reduced phlorotannin concentration, metabolites formed during digestion may exert their effects through modulating intracellular signaling mechanisms.

In summary, this study sheds light on the journey of phlorotannins through the gastrointestinal tract. While lacking an evident prebiotic effect, phlorotannins demonstrated positive modulatory effects on gut microbiota, contributing to overall gastrointestinal health. The susceptibility of phlorotannins to structural transformations, leading to the loss of specific compounds, does not necessarily compromise their bioactivity, particularly anti-inflammatory effects, most likely owing to the potential bioactive effects of the metabolites resulting from the transformation of these phenolics.

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Acknowledgments

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Keywords : Phlorotannins, Gut microbiota, Metabolites, Seaweeds, INFOGEST

ICFD 2024

POSTER PRESENTATIONS

Portugal | April 9-11th, 2024

ICFD 2024

TOPIC 1

ORAL PROCESSING AND SENSORY PROPERTIES OF FOODS

Portugal | April 9-11th, 2024

Topic 1: Oral processing and sensory properties of foods | Poster

(21474) - INFLUENCE OF EATING CAPABILITY AND ORAL PROCESSING DIFFICULTIES ON NUTRIENT INTAKE.

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Abstract

Food oral processing is frequently compromised by aging-related limitations, including the loss of teeth and declines in oro-masticatory muscle performance[1]. These factors contribute to chewing difficulties and, consequently, malnutrition[2]. Despite these known effects, the quantitative relationship between chewing difficulty, instrumentally measured oro-muscular forces, denture status, and their associations with nutrient intake and overall nutritional status are rarely investigated.

Aims: To explore the associations of denture status with objectively assessed biting force, self-perceived chewing difficulties, nutrient intake, and nutritional status of community-dwelling older adults in Thailand.

A total of 148 older adult, aged 64 to 90 years old (mean: 71.9 ± 6.0 years), within the Bangkok Metropolitan Region and Chonburi Province completed a self-assessment of chewing ability The chewing difficulty of 20 common food items (five difficulty grades of texture) were rated using a 3-point Likert scale. The maximum biting force of posterior teeth was measured as the objective assessment, and denture status was self-reported. Nutritional status was assessed based on the body mass index of the participants, while nutrient intakes were obtained using 24-hour dietary recalls.

The results indicate that participants who did not wear dentures and had more than 11 natural teeth rated their chewing ability significantly higher than those without dentures but had fewer than 11 natural teeth ($p < 0.001$) and those with complete dentures ($p < 0.001$), particularly for food items with a difficulty grade higher than three. Similarly, the maximum biting force was significantly lower in these groups ($p < 0.001$) compared to those with more than 11 teeth, implants, and partial dentures. While chewing ability showed a weak positive correlation with the maximum biting force among older adult participants, there was no correlation with nutritional status. Positive correlations with the maximum biting force were found for daily energy intake, protein intake, and fat intake, although these correlations were negligible.

The findings suggest an association between denture status, self-perceived mastication ability, and maximum biting force. The reduced biting force in individuals with dentures may limit the consumption of food groups with certain textures, potentially affecting the overall nutrient intake in this vulnerable group.

References

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Acknowledgments
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Keywords : Older adults, Nutrition, Denture status, Biting force, Chewing difficulty

Topic 1: Oral processing and sensory properties of foods | Poster

(22624) - IN VIVO STUDY COMBINING SENSORY ANALYSIS AND ULTRASOUND IMAGING TO INVESTIGATE FOOD TEXTURE PERCEPTIONS RELATED TO TONGUE BIOMECHANICS

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Abstract

All along food oral processing, the tongue continuously evaluates the mechanical and structural properties of foods, all by guiding them towards the different organs involved in the formation of the bolus. The tongue thus steers the oral strategy, adapting to food type, individual preferences, and physiological constraints, playing an important role in sensory experience and swallowing safety.

In the present study, sensory analysis was coupled with ultrasound (US) imaging to better understand the links between tongue motions and texture perceptions. Six commercially available semi-liquid foods (chocolate desserts) were selected for their diverse textures (including mousses, gels and creams). Sensory profile and temporal dominance of sensations were investigated with a trained panel of 16 volunteers. The mechanical interactions between food and an artificial tongue and palate were characterized on a biomimetic test bench embedding multi-axes force sensors, an accelerometer, and a linear US imaging probe. Feasibility US imaging measurements were performed on 10 volunteers, using a convex US probe positioned under the chin.

Highly contrasting mechanical and sensory properties could be confirmed across the different food products. Image processing methods were developed on *in vitro* US imaging datasets to track the evolution of the dorsal surface of the artificial tongue (interface tracking algorithm) and characterize the deformation fields within the artificial tongue (particle image velocimetry). Tongue movement profiles were linked to food rigidity, while shear velocity fields in the bulk of the tongue correlated with friction and adhesion phenomena between the tongue and the palate. High inter-individual variability was reported in the morphology and contrast level of the US images from *in vivo* experiments. Even though the implementation of image analysis processing methods was challenging, the feasibility of tracking the dorsal surface of the tongue has been demonstrated. The type and number of tongue movements as well as the duration of the oral processing before food swallowing were characterized.

The results underscore the potential of US imaging for monitoring and characterizing tongue biomechanics during oral processing, providing valuable insights into the diverse mechanical responses of different food products and their impact on sensory experiences.

Keywords : Texture, Tongue, Biomechanics, Ultrasound imaging, Sensory analysis

Topic 1: Oral processing and sensory properties of foods | Poster

(22632) - MICROSTRUCTURE OF WHEY PROTEIN GELS INFLUENCES IN VITRO GASTRIC PROTEIN DIGESTION AFTER ORAL PROCESSING

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Abstract

Gastric protein digestion is influenced by food structure and oral processing. The microstructure and mechanical properties of whey protein gels affect *in vitro* gastric protein digestion. However, after mastication, the extent to which these gel characteristics influence digestion remains unclear. We hypothesised that the impact of microstructure on *in vitro* whey protein gastric digestion is reduced after mastication due to the increase in total surface area caused by macrostructural breakdown being the determinant of protein digestion. This study investigated the influence of oral processing of whey protein gels differing in microstructure and mechanical properties on *in vitro* gastric digestion. Whey protein isolate was mixed with κ-carrageenan at various concentrations to obtain heat-induced gels with four distinct microstructures (homogeneous, coarse stranded, protein continuous, bi-continuous). Gel boli at the moment of swallowing were pooled from 14 healthy adults and characterized for number and size of bolus fragments. Static *in vitro* gastric digestion of gels before mastication and expectorated gel boli after mastication were conducted following the INFOGEST 2.0 protocol with minor modifications. Before mastication, coarse stranded gels showed the highest digestion rate (1.57 mmol·L^{-1}·g dry $matter^{-1}$/h), while homogeneous, protein continuous and bi-continuous gels showed significantly ($p < 0.05$) lower and similar digestion rates (1.10-1.23 mmol·L^{-1}·g dry $matter^{-1}$/h). After mastication, the total surface area of coarse stranded gels increased 7.9-fold leading only to a 1.6-fold increase in digestion rate. In contrast, a 3.4-fold increase in total surface area of bi-continuous gels caused a 2.4-fold increase in protein digestion. For homogeneous and protein continuous gels, the total surface area increased 3.1- to 3.6-fold upon mastication resulting in a 1.5- to 1.7-fold increase in digestion rate. This study emphasised the role of microstructure in protein gastric digestion after oral processing.

Keywords : protein digestion, microstructure, oral processing

Topic 1: Oral processing and sensory properties of foods | Poster

(22638) - EFFECT OF MASTICATION ON IN VITRO PROTEIN DIGESTIBILITY OF PEA PROTEIN-BASED MEAT ANALOGS

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Abstract

Background and objectives: Chewing contributes to determining nutritional benefits of foods by affecting gastro-intestinal digestion (1). Chewing creates different size and type of bolus particles depending on the food type (2, 3). It has been shown that chewing times and bolus properties of a soya-based chicken analog significantly affected *in vitro* digestion of protein (4). We aimed to study whether incorporation of a mastication phase instead of mechanical cutter influences subsequent *in vitro* degree of protein hydrolysis (DH %) of pea protein-based meat analogs.

Methods: Three extrudates and one baked sample were prepared as follows: Pea protein isolate (PPI) was wet extruded using a temperature profile of either 140/160/160/140/100/80/70/60 °C (**PPI140**), 125/150/150/135/100/80/70/60 °C (**PPI125**), or 90/100/100/100/100/80/70/60 °C (**PPI90**). PPI was also mixed with water (30:70) (**PPIpwd**) and baked in oven until reaching inner temperature of 90°C. Chicken fillet was boiled and acted as a control. For the *in vitro* oral phase, all the food samples were manually chopped into small pieces using a cutter to simulate mastication. Three replicates of the samples were normalized to a protein content of 17 % in 5 grams by diluting with water and digested using the standardized INFOGEST model with oral, gastric, and intestinal phases (5), without amylase in the oral phase.

Five selected masticators with healthy dental status, and predetermined average chewing times to decrease individual variation, chewed 4 grams of each food sample until feeling ready to swallow. The expectorated boluses were pooled and normalized to a protein content of 8.5 % in 10 mL by diluting with water. Then, digestion was continued starting from the gastric phase of the INFOGEST model. DH % was calculated based on released amino groups in *o*-phthalaldehyde (OPA) measurement.

Results: There was no difference in DH % among the food samples when using the standardized *in vitro* oral phase. After the mastication phase, the DH was the lowest (20 %) for the PPI90 with significant differences to PPI125 (28.6 %, p=0.028; paired samples t-test) and to chicken (22.7 %, p=0.015). Also, the DH of PPI125 differed from that of chicken (p=0.034). For chicken, the DH was 33.4 % and 22.7 % after *in vitro* and *in vivo* oral processing, respectively (p=0.013; one-way ANOVA followed by Tukey's *post hoc* test). The differences were not explained by chewing duration or amount of excreted saliva.

Conclusions: Mastication of meat analogs and chicken had different influence on DH % from *in vitro* oral processing in the INFOGEST model. The relatively low DH percentages may be explained by saturation of digestive enzymes in the model. Microscopy of the foods and particle size of the boluses is under investigation to elucidate if structural differences and consequent particle size after chewing explain the observed effects in protein digestion.

References

Kim et al 2022; Minekus et al 2014; Vanhatalo et al 2022; Chen et al 2021; Brodkorb et al 2019

Acknowledgments

We thank Eero Päiväkumpu for extrusions, sample preparation, and assistance in the mastication, and Tarja Wikström for performing in vitro protein digestibility based on INFOGEST model.

Keywords : chewing, bolus, protein digestibility, meat analog, particle size

Topic 1: Oral processing and sensory properties of foods | Poster

(22704) - DEVELOPMENT AND VALIDATION OF AN IN VITRO COLON MODEL FOR EFFICIENT GUT MICROBIOME INTERVENTION SCREENING: A CASE STUDY WITH ENGINEERED ANTI-TNFA PRODUCING E. COLI

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Abstract

Abstract

In recent years, the impact of the human gut microbiome on human health and disease has been acknowledged. Consequently, novel *in vitro* and *in vivo* methods have been developed to explore the potential of treating gut microbiome-related diseases. In this context, we introduce an *in vitro* colon model designed to serve as an economical and rapid screening tool in microbiome research. The model comprises of 12 parallel anaerobic reactor units, each with a working volume of 10 mL. The fermentations are supplemented with nutrient-rich colon media and incubated with fecal matter. Temperature is kept stable at 37°C and magnetic stirring ensures homogeneity. pH is closely monitored and regulated for each fermentation by the addition of NaOH. Additionally, within each fermentation unit, a small plastic compartment coated with mucin-soft agar is submerged in the fecal slurry. This allows the formation of a distinct microbiological environment, mimicking the mucus layer in the intestine. As an additional feature of the model, three parallel continuous fermentations enable the investigation of interventions on the gut microbiome over an extended period, providing insights into more long-term effects.

Using this *in vitro* colon model, both batch and continuous fermentation experiments were conducted to assess the colonization capability of an engineered probiotic *E. coli* strain within a complex microbiome obtained from a healthy human fecal donor. It was demonstrated that the strain was able to produce a human anti-TNFa compound. These findings were comparable to an *in vivo* mouse study, confirming this *in vitro* colon model is suitable as a pre-screening tool for *in vivo* mouse studies.

Keywords : gut microbiome, in vitro colon model, Anti-TNF-alpha, E. coli

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(22710) - (GLYC)OXIDATION DURING BARBECUING AND IN VITRO GASTROINTESTINAL DIGESTION OF HME-PEA AND PORK BALLS

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Abstract

Introduction

Plant-based meat alternatives have become increasingly popular for various reasons, however, their nutritional quality and possible health implications are poorly investigated. Oxidative reactions occur during thermal processing and gastrointestinal digestion of foods, leading to the formation of various biologically active metabolites derived from lipid- and protein oxidation and glyc(oxid)ation. In this study, we investigated the oxidative and glyc(oxid)ative stability of model extruded-pea balls and pork balls during heating with various sugars and/or herbs, and during subsequent simulated gastrointestinal digestion.

Materials and methods

Ground high-moisture extruded pea isolate (HME-pea) was mixed with 5% coconut oil, 10% rapeseed oil and 9% starch. Minced pork shoulder was mixed with 13% lard and 2% starch. Thereafter, 3% glucose, sucrose, or starch and 0% or 0.5% herbs mixture were added respectively. Equal-sized balls of each treatment were manufactured manually, and barbecued at 260°C for 16 min with flipping every 4 min. The heated samples were then subjected (in quadruplicate) to an *in vitro* gastrointestinal digestion model. Raw, heated and digested samples were assessed for 1) Glyc(oxid)ation (α-oxoaldehydes: glyoxal (GO), methylglyoxal (MGO), 3-deoxyglucosone (3-DG), UPLC-MS/MS; protein-bound and free N^{ε}-(carboxymethyl)lysine (CML), N^{ε}-(carboxyethyl)-lysine (CEL) and N^{d}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1), UPLC-MS/MS; protein-bound pentosidine (PEN), HPLC-FLD); 2) Lipid oxidation (4-hydroxy-2-nonenal (4-HNE) and hexanal (HEX), HPLC-FLD; thiobarbituric acid reactive substances (TBARS), spectrophotometry); 3) Protein oxidation (protein carbonyl content (PCC), spectrophotometry).

Main findings

Gastrointestinal digestion of HME-pea and pork balls resulted in increased levels of α-oxoaldehydes, free AGEs, protein-bound PEN, 4-HNE, HEX and TBARS, while other protein-bound AGEs were decreased, and no changes were observed in PCC levels. Compared to pork ball digests, HME-pea ball digests were high in MGO, GO, protein-bound PEN, free AGEs, PCC and TBARS, while comparable in other protein-bound AGEs, 4-HNE and HEX. During heating and digestion, glucose promoted α-oxoaldehydes formation in all samples, it also led to the highest formation of protein-bound AGEs in HME-pea balls, whereas starch contributed the most to levels of protein-bound AGEs in pork balls. Other effects of sugars were observed on the levels of free AGEs, PCC, 4-HNE and HEX, but were perceived to be relatively minor. Herbs addition resulted only in a minor reduction of 4-HNE and HEX levels in heated and digested pork balls.

Conclusions

The present study indicates that oxidation and glyc(oxid)ation of extruded-pea balls during gastrointestinal digestion was generally higher than pork balls subjected to similar heating conditions.

Acknowledgments

This study was financially supported by the China Scholarship Council (grant 202006850008) and VLAIO (HBC.2021.0546).

Keywords : Plant-based pea protein, glyc(oxid)ation, oxidation, in vitro gastrointestinal digestion

Topic 1: Oral processing and sensory properties of foods | Poster

(22742) - NEW FINDINGS IN THE REGULATION OF INTESTINAL GLUCOSE ABSORPTION BY DIETARY PROTEINS

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Abstract

Purpose: Numerous studies have established the positive impact of high-protein diets on glucose homeostasis. However, the specific mechanisms responsible for this effect remain unclear. Preliminary investigations indicate that different digested proteins enhance glucose tolerance while reducing glucose absorption and the activity of the glucose transporter GLUT2[1]. We hypothesise that a "cross-talk" between the oligopeptide carrier PepT1 and glucose transporters GLUT2 and SGLT1 is at the origin of this regulation. This work thus investigated the cellular and molecular mechanisms regulating glucose absorption induced by digested proteins from various food sources. **Method:** Fish gelatine, caseins and pea protein preparations were subjected to digestion using the INFOGEST static gastrointestinal digestion protocol. Digested proteins were subsequently incubated with glucose (25 mM) in an intestinal barrier model (Caco-2/HT29-MTX coculture) with or without a PepT1 inhibitor. RT-qPCR, western blot and immunofluorescence evaluated the mRNA and protein expression of SGLT1 and GLUT2**. Results:** Digested proteins reduced the expression of GLUT2 mRNA and SGLT1 protein expression at the apical side of enterocytes. These findings further support the notion that peptides and amino acids derived from digested proteins play a crucial role in regulating glucose homeostasis, emphasising their significance in intestinal glucose absorption.

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Acknowledgments

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Keywords : dietary proteins, intestinal glucose absorption, PepT1, SGLT1, GLUT2

Topic 1: Oral processing and sensory properties of foods | Poster

(22761) - INFLUENCE OF PROTEIN FRACTIONS ON THE TRACE ELEMENT BIOACCESSIBILITY OF TURNIP TOPS (BRASSICA RAPA) GROWING UNDER MEDITERRANEAN CONDITIONS

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1 - University of Cordoba

Abstract

Vegetable species belonging to the *Brassicaceae (*formerly *Cruciferae*) family are some of the most economically important plant groups for humans. A previous study [1] has shown that unlike other green leafy vegetables (such as spinach or Swiss chard), the trace elements bioaccessibility present in cruciferous vegetables is high, similar in some cases to that of powdered milk. This is due to a low content of some anti-nutritional compounds such as oxalates. *Brassica rapa L.* is another species belonging to the *Brassicaceae* family. In northwest Spain and Portugal, there has been a long tradition of cultivating *B. rapa subsp. rapa* to obtain turnip tops [2].

The aim of this work was to study the influence of three protein fractions (casein, lactalbumin and soy) on the trace elements bioaccessibility (Fe, Mn, Ni, Se and Zn) of turnip growing under Mediterranean conditions. The purpose was the use of this vegetable not only for direct fresh consumption but also as a main ingredient in the development of food mixtures. The assays were conducted with different protein fractions (casein, lactalbumin and soy), increasing amounts of the aforementioned compounds to represent 5, 15 and 25% in the final mixture.

The influence of casein on the bioaccessibility of Fe and Mn in turnip tops was negligible. In the case of Zn, the improvement in its bioaccessibility was only effective as from the highest dose (25%). Caseins had no effect on Mn bioaccessibility either, which is somewhat justifiable considering the low Mn content in milk. On the other hand, it was highly obvious that these proteins caused an important increase in the Se bioaccessibility. This was noted to be from 18% (control) to 96% as from the lowest dose (5%).

The effect of lactalbumin on the trace elements bioaccessibility present in turnip tops was very similar to that found for casein. There was none for Fe, Mn and Zn; a slightly decrease for Ni and a remarkable improvement in Se bioaccessibility (reaching 85%) as from the lowest protein dose (5%)

Finally, unlike the two previous protein fractions, soy protein improved the bioaccessibility of most of the trace elements studied (with the exception of Ni for which no effect was observed). The bioaccessibility percentages increased from 32% to 93% for Fe; from 50% to 88% for Mn; from 17% to 100% for Se; and from 36% to 100% for Zn.

The results obtained here can be taken into consideration when selecting different ingredients for formulating new foods developed from cruciferous vegetables.

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Keywords : Anti-nutrional compounds, Bioavailability, Caseins

Topic 1: Oral processing and sensory properties of foods | Poster

(22806) - A METABOLOMIC STUDY ON THE DIGESTION PRODUCTS OF EXTRA VIRGIN OLIVE OIL AND THEIR IN VITRO BIOACTIVITY

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Abstract

Extra-virgin olive oil (EVOO), chemically characterized by a high content of fatty acids and minor compounds such as phenolic compounds (e.g., hydroxytyrosol, HT, oleuropein aglycone, OLE-A, and elenolic acid, EA), is known as the main seasoning ingredient in Mediterranean countries [1]. The richness in phenolic compounds has been positively associated with the prevention of cardiovascular diseases [1]. Recently, efforts have been made to achieve a deeper understanding on the link between EVOO consumption and the prevention of colorectal cancer (CRC), one of the main causes of mortality worldwide. Although epidemiological, cellular, and animal evidence suggest positive effects, so far, there are a lack of studies reporting the impact of metabolites derived from EVOO digestion on CRC cells. To overcome this, the present study seeks for a deeper understanding on the metabolomic composition of the EVOO digestion products and its impact in *in vitro* cell lines.

In this study, a Simulator of Human Intestinal Microbial Ecosystem (SHIME®) model was used to dynamically access the bio accessibility of phenolic compounds present in an extract of EVOO, throughout the gastrointestinal tract [2]. This approach also simulated the colonic *in vivo* conditions including the microbial diversity responsible by the fermentation of the undigested fraction. Both EVOO extract and the products of digestion were analysed by mass spectrometry using a Q ExactiveTM Focus Hybrid Q-Orbitrap spectrometer following an untargeted and targeted approaches. The bioactivity of the final digestion products and available standards were tested for their anti-proliferative effect in HT29 cell line.

Data showed that gastrointestinal digestion, changed the metabolomic profile of the EVOO extract, being responsible by a decrease of phenolic compounds (e.g., OLE and EA), namely due to enzymatic cleavage, dialysis, and colonic fermentation. In this last gastrointestinal section, the more prevalent compounds were simple organic acids, amino acids and sugar moieties produced by propionate-producing bacteria (such as *Bacteroides*, *Veillonella* or *Megamonas* spp.) at the expense of butyrate-producing micro-organisms (such as *Anaerostipes caccae*, *Anaerostipes hadrus* or *Eubacterium hallii*). The cell-based studies using HT29 cell lines showed a slight anti-proliferative effect of the colonic fermentation product at 0h. Studies regarding the targeted metabolomic and its evaluation in HT29 cell line are ongoing.

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Keywords : Metabolomics, Bioactivity, SHIME, Digestion, Extra-Virgin Olive Oil

ICFD 2024

TOPIC 2

FOOD STRUCTURES, DIGESTION AND IMAGING TECHNOLOGIES

Portugal | April 9-11th, 2024

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21368) - GASTROINTESTINAL STABILITY OF POLYPHENOL-FUNCTIONALIZED SELENIUM NANOPARTICLES

Golub, Nikolina (Croatia)[1]; Galić, Emerik (Croatia)[1]; Radić, Kristina (Croatia)[1]; Vitali Čepo, Dubravka (Croatia)[1]; Conway, Róisín (Ireland)[2]

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Abstract

Selenium nanoparticles (SeNPs) are hypothesized to present a novel prospect for nutritional supplementation due to supposed lower toxicity and higher bioavailability in comparison to inorganic selenium forms. Because of particular surface characteristics, SeNPs also represent efficient vehicle for targeted delivery of biologically active compounds.

In this work we developed the procedure of green synthesis of polyphenol-functionalized selenium nanoparticles (PF-SeNPs) to obtain spheric-shaped particles with adequate size range and acceptable polydispersion index. Tomato-pomace derived pectin was used as coating/stabilizing agent while olive pomace extract (OPE), rich in hydroxytyrosol derivates, was used for surface functionalization. The aim was to create a formulation that will protect selenium and OPE during their passage through the harsh acidic environment of the stomach and facilitate controlled release in the intestine.

To investigate gastrointestinal stability, an in vitro simulation of the digestive process was conducted according to the procedure of Brodkorb and co-workers (1) with slight modifications. PF-SeNPs were characterized using various techniques, including dynamic light scattering (DLS) and electrophoretic light scattering (ELS), prior and after the simulation of gastrointestinal digestion, in order to investigate the impact of gastrointestinal system conditions on SeNP features such as zeta potential and size distribution. Selenium content was determined using atomic absorption spectroscopy (AAS). Free radical scavenging properties of PF-SeNPs were determined to investigate the impact of simulated digestion on stability/release of hydroxytyrosol derivates used for PF-SeNP surface modification.

PF-SeNPs nanoparticles demonstrated resistance to the acidic conditions in the stomach, preventing premature degradation and showing strong antioxidant activity. This research highlights the potential of pectin-coated polyphenol-functionalized SeNPs as a strategy to potentially improve the selenium supplementation and its applications in the prevention and treatment of various health conditions.

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Acknowledgments
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Keywords : selenium, nanoparticles, antioxidant capacity, bioaccessibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21398) - CANDIDA TROPICALIS CMGB165 – FROM INDUSTRIAL WASTES TO BIOCOMPOUNDS FOR FOOD AND MEDICINE

Csutak, Ortansa (Romania)[1,2]; Nicula, Nicoleta-Oana (Romania)[3,4]; Lungulescu, Eduard-Marius (Romania)[3]; Corbu, Viorica Maria (Romania)[1,2]

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Abstract

The intensive pollution of soil and water with industrial / household wastes and emerging microbial pathogens, urged the development of new technologies aimed to remove the contaminants and to restore a clean, safe climate. Numerous yeast species and strains belonging to *Candida, Rhodotorula or Yarrowia* genera are able to degrade these pollutants and to convert them into biocompounds of biotechnological interest, based on their intrinsic metabolic characteristics.

The yeast strain *Candida tropicalis* CMGB165 was cultivated on Yeast Peptone (YP) medium with 1% *n*-alkanes (decane, dodecane, tetradecane, hexadecane), o-/p-xylene, petroleum, toluene, benzene and fried sunflower oil. The variation of the growth curve dynamics depended on the nature of the carbon source, the highest growth being recorded on fried vegetable oil, with an upward trajectory throughout the entire incubation period (six days). However, within the first 24 hours, the maximum growth rate was obtained on *n*-tetradecane, after which the cultured entered in the stationary phase until the end of the experiment.

The biosurfactant obtained on previous tested growth media, was characterized for the emulsification activity (E24%) against *n*-hexadecane, best values being determined for *n*-tetradecane - 48% in 6 days, respectively, for old fried sunflower oil (41%) and *n*-dodecane (32%) in 3 days.

The UV-Vis spectrum of the biosurfactant obtained on *n*-tetradecane, showed an absorption peak around 260 nm, indicating most probably the presence of aromatic amino acids, while the FTIR analysis revealed characteristic peaks confirming the mannan-lipid-protein structure of the biosurfactant.

The crude (cell free phase), [50X] and [25X] concentrated biosurfactants were tested for antimicrobial activity against nine pathogenic and potential pathogenic microbial strains: *Candida* (*C. krusei* CMGB-Y8, *C. krusei* CMGB 94, *C. parapsilosis* CBS 604, *C. parapsilosis* CMGB-Y3) and bacteria (*Salmonella typhymurium ATCC 14028*, *Bacillus subtilis CMGB*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa ATCC 27853*, *Listeria monocytogenes CMGB 333*). While no activity was observed against the bacterial strains, the [25X] biosurfactant significantly inhibited the growth of the pathogenic strain *C. krusei* CMGB-Y8 within the first four to eight hours of incubation.

The crude biosurfactant strongly inhibited the adherence of *C. krusei* CMGB 94 biofilm (88.95%), without a significant effect on the adherence capacity of *C. krusei* CMGB-Y8.

In conclusion, *C. tropicalis* CMGB 165 presents a high potential for developing new strategies aimed to functional reintegrate harmful compounds by converting them into biosurfactants with potential beneficial effects for food industry and human health.

Acknowledgments

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Keywords : Candida tropicalis, wastes, biosurfactants, antimicrobial, human pathogens

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21400) - CURCUMINOIDS REDUCE INTESTINAL EPITHELIAL GLUCOSE TRANSPORT

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Abstract

In 2022, approximately 60% of the European adults above 18 years is overweight or obese. Physical activity in combination with consuming low fat and low sugar diets could contribute to the prevention of overweight/obesity. However, for most people with obesity it is difficult to adhere to such lifestyle changes and, hence, alternative approaches to tackle body weight gain are needed. The aim of this study was to identify substances that could lower the intestinal absorption of saccharides. In people with obesity, the expression of intestinal glucose SGLT1 transporter involved in saccharide uptake is increased (1). Could curcuminoids decrease intestinal monosaccharide transport by decreasing monosaccharide transporter expression? It is hypothesized that curcuminoids reduce intestinal monosaccharide transport by decreasing monosaccharide transporter expression.

Methods: A model system of differentiated Caco-2 cells in a transwell set-up was exposed to 400 µM of dibenzoylmethane, 200 µM curcumin or 200 µM bismethoxycurcumin for 4 hours. Subsequently, 1 mM glucose and 0.5 mM fructose were added for 30 minutes and basolateral glucose and fructose concentration was determined using the Amplex Red glucose assay and NBD-fructose. mRNA expression of SGLT1 and GLUT5 was determined by QPCR. All experiments were performed in N≥2. Statistical differences were assessed using a Kruskal-Wallis test.

Results:
Dibenzoylmethane (101.7 to 53.61 µM, P<0.0001), curcumin (101.7 to 28.54 µM, P<0.0001) and bismethoxycurcumin (101.7 to 64.33 µM, P<0.0001) decreased intestinal epithelial glucose transport. This decrease was associated with a decrease in the expression of apical SGLT1 transporter (Fold change = 0.44, 0.77, 0.29;P value= 0.0002; 0.0047; <0.0001, respectively). Dibenzoylmethane had no effect on the fructose absorption, while curcumin (8.24 to 19.66 µM, P<0.0001) and bismethoxycurcumin (8.24 to 14.13 µM, P=0.0026) increased the intestinal fructose absorption. None of the investigated curcuminoids modulated GLUT5 expression.

Conclusion and discussion:
In conclusion, curcuminoids reduce the intestinal glucose absorption, at least in part by decreasing SGLT1 expression. However, for curcumin and bismethoxycurcumin this decrease in glucose uptake was accompanied by an increase in intestinal epithelial fructose transport. Since fructose may contribute to (hepatic) insulin resistance and metabolic consequences of obesity (2,3), it is recommended to further investigate the potential of dibenzoylmethane for decreasing intestinal saccharide absorption. These findings should be validated by measuring the effects on SGLT1 with immunofluorescence and determining the effects on sugar absorption in an *in vivo* study.

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Acknowledgments
Not applicable.

Keywords : intestinal saccharide transport, obesity, curcuminoids, SGLT1, GLUT5

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21402) - IMPACT OF PEA DIETARY FIBRES ON PROTEIN DIGESTIBILITY AND INTESTINAL CELL INTEGRITY

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Abstract

Dietary fibres are important components of human and animal diets (1). However, they can decrease protein digestibility and absorption, and thus the nutritional value of a food (2). The aim of this study was to investigate a specific fibre mechanism, nutrient encapsulation, which is known to decrease the digestibility of protein and absorption of proteolysis products. To this end, an *in vitro* and ex-vivo study was conducted to evaluate the impact of cell wall integrity in pea i) on proteolysis and ii) on the viability and permeability of a jejunum porcine cell line (IPEC-J2). Two pea flours, with either intact (R1) or ruptured (R2) cell walls, pea fibres and pea protein were analysed, before and after digestion, using a variety of biochemical and biophysical methods. The pea materials were digested *in vitro* and the digesta obtained applied onto IPEC-J2 cells. Cell viability and integrity were analysed by transepithelial electrical resistance (TEER) measurement, colorimetric assay (MTS tetrazolium reagent), and immunohistochemistry for tight junction proteins (Zonula occludens-1, ZO-1). Additionally, monitoring of the diffusion of FITC-dextran (FD4) through the cells was performed. Overall, our digestibility data showed a 20 % ($P < 0.05$) increase in protein digestibility between R1 and R2. As expected, the cell wall integrity influenced how well digestive enzymes could access their substrate. Regarding the experiments performed on IPEC-J2, the pea components affected the viability and permeability of the IPEC-J2 differently depending on their level of complexity. For instance, pea protein and fibre digesta led to a greater diffusion of FD4 after 2 h of incubation than R1 and R2. Similarly, the pea protein or fibres resulted in a significant reduction in ZO-1 labelling intensity compared to the control, R1 and R2 ($P < 0.05$). This study confirms that the structure of the pea influences protein digestion and intestinal physiology, particularly cell wall integrity. Further work is still required to fully understand the impact of pea dietary fibres on IPEC-J2 integrity.

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Acknowledgments
This work was funded by INRAE (PHASE division), France.

Keywords : Dietary fibres, Pea, Protein digestion, IPEC-J2, Food structure

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21405) - EFFECT OF AMAZONIAN FRUIT FLOUR PROCESSING ON THE PROTEIN DIGESTIBILITY OF COOKIES

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Abstract

Pupunha (Bactris gasipaes) is a palm plant that is native to the Amazon region and produces fruits rich in phenolic compounds and starch. Despite the consumption and uses in local communities, it has not been widely explored scientifically or commercially. Therefore, the production of pupunha flour (PF), rich in phenolic compound content, can be an important strategy for its use in other regions. Previous research has shown that the addition of freeze-dried fruit flour to yogurt results in a decrease in protein digestibility, possibly because of the concentration of bioactive compounds. This study aimed to evaluate the impact of different PF production methods on protein digestibility and bioactive compounds. PF was produced using two methods: freeze-drying flour (FDF) and cooking with freeze-drying (CFDF). Cookie formulations containing 100% PF were evaluated regarding the phenolic compound content, tannin concentration, and protein digestibility. The presence of tannins in both flour is accentuated, with values of 19.58a ± 2.92 and 15.04b ± 1.34 mg of catechin equivalent/g of dry residue for FDF and CFDF, respectively. About phenolic compounds, the same comportment was found, with CFDF presenting higher phenolic content than the FDF (4.28a ± 0.23 and 3.87b ± 0.03 mg of gallic acid equivalent/g of dry residue, respectively). Although there is evidence of the negative effects of bioactive compounds, especially tannins, on protein digestibility, there were no significant effects in either sample (FDF: 37.76a ± 4.9; CFDF: 26.20a ± 0.8 mg/L of free amino acids). Given these results, we concluded that, under the experimental conditions studied here, there was no influence of bioactive compounds on protein digestibility.

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Acknowledgments

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Keywords : Pupunha fruit, freeze-dried, cooking, tannins, phenolic compounds

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21415) - INFLUENCE OF FOOD COMPOSITION ON PROTEOLYSIS OF SPI GELS IN ADULT AND ELDERLY CONSUMERS

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Abstract

The challenge of meeting the protein demands, arising from projected global population growth (9.7 billion by 2050), leads to the need to transform the food system to provide enough protein to meet the needs of a healthy diet. These needs encourage researchers in sustainable production and use of alternative proteins in foods, such as plant proteins. Soy protein isolate (SPI) was used to produce heat-set high protein gels (total protein 15% in mass). Semi-dynamic *in vitro* digestion (IVD) was used to obtain data about the bioaccessibility of peptides and amino acids. The main objective of the present work was to evaluate the influence of other nutrients on proteolysis for two conditions, considering the impact of ageing on IVD. For this purpose, four digestion conditions were carried out simulating the conditions of adult and elderly people, as well as the ingestion of a meal containing only proteins and a complete meal (including carbohydrates and lipids). The protein content in the meals used for IVD was normalized to 4% (in mass). The digestions of a complete meal were performed with the SPI gel and a protein-free cookie. The parameters used in the semi-dynamic method to simulate the elderly digestion were adapted from the established by INFOGEST international network for static IVD model. Digesta samples were analyzed by confocal laser scanning microscopy and SDS-PAGE gel electrophoresis, and the results showed that proteolysis by pepsin was gradual, but its effect was not as strong as the proteolysis by pancreatin. Protein release and protein hydrolysis (determined by total protein content and free amino groups) indicated similar patterns at the beginning of the gastric phase for the 4 digestions, but after half-time of digestion the hydrolysis increased significantly. The molecular weight distribution of soluble fractions and the free amino acids results were similar with higher concentrations of free amino acids and released peptides (below 1000 Da) for adult than elderly models. Higher concentrations of free amino acids were detected for the complete meal than for the protein-only meal. The adult gastrointestinal (GI) conditions showed higher proteolysis compared to the elderly. IVD simulating a complete meal showed higher proteolysis compared to a protein-only meal, for adult and elderly IVD model. Therefore, proteolysis was highly affected by the GI changes occurred due to aging, and it was also affected by the meal composition. The results obtained can be very useful for the development of plant-based foods and protein-rich products capable of delivering adequate amounts of protein.

Acknowledgments

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Keywords : elderly, semi dynamic, proteolysis

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21417) - THE USE OF MULTIPLE EMULSIONS FOR DELIVERING BIOACTIVE COMPOUNDS

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Abstract

Multiple emulsions are complex structures that enable to decrease of the fat content in food, mask the undesired taste, and deliver bioactive compounds. In particular, w/o/w multiple emulsions can be used as systems for the protection of sensitive bioactive compounds such as bioactive proteins that can be encapsulated in the internal water phase. The complex structure of multiple emulsions preserves the bioactivity of encapsulated proteins in the stomach during the digestion process and ensures their target release in the intestine. The encapsulation efficiency of bioactive proteins within the structure of multiple emulsions which was around 50% after 4 week storage period has been confirmed by microscopic observation during the in vitro simulation of the gastrointestinal tract and ELISA technique. Based on these results, multiple emulsions may be a promising tool for delivering bioactive compounds and increasing their bioaccessibility.

References

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Acknowledgments

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Keywords : Multiple Emulsions, Encapsulation, Bioactive Compounds, Immunoglobulins, Target Delivery

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21418) - EFFECTS OF IN VITRO DIGESTION ON BEEF AND VEGAN BURGERS: A COMPARATIVE ANALYSIS

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Abstract

Introduction and objective:

For several health, sustainable or ethical reasons, the demand of plant-based meat analogs has increased considerably over the last years. In this study, beef and vegan burgers with fat content of 21.2 and 16.7% and total protein content of 16.25 and 17.08%, respectively, were submitted to *in vitro* digestion and its effect on protein and lipid fractions was analyzed.

Materials and methods:

Beef and vegan commercial burgers were cooked on microwaves at 900W during one minute for each side. *In vitro* digestion following the INFOGEST method (Brodkorb *et al.*, 2019) was performed, and the resulting digestion products were analyzed. The degree of oxidation was determined through the TBARs method for the lipid fraction and by the measurement of carbonyls for proteins. Total protein was determined by the Kjeldhal method and total peptides by spectrophotometry. The fatty acid profiles were obtained by GC-FID and the antioxidant activity of the digested samples was measured by the DPPH method.

Results:

Digested vegan burgers showed lower fat amounts than beef burgers (75 and 90% from the total fat of cooked samples, respectively). PUFAs were the most abundant fraction in vegan digested samples and SFAs in beef digested samples. The degree of lipid oxidation measured by TBARs was higher in digested vegan samples, but the final MDA content was significantly lower (1.17 and 1.21 µg/ml digestion extract, for beef and vegan, respectively), as a consequence of the lower fat amount in the vegan samples.

In relation to the protein fraction, although the amount of protein in the cooked samples was similar for both types of samples, some differences were found after digestion. Protein content was similar (1.09 and 1.17 g protein/ml of digestion extract in beef and vegan digested samples, respectively) but the total amount of carbonyls was much lower in digested vegan samples (80 nmol/mL), as compared to that of the beef samples (106 nmol /mL digestion extract). Regarding the peptide content, vegan digested sample had significantly higher amount (5.02 and 4.04 mg tyrosine/ml digestion extract).

Finally, the antioxidant activity was slightly lower in vegan digested sample than beef digested sample (4.9 and 5.4 µg/ ml digestion extract, respectively).

Conclusions:

Obtained data pointed out to a higher intensity in the lipid's oxidation and lower intensity in the protein's oxidation in the vegan products than in conventional ones during the gastro-intestinal *in vitro* digestion process. The fatty acid profile of digested samples corresponds with the expected results: higher PUFAs in vegan samples and higher SFA in conventional ones. The addition of E-301 and E-331 to meat burgers would explain the slightly higher DPPH values for these samples.

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Acknowledgments

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Keywords : INFOGEST, MEAT ANALOGUE, PROTEIN AND LIPID

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21419) - SIMULATED DIGESTION OF BLACK RICE AFTER COOKING: BIOACCESSIBILITY OF PHENOLIC COMPOUNDS

Disca, Vincenzo (Italy)[1]; Colasanto, Antonio (Italy)[1]; Jaouhari, Yassine (Italy)[1]; Travaglia, Fabiano (Italy)[1]; Bordiga, Matteo (Italy)[1]; Coisson, Jean Daniel (Italy)[1]; Arlorio, Marco (Italy)[1]; Locatelli, Monica (Italy)[1]

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Abstract

The Italian Artemide black rice (*Oryza sativa* L.) is a pigmented rice variety obtained from the breeding between Venere black rice and a white *indica* variety. It is particularly rich in healthy polyphenols, mainly anthocyanins and phenolic acids, which make it a sort of natural functional food [1]. Cyanidin-3-O-glucoside represents the most abundant anthocyanin quantified in the Artemide black rice, as in other black rice varieties, representing on average about 83% of total anthocyanins. Besides anthocyanins, the phenolic acids mostly present in black rice in free form are cinnamic, protocatechuic and gallic acids, moreover the main represented bound (insoluble) forms of phenolic acids are ferulic, coumaric and caffeic acids[1,2]. Other classes of compounds present in black rice are non-anthocyanin flavonoids of which flavonols and flavan-3-ols[1]. However, polyphenols and, particularly, anthocyanins are strongly affected by thermal treatment[3], so the cooking, necessary for the rice consumption, further decreases the available amount for the absorption.

The aim of this work was to evaluate the impact of digestion process on the polyphenolic fraction of cooked ("risotto" mode) Artemide black rice, through the application of the *in vitro* INFOGEST simulated static digestion protocol. Anthocyanins, other flavonoids and phenolic acids were determined by RP-HPLC-DAD following digestion at oral, gastric and intestinal level, in both soluble (bioaccessible) and insoluble (undigested) portions. In general, anthocyanins were found to be stable up to the gastric level, while their concentrations decreased significantly in the intestine, with a consequent reduction also in the bioaccessibility values. The free fraction of phenolic acids showed a reduction in their concentration in the insoluble portion and a simultaneous increase in the soluble one during digestion. The bound phenolic acids, determined on insoluble portions, were detected almost unchanged, after digestion. The flavonoids free fraction showed an increase in the concentration, with a consequent increase in their bioaccessibility, during the digestion. These results allowed to highlight the main changes in the polyphenolic composition of one variety of black rice after cooking and digestion.

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Acknowledgments

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Keywords : anthocyanin, antioxidant, bound phenolic fraction, RP-HPLC-DAD, phenolic acids

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21420) - INTEGRATED SAMPLE PREPARATION FOR SIMULTANEOUS DETERMINATION OF NUTRIENT DIGESTIBILITY

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Abstract

Infogest is a well-established method for simulating human digestion. However, interpretation and comparison of results is difficult because of the variability in the analytical protocols used. In addition, the assessment of macronutrient digestibility is usually performed separately and is based on the selective isolation of the analytes of interest (amino acids, fatty acids and sugars) after sampling the digesta. Analytical protocols are available to assess the digestibility of proteins[1], lipids[2] and carbohydrates[3], all based on different sample preparation methods. Overall, assessing the digestibility of multiple macronutrients simultaneously, or mapping interactions between nutrients during digestion, requires extensive sample preparation or multiple parallel digestion simulations.

Our hypothesis was that the above methods share common characteristics. An attempt has been made to combine and unify the above methods in order to improve the throughput of the Infogest method and make the assessment of nutrient digestibility more systematic.

The Bligh and Dyer method was chosen as the basis from which, with minor modifications, bioaccessible nutrients could be selectively isolated, identified and quantified via chosen (or available) analytical techniques.

To test the validity of the integrated sample preparation method, several analytes (BSA and amino acids, TAG and FFA, mono-, di-, oligosaccharides) were selected for recovery experiments, which were performed both on blank digests and on digests of canned chickpeas and wheat bran cereals. Recovery, end-point digestibility and digestion kinetics were measured by several analytical methods (amino acid profiling, TN, free amino group content, SEC, total FA and free FA content, free glucose content, sum of hydrolysable carbohydrates and sugar composition). The results of the end-point digestions were compared with data obtained from the available methods mentioned above.

The results show that the sample preparation method is suitable for the selective isolation of the bioaccessible fraction. The analytes tested all showed high recoveries (70-120%). In addition, protein fractionation demonstrated the suitability of protein isolation after Bligh and Dyer extraction on the bioaccessible protein fraction. Therefore, the digestibility and bioaccessibility of macronutrients could be determined after Infogest digestion simulation using the integrated sample preparation method presented here.

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Acknowledgments

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Keywords : Infogest, Macronutrient digestion

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21424) - EFFECTS OF TANNIN PREPARATIONS ON FATTY ACID DIGESTIBILITY IN BEEF

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Abstract

Three commercially available tannin extracts (**Q**uebracho tree, **C**hestnut tree, **T**ara pod) with different chemical composition (condensed tannin, hydrolysable – ellagitannin, hydrolysable – gallotannin, respectively) were tested in their ability to affect lipid digestibility in two steps. First, *in vitro* enzyme activity tests[1] on gastric and pancreatic lipase were conducted at three levels, 0,3%, 0,6% and 0,9% and enzyme inhibitory effect was calculated. In addition, protein precipitation capability of tannins was assessed[2]. In the second phase, the tannin extracts were added to baked beef (with 17% fat content) in the same concentration as before, then Infogest *in vitro* digestion simulation was conducted and lipid digestibility was measured through free fatty acid release[3].

Based on the results of the *in vitro* enzyme assays, tannins C and T have significant concentration-dependent inhibitory effect on gastric lipase (p(C)=0.036; p(T)=0.045) and on pancreatic lipase (p(C)=0.027) whereas tannin Q showed high inhibitory effect without concentration dependency in the studied range. Furthermore, hydrolysable tannins showed higher protein precipitation effect, but the effect was lower for the digestive enzymes as for the reference protein (BSA).

Controversially, during the *in vitro* digestion simulation these effects could not be fully expressed. In spite of the fact that significant inhibition was reached with tannin T at 0.3% and 0.9% (4.5%; p=0.007; 7.2%; p=0.003; respectively), and tannin C at 0.6% (5.5%; p=0.003), the highest inhibition was observed with the addition of the tannin Q at 0.6% (20.6%; p<0.001). Other compositions showed no effect on the fatty acid content released.

Based on the enzyme assays it appears that hydrolysable tannins may be better options for lipolysis inhibitors. However after simulated digestions this potential could not be reached. One explanation may lay in their (hydrolysable) nature. During digestion, they could be 'deactivated' by acid hydrolysis, and no or low effect could be observed after simulated digestion. In contrast, this issue does not seem to be prominent for the condensed tannin which could exert its inhibitory effect despite the acidic environment during digestion.

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Acknowledgments
Financial support of the OTKA K135294 grant is kindly acknowledged.

Keywords : infogest, tannin extracts, lipid digestibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21428) - GRAIN PROPERTIES AND THEIR EFFECTS ON STARCH DIGESTIBILITY IN SWEET BAKERY PRODUCTS.

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Abstract

In 2021, the International Diabetes Federation reported 537 million adults with diabetes and is predicted to reach 783 million by 2045 if unaddressed. Cross-disciplinary interventions may help reduce this projected increase.

Dietary intake plays a key role in managing and preventing non-communicable diseases (NCDs), particularly type 2 diabetes, by influencing postprandial blood sugar excursions caused by carbohydrate metabolism. Sweet bakery products are an interesting product category for moderating carbohydrate impact due to their wide consumption across diverse demographic groups. In the UK, sweet bakery products, are consumed by more than 70% of the population. In these products, extrinsic sugar, and starch significantly influence glycaemic responses. However, substituting or reducing sugar in them without affecting other crucial qualities is challenging as the sugar in the products is known to perform other technological functions aside from sweetening. Nevertheless, ingredient modification provides opportunities to limit the digestibility of starch within the matrix, aiming to decrease glycaemic response without compromising the functional properties associated with sugar in these products. We hypothesised that the glycaemic effect of sweet bakery products can be attenuated by altering the physicochemical properties of the flour component using grains with starches exhibiting a lower hydrolysis rate.

To optimize flour selection for in-depth investigations, the in-vitro starch digestibility of several whole flours was screened using a single system α-amylase assay. This assay ensures a constant enzyme-substrate ratio for all samples to allow for an accurate comparison. The whole flours that were investigated underwent hydrothermal processing, and their starch digestibility was compared to the raw form. Flours with contrasting starch digestibility were identified. This comprised flours of mainstream cereals, pseudocereals and millets. We used light microscopy, differential scanning calorimetry and particle size analysis to gain insights into the mechanisms underpinning the contrasting amylolysis profiles of the raw and processed flours.

These insights will be used to guide the development of sweet bakery products containing flour with different properties. The in-vitro starch hydrolysis of the flours in a complex matrix will be examined. Products that exhibit superior in vitro starch digestibility compared to a control product will be characterized and advanced to a human study to confirm their efficacy. Our research aims to recommend flours with favourable glycaemic effects and elucidate mechanisms by which these flours exhibit lower hydrolysis within a complex baked food matrix. This will aid food innovation strategies in developing sweet bakery products with improved glycaemic responses, contributing to the long-term prevention of some NCDs.

Acknowledgments

This research was funded by the UKRI Biotechnology and Biological Sciences Research Council Collaborative Training Partnership Award [Grant number BB/P011640/1] in collaboration with Mondelēz UK R&D Limited.

Keywords : Glycaemic response, Grains, Sweet bakery products, Processing, Starch digestibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21452) - GLUTENOMICS: TRACKING GLUTEN IMMUNOREACTIVE PEPTIDES AND GLUTEN DIGESTIBILITY

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Abstract

Up to 7% of adults suffer from wheat-related disorders (WRD), which have become more common over the past 50 years [1,2]. They include celiac disease, non-celiac gluten sensitivity, and wheat allergy caused by eating wheat and other gluten-containing grains. Gluten proteins are storage proteins found in the endosperm of wheat, rye, and barley. Their repetitive amino acid sequences with high glutamine and proline content cause gluten peptides to be resistant to gastrointestinal digestion (GID). Yet, as long as their exact chemical structures are unknown, it is impossible to track the fate of immunoreactive gluten peptides (GIP) in the human body. Developing new therapies and preventive strategies for WRD requires a deep comprehension of the interplay of GIP, the human immune system, and other yet unknown factors.

Previous studies on gluten digestibility rely on isolated proteins or single foods and peptide analysis of digesta is often limited to gel electrophoresis or immunoassays. Food processing, e.g. baking, affects protein digestibility due to protein refolding, aggregations, and binding to other food components. However, detailed information on how food composition and processing might affect the GIP profile is missing.

Our project investigates the effect of raw materials, food processing, and varying food constituents on the GIP profile after GID. To predict GIP *in silico*, a comprehensive database comprising all known and potentially immunoreactive gluten proteins is created. Utilizing the INFOGEST 2.0 static *in vitro* digestion model, flours from different raw materials and variously processed model foods undergo simulated GID. Wholemeal and white flour from popular wheat, barley, and rye cultivars are used to create model foods (yeast-leavened bread, sourdough bread, cakes), allowing the investigation of different raw materials, bran constituents, food ingredients, and processing parameters on gluten digestibility.

GIP in the digesta will be primarily analyzed using liquid chromatography-tandem mass spectrometry. An optimized approach to identify and quantitate GIP via LC-MS/MS will be developed, employing untargeted data-dependent acquisition and data-independent acquisition MS methods to maximize GIP discovery. Subsequently, targeted MS analyses will be used to quantify relevant GIP. These results aim to enhance our understanding of the fate of GIP during and after GID and to predict gluten digestibility of various foods, which is crucial for the development of novel treatments for WRD patients.

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Scherf K. A. *et al.* 2020 In *Front Nutr* 7 p. 29

Keywords : gluten, digestibility, food processing, wheat-related disorders, celiac disease

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21453) - MICRONUTRIENT & PHYTOCHEMICAL FATE DURING IN VITRO DIGESTION OF PROCESSED GREEN VEGETABLES

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Abstract

Vegetables, integral to healthy diets, undergo various processing steps before consumption. However, their functionalization extends beyond ingestion as the human digestive tract subjects vegetables to diverse conditions, such as elevated temperature, pH alterations, enzymatic activity, electrolytes and mechanical disintegration. These conditions can significantly impact the concentration of essential micronutrients and phytochemicals. Apart from how well these compounds endure the challenges presented by digestive conditions, it is equally crucial to understand the accessibility of these compounds for potential absorption by the body. Despite the significance of this aspect, comprehensive knowledge regarding the fate of micronutrients and phytochemicals in processed green vegetables during digestion remains elusive.

To bridge this knowledge gap, we investigated the stability and bioaccessibility of key micronutrients (vitamin C, vitamin K1) and phytochemicals (glucosinolates, S-alk(en)yl-L-cysteine sulfoxides (ACSOs), carotenoids) during static *in vitro* digestion of Brussels sprouts (*Brassica oleracea* var. *gemmifera*) and leek (*Allium ampeloprasum* var. *porrum*). The few *in vitro* digestion studies considering the stability and/or bioaccessibility of these compounds are all performed on fresh vegetables (Hwang et al., 2019; Martínez-Castro et al., 2023; Scrob et al., 2019; Vallejo et al., 2004). In these cases, the plant endogenous enzymes present can continue to exert their actions along the digestive tract. However, vegetables are mainly consumed after a heating process inactivating most of these enzymes. Therefore, we performed *in vitro* digestion on heated Brussels sprouts and leek.

Our study revealed distinct behaviors of water-soluble (vitamin C, glucosinolates, ACSOs) and lipid-soluble (vitamin K1, carotenoids) compounds during digestion. Water-soluble compounds exhibited higher reactivity but achieved complete bioaccessibility. Notably, vitamin C was very unstable in the small intestinal phase, which could be linked to several digestive factors (oxygen, bile salts, pancreatic enzymes). In contrast, lipid-soluble compounds displayed lower reactivity during digestion but were bioaccessible ranging from 26% to 81% when an oil-in-water emulsion was added to the digestive tract.

These findings provide valuable insights into the often overlooked fate of micronutrients and phytochemicals in processed green vegetables during digestion, contributing to understanding of the functionalization of these health-related compounds as part of the human body.

References

Hwang et al., 2019; Martínez-Castro et al., 2023; Scrob et al., 2019; Vallejo et al., 2004

Keywords : vitamin C, vitamin K1, glucosinolates, S-alk(en)yl-L-cysteine sulfoxides, carotenoids

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21458) - UNRAVELLING THE BIOAVAILABILITY OF AAS AND BAPS FROM COLLAGEN HYDROLYSATE IN COFFEE

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Abstract

The availability of amino acids (AA) and bioactive peptides (BAPs) from collagen hydrolysates (CH), determined by how effectively they are absorbed into the bloodstream, is a crucial prerequisite to exert beneficial effects on human health. Different factors influence the bioavailability of AA, including the form in which they are consumed (e.g. as isolated or hydrolyzed protein, alone or in a complex food matrix), the efficiency of the digestive processes that break the parent protein down, and the ability of the AAs and BAPs to be absorbed by the intestinal lining (Nuñez et al., 2020). Coffee, a common part of daily diets, presents a paradox due to its ambivalent effects on health, encompassing both positive and negative aspects. Despite potential harmful effects, coffee consumption has been linked to various health benefits such as neuroprotection, anti-inflammatory properties, immune system stimulation, antihypertensive effects, and a cholesterol-lowering impact (Machado et al 2023).

It is widely recognized that dietary polyphenols, have a strong tendency to interact with proteins, specifically those rich in proline residues (PRPs). This interaction is influenced by the structure of the polyphenol and the protein, and it can be reversible or irreversible. This information opens up a new area of study for nutritionists to explore how these interactions might impact health, especially considering the roles of polyphenols in controlling gene expression related to proline-rich proteins. (Perez-Gregorio et al., 2020)

So far, the impact of a more complex matrix like coffee on the bioavailability of AA and BAPs of CH has not been elucidated yet. This study aimed to compare the bioavailability of the key AAs and di- and tri- peptides from bovine CH (average molecular weight 2000 Da) in coffee in healthy human volunteers.

The clinical trial was carried out as double-blind, randomized, cross-over design. CH were dissolved in water or coffee respectively and provided as single dose in fasting state. The uptake of the CH signature AA hydroxyproline (Hyp), glycine (Gly) and proline (Pro) and di and tri-peptides (Pro-Hyp, Hyp-Gly, Gly-Pro-Hyp) into the bloodstream was followed over a period of 6 hours. Plasma concentrations were determined with UPLC-MS/MS and pharmacokinetic endpoints were calculated from concentration-time curves.

The peak concentrations of AAs and BAPs in water occurred between 60 to 130 minutes. Notably, there were significant variations in the absorption rates of Hyp and Pro compared to other AAs. However, when considering absorption kinetics such as AUCs, Cmax, and Tmax, the uptake of bioactive peptides demonstrated high similarity between water and coffee

Ongoing research in this domain is delving into the relationships between food matrices, bioactive peptides, and human health.

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Keywords : collagen hydrolysate, food supplement, bioavailability

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21463) - THE ENCAPSULATION OF THE DHA OIL PROFOUNDLY MODIFIES THE METABOLISM OF DHA IN VIVO.

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Abstract

An oil consisting of triglycerides highly enriched in docosahexaenoic acid (DHA), a valuable omega 3 fatty acid, was encapsulated with whey proteins to form Pickering emulsions. The emulsion or crude DHA oil was then cooked in an omelette. In vitro digestion showed that the bioaccessibility of DHA was significantly improved by the encapsulation of DHA oil. As much DHA was released during digestion after one hour of lipase activity when the DHA oil was encapsulated, as after two hours of digestion when the DHA oil was not encapsulated. On the basis of this observation, 3g of omelettes containing encapsulated or non-encapsulated DHA oil (25 mg DHA per day) were fed to young rats for 4 weeks to observe the metabolic effects.

Firstly, the encapsulation of DHA oil tended to increase the bioavailability of DHA, although plasma levels were not significantly different with the encapsulation of DHA oil. It also modified feeding behaviour by stimulating rodent chow consumption. This effect promoted the animal's growth.

Secondly, administration of DHA oil significantly altered the profiles of oxygenated fatty acid derivatives, drastically reducing the overall levels of omega-6-derived oxylipins in the plasma and the heart, but not in the brain. This effect was greatly accentuated when the DHA oil was encapsulated. On the other hand, DHA-derived oxylipins were increased overall in the heart and brain, even more so when the DHA oil was encapsulated.

Thirdly, administration of DHA oil also modified the profiles of endocannabinoid derivatives of fatty acids. Endocannabinoids and N-acylethanolamides were greatly reduced in plasma and brain, but without the impact of DHA encapsulation. The heart showed a different pattern, with an increase in DHEA from DHA, specifically when DHA oil was encapsulated.

In conclusion, these results show that modifying the food structure allows a nutrient to be delivered differently, and thus to modify not only its digestion process but also its subsequent metabolism. They also highlight the fact that the impact of the food structure may not really influence the levels of the target nutrient in the body, but may completely affect its metabolism into lipid derivatives, which must be investigated and quantified.

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Acknowledgments

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Keywords : DHA oil, encapsulation, metabolism, endocannabinoids

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21466) - POLYPHENOL HIERARCHICAL CLUSTERING USING STATIC AND SEMI-DYNAMIC INFOGEST BY LC-QTOF-MS

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Abstract

Binding interactions between polyphenols (PPs) and plant cell walls (PCWs) could be strong enough to result in minimal extraction of PPs during upper intestinal digestion and, hence, diminish their bioactivity in the upper gut while enhancing their effects in the colon. Yet, only binding studies using pure PP standards in aglycone form or using extracts with only a limited number of polyphenols quantified by simple analytical procedures (e.g., LC-DAD, colorimetric) exist. Moreover, there is a lack of standardization of the *in vitro* digestion protocols used. Undoubtedly, the static INFOGEST 2.0 is gaining popularity for the study of the digestion of dietary macromolecules, such as proteins. Furthermore, a semi-dynamic INFOGEST protocol has been recently developed to account for a calorie-driven gastric emptying and a transient pH during gastric digestion (Mulet-Cabrero et al., 2020), which could in principle provide more accurate information about the biotransformation and bioaccesibility of PPs considering the sensitivity of PPs to pH and oxygen. In this study, we firstly develop a procedure to perform a non-targeted screening of PPs and their semi-quantification by subjecting the aliquots taken from the INFOGEST digestion models to the physical simulation of transepithelial absorption via re-solubilization, centrifugal filtration, and UHPLC-ESI-QTOF-MS/MS analysis. The static INFOGEST proved to be just as convenient and reproducible as the semi-dynamic method. Moreover, the semi-dynamic model posed difficulty to select gastric emptying considering the negligible caloric content of PCWs (dietary fiber). Digestion of apple pomace (and its corresponding matrix-free extract) clustered 45 PPs into five main groups according to their interaction with PCWs during each digestion phase. This grouping was not reproduced in apple pomace, which exhibited a greater matrix effect than whole apple during oral and gastric digestion. Nevertheless, the interaction between most polyphenol groups, including dihydrochalcones, flavanols and hydroxycinnamic acid derivatives, and pomace PCWs was lost during intestinal digestion. Overall, results could facilitate the comprehension about the interactions of polyphenols, as found in real fruit matrices such as in apple cold-pressing fractions, with the gastrointestinal tract, and propose strategies to improve their likelihood of reaching optimal target sites along the gastrointestinal tract.

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Keywords : apple polyphenols, simulated digestion, INFOGEST, semi-dynamic, mass spectrometry

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21467) - BIOACCESSIBILITY OF BIOACTIVE COMPOUNDS AFTER SIMULATED DIGESTION OF HIGH-POLYPHENOL COCOA

Carrà, Francesca (Italy)[1]; Disca, Vincenzo (Italy)[1]; Locatelli, Monica (Italy)[1]; Bordiga, Matteo (Italy)[1]; Coisson, Jean Daniel (Italy)[1]; Arlorio, Marco (Italy)[1]; Travaglia, Fabiano (Italy)[1]

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Abstract

Cocoa and cocoa-derived products are obtained from the beans of the evergreen tree *Theobroma cacao* L. Cocoa is naturally rich in bioactive compounds like methylxanthines and, particularly, polyphenols, a heterogeneous group of phytochemicals with anti-inflammatory and antioxidant anti-inflammatory properties, desirable for their positive effect on human health. The main polyphenols in cocoa beans are the flavan-3-ols, epicatechin and catechin, present in both monomeric and polymeric (up to 11-mers) form[1]. Concerning their specific activity, cocoa flavanols help to maintain the normal endothelium-dependent vasodilatation, thus contributing to normal blood flow; this beneficial effect can be obtained consuming daily 200 mg of cocoa flavanols.[2] Despite their high concentration in raw cocoa beans, different technological processes before being transformed into chocolate decrease their content. Furthermore, these bioactive molecules have low bioaccessibility and bioavailability and they are poorly absorbed in the intestine. The metabolic fate of these bioactive compounds is affected by both the intrinsic characteristic of the food matrix and the variability of digestion/absorption between different individuals. All these aspects modulate the rate of absorption, metabolism and distribution[3].

This research work aims to evaluate the impact of simulated in vitro digestion on a high- flavanols cocoa powder (Acticoa™, Barry Callebaut) in comparison with a conventional medium alkalinized cocoa (SAANDAM). After simulated digestion (INFOGEST protocol), both gastric (G) and gastrointestinal (GI) phases have been evaluated and the characterization of soluble (bioaccessible) and insoluble fractions of cocoa powders has been obtained by spectrophotometric and chromatographic methods (RP-HPLC-DAD), evaluating on polyphenols and methylxanthines.

Following simulated digestion, the concentration of bioactive compounds in the soluble fractions increased from G to GI phase. However, the total recovery of the main polyphenols identified, expressed as percentage of total digested (sum of soluble and insoluble fractions) respect to the content before digestion, evidenced a general decrease of phenolic component; on the contrary, the recovery of methylxanthines reached about 100%.

Finally, in accordance with a major phenolic concentration in the high-flavanols cocoa powder, the digestion of Acticoa™ resulted in a higher amount of bioaccessible polyphenols respect to the one obtained from SAANDAM.

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Keywords : cocoa, antioxidant, phenolic fraction, simulated digestion, RP-HPLC-DAD

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21471) - ENHANCED CURCUMIN BIOACCESSIBILITY: POSSIBLE SYNERGISTIC EFFECT OF Γ-CD-MOFS WITH MICELLES

Oh, Jia Xin (United Kingdom)[1]; Murray, Brent (United Kingdom)[1]; Mackie, Alan (United Kingdom)[1]; Ettelaie, Rammile (United Kingdom)[1]; Holmes, Melvin (United Kingdom)[1]

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Abstract

Biocompatible γ-cyclodextrin metal-organic frameworks (γ-CD-MOFs), consisting of γ-cyclodextrin and alkali metal ions, are classed as a multifunctional porous framework. They have been reported to improve the apparent solubility and bioavailability of encapsulated bioactive compounds, including curcumin, with the potential of more sustained delivery. Several modelling studies as well as Fourier transform infrared spectra (FT-IR) [1] have suggested that not only does curcumin sit within the hydrophobic cavity of γ-CD-MOFs, but also exists in between the γ-CD pairs. It was inferred that improved apparent solubility was due to the interaction between curcumin and γ-CD pairs because the γ-CD-MOFs disintegrate in an aqueous environment, whereas the "release" of trapped curcumin from within the cavity leads to its insoluble crystallised form. The aim of this study was to assess whether the presence of oleic acid micelles (i.e., at a concentration above its maximum solubility) or artificial micelles would be able to take up the "released" curcumin from γ-CD-MOFs and thereby further increase its apparent solubility. Release in the presence of a micellar phase was compared to an environment of just curcumin encapsulated within γ-CD-MOFs. Post-characterisation, each sample was subjected to in vitro digestion (INFOGEST method [2]) to access the bioaccesibility of curcumin. This research enabled the evaluation of γ-CD-MOFs' functionality for delivery and controlled release, with the possibility of an emulsion system boosting the fraction of curcumin that would potentially be bioavailable.

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Keywords : Cyclodextrin-metal-organic frameworks, Encapsulation, INFOGEST, Bioaccessibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21473) - IN VITRO GUT ANTI-INFLAMMATORY EFFECT OF PLANT STEROL FOOD SUPPLEMENT AND ENRICHED FOOD

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Abstract

Previous research has demonstrated the capacity of plant sterols (PS) to modulate inflammatory response at gastrointestinal level. However, it remains unclear whether the incorporation of PS into the food matrix could alter their anti-inflammatory effect. This study aimed to assess the anti-inflammatory potential of PS from food supplement and PS-enriched beverage and rye bread. An *in vitro* model of intestinal inflammation involving the co-culture of 8-day-differentiated Caco-2 intestinal epithelial cells (apical compartment) with RAW264.7 murine macrophages (basolateral compartment) was used (1). The bioaccessible fractions (BF) of the samples were obtained after gastrointestinal digestion using the standardized INFOGEST 2.0 model (2). Initially, an assay with different dilutions of the BF (ranging from 1:2 to 1:30, v/v) with DMEM to check potential cytotoxicity of the samples (MTT test) was carried out. For the three samples tested, the 1:20 (v/v) dilution of BF showed no cytotoxic effect and was added into apical compartment for 90 min, using a blank of digestion as a control. Additionally, the impact of the drug budesonide (1 μM) was examined, both independently and in combination with the samples, to evaluate possible synergistic or additive effects. A pro-inflammatory stress was induced using 1 μg/mL of lipopolysaccharide (LPS) on the basolateral side for 24h. Then, pro-inflammatory mediators were evaluated in apical (IL-8) and basolateral medium (TNF-α, IL-6, PGE_2, and NO), as well as intracellular levels of ROS in Caco-2 cells. Regarding the concentration of cytokines TNF-α, IL-6, and IL-8, no statistically significant differences ($p > 0.05$) have been observed after pre-treatment with the BF of beverage (1708, 1865, and 3659 pg/mL) and bread (1785, 1703, and 4475 pg/mL), compared with digestion blank + LPS (1788, 1662, and 3910 pg/mL, respectively). Nevertheless, pre-treatment with BF of PS food supplement led to a significant reduction ($p < 0.05$) in the concentration of TNF-α (1656 pg/mL), IL-6 (765 pg/mL), and IL-8 (2798 pg/mL), compared to digestion blank + LPS. Additionally, the anti-inflammatory effect of BF of PS food supplement was confirmed by a reduction (*vs*. digestion blank + LPS) in the levels of NO (13.3 *vs*. 18.9 μM), ROS (2.2- *vs*. 4.0-fold change over LPS-untreated cells) and PGE_2 (401 *vs*. 530 ng/mL). Concerning the interaction between PS food supplement and the budesonide, an empowerment effect was exclusively noted in IL-8. The reduction in this cytokine was markedly more pronounced with the combined treatment (1763 pg/mL), surpassing the impact of the individual treatments with PS food supplement (2798 pg/mL) and budesonide (2705 pg/mL). The findings of this study underscore the importance of considering the ingestion form of PS, since their anti-inflammatory efficacy may be reduced by their incorporation into foods.

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Acknowledgments

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Keywords : beverage, cell culture, food matrices, INFOGEST, rye bread

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21477) - THE FATE OF SALIVARY PROTEINS-APPLE POLYPHENOLS COMPLEXES DURING GASTRIC DIGESTION

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Abstract

Despite recognized health benefits, some polyphenols referred to as tannins exhibit anti-nutritional properties. Binding of tannins by proteins prior to the stomach may therefore act as a protective mechanism against their deleterious effects on digestion[1]. Illustration is provided by the particular case of salivary proteins, especially Proline-Rich Proteins (PRPs) with high affinity for tannins. Polyphenols-salivary proteins complexes are formed in the oral cavity, but it is not entirely clear how they behave in the harsh digestive environment. The overall objective of this study was to describe the interactions between salivary proteins and apple polyphenols, and the impact of gastric digestion on such interactions.

A polyphenol extract was obtained from Dous Moën cider apples and mixed with pooled human saliva to reach different ratios of polyphenols to saliva proteins. In parallel, saliva, polyphenols extract or mixtures were subjected to the gastric phase of the INFOGEST static *in vitro* digestion procedure. Samples were centrifuged at 10000 g, 4 °C for 10 minutes. Protein profiles in supernatants and pellets were analyzed by SDS-PAGE. Proteins contained in bands of interest were identified by nano-LC-ESI MS/MS after in-gel trypsinolysis, and by staining of PRP with Coomassie blue R-250. Native polyphenols were quantified in supernatants by UPLC-UV-MS.

Before digestion, increasing the polyphenols load resulted in a rise of turbidity of the mixtures suggesting the formation of progressively larger aggregates. Supernatants showed a protein profile similar to that of saliva while four bands were enriched in pellets. These proteins, identified as carbonic anhydrase 6, PRP and S100-A8, formed insoluble complexes with apple polyphenols. At low polyphenols load, the interaction with saliva induced a significant ($p<0.05$) concentration decrease in the supernatants of procyanidin oligomers (dimers PA-B1, PA-B2, PA-B5, trimer PA-C1, tetramer DP4), catechins and hydroxycinnamic acids. At a higher polyphenol load, the only affected compound was DP4, indicating that when polyphenols are abundant, highly polymerized procyanidins are preferentially complexed with proteins.

After digestion of saliva, samples containing the highest polyphenols load showed specificities, namely the persistance in pellets of two bands at 60 kDa (identified as α-amylase) and at 20kDa (containing PRP). In addition, digestion of low polyphenols-saliva mixtures resulted in a large decrease of procyanidins PA-B5, PA-C1 and DP4 from the soluble fractions of digests compared to digestion of polyphenols alone. This suggests that insoluble complexes between salivary proteins and those tannins are formed and remain stable during gastric digestion.

This study supports that saliva modulates the nature and amount of polyphenols that reach the digestive tract in free or soluble form.

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Keywords : apple polyphenols, tannins, saliva, gastric digestion

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21489) - UNLOCKING APPLE POLYPHENOLS BIOACCESSIBILITY DURING THE IN VITRO DIGESTION

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1 - LAQV/REQUIMTE; 2 - CEB; 3 - LABBELS

Abstract

Fruits and vegetables are responsible for about 22% of food losses and wastes along the supply chain. However, these by-products are still rich in valuable components such as dietary fibers and polyphenolic compounds, thus potentially bringing value to the food industry due to health benefits, nutritional properties, and technological functionality. In the last years, there has been considerable interest in understanding and exploiting the functions and health benefits of fruit and vegetable pomaces, 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, apple pomace, a waste generated from the fruit juices production, was used to produce a flour rich in dietary fibers and polyphenols, aiming to upcycle this by-product into novel food. Chlorogenic acid, (-)-epicatechin, and phoretin derivative were found as the most abundant phenolic compounds in apple pomace flours. The raw material was subjected to an *in vitro* simulated gastrointestinal digestion, according to the INFOGEST protocol. The hydrolysis of carbohydrates and release of polyphenols during digestion was investigated. Regarding the bioaccessibility of phenolic compounds, there was an observed increase in their amount from the gastric to the intestinal phase. 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 2h period using a 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

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Keywords : Apple, Bioaccessibility, Polyphenols, caco-2

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21495) - RELEASE OF VITAMINS D3 AND B12 IN LIPOSOMES INCORPORATED IN STRAWBERRY YOGURTS

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Abstract

The use of liposomes in foods has been increasing due to some interesting characteristics of these systems, as their low toxicity and the possibility of increasing the bioaccessibility and/or bioavailability of encapsulated bioactives. In this work, vitamins D3 and B12 were coencapsulated in liposomes, which were afterwards incorporated in strawberry yogurts. Liposomes were produced by ultrasonication and lyophilized, and sucrose was used as a cryoprotectant. Two types of phospholipids were used to produce the vesicles, Phospholipon 90G (hydrogenated, at least 90% phosphatidylcholine) and Lipoid S45 (non-hydrogenated, at least 45% phosphatidylcholine). Lyophilized liposomes contained between 2.5 and 2.6 µg D3/mg and between 0.84 and 1.0 µg B12/mg after lyophilization, and they were incorporated in whole milk yogurts in the amounts of 1 and 2% (in mass). The yogurts were also produced with or without the incorporation of 3% (in mass) lyophilized strawberry. *In vitro* static digestion was carried out using adult conditions, by protocol INFOGEST 2.0. For vitamin D_3, after gastric step there was a strong influence in the release of vitamin of the type of phospholipid used and also of the presence of lyophilized strawberry in the yogurts. Vitamin D3 was not released after gastric step in yogurts incorporated with Lipoid S45 liposomes, nor in yogurts with strawberry pieces. Apparently the presence of the solids of strawberry delayed the release of vitamin D3 after gastric step. After intestinal step, the release of vitamin D3 ranged between 30 and 50% for all yogurts. On the other hand, the release of vitamin B12 was not affected by any of these factors, as it was released totally after gastric phase, independently of the type of liposome or the presence of lyophilized strawberry. Therefore, the yogurt formulation was highly relevant to determine the release of the hydrophobic vitamin, but not the release of the hydrophilic micronutrient.

Acknowledgments
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Keywords : microstructure, yogurt, vitamins

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21496) - ENHANCING PHENOLIC STABILITY IN OLIVE LEAF-ENRICHED BISCUITS

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Abstract

The olive leaf, renowned for its rich content of phenols and flavonoids, including oleuropein and luteolin, holds promise for conferring functional and health-related benefits in food formulations. However, the susceptibility of these bioactive compounds to chemical instability during processing and degradation within the digestive system can compromise their bioavailability, leading to reduced absorption. In this investigation, we aimed to assess the phenolic profile of olive leaf extract encapsulated at both micro- and nano-scales within biscuits, by subjecting the samples to the INFOGEST static in vitro digestion model. Our primary objective was to enhance stability and sensory characteristics, crucial for the successful development of functional food products.

The olive leaf extract was obtained by ultrasound-assisted extraction and characterized through liquid chromatography. Subsequently, two encapsulation techniques were employed: micro-encapsulation using spray drying with maltodextrin-glucose blend as coating agent, and nano-encapsulation employing a tailored blend of maltodextrin, whey protein isolate, and arabic gum. To evaluate the efficacy of these strategies, we conducted microscopic analyses, including transmission electron microscopy (TEM) and scanning electron microscopy (SEM), along with encapsulation efficiency assessments for the various formulations.

Our results reveal that both micro- and nano-encapsulation techniques effectively enhance the functionality of biscuits by preserving phenolic stability throughout the digestive process. However, it is noteworthy that the concentration of encapsulated phenolics significantly impacts sensory and textural parameters. The highest concentration, while maximizing health benefits, negatively influences these attributes. These findings provide valuable insights into the development of functional food products enriched with bioactive compounds. Our research aims to strike a delicate balance between bolstering health benefits through improved bioaccessibility and maintaining optimal sensory attributes, contributing to the advancement of consumer-friendly, health-promoting food options.

Keywords : functional food, bioactive compounds, industrial by-products valorization

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21498) - IN VITRO DIGESTIBILITY OF ALLERGENIC ARGININE KINASE FROM HERMETIA ILLUCENS EDIBLE INSECT

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Abstract

Arginine kinase (AK) is an important enzyme for energy metabolism of invertebrate cells by participating in the maintenance of constant levels of ATP. However, AK is also recognized as a major allergen in insects and crustaceans capable of cross-reactivity with sera of patients sensitized to orthologous proteins. In the perspective of introducing insects or their derivatives in the human diet in Western world, the evaluation of possible risks for allergic consumers is essential. In this work we reported the identification and characterization of AK from *Hermetia illucens* (Black Soldier Fly), a promising insect for human consumption. Recombinant AK X4 isoform was produced in E. coli by using the pET expression system and purified by affinity chromatography. To evaluate allergenicity of AK, linear and conformational epitopes were identified by bioinformatics analyses and Dot-Blot assays were carried out by using sera of patients allergic to shrimp or mites to validate the cross-reactivity. *In vitro* digestion of AK X4 was performed in duplicate using Simulated gastrointestinal digestion (SGD) INFOGEST protocol, with some modifications, consisting of a gastric and an intestinal phases (1). The digestate solutions were studied through SDS PAGE and LC-HRMS analyses. The electrophoretic profile showed that after digestion, no bands around 40 kDa were present. Sixty-eight and fifty-six peptides were identified by MS-spectrometry in the two digestion replicates, for a total protein coverage of 69 and 63%, respectively, and were analyzed by bioinformatic tools and only one out of 18 epitopes (WPTGRGIYHNDNKTF, IEDB ID 418907) was still detected after the SGD. Moreover, conformational epitopes are susceptible of rupture in acidic and proteolytic environments, such as those occurring during GD (2). Therefore, those identified here can probably be lost during GD. To assess the IgE-reactivity of AK X4 after SGD, the treated protein was tested for its IgE-cross-reactivity by using sera of a patient positive to shrimp and mites and of a patient negative to them. The result of the Dot-Blot experiment showed a significant decrease of IgE reactivity for the digested AK X4 compared with the AK X4 used as a control in the SGD. In conclusion, current results indicate that the risk of allergy due to AK must be taken in serious consideration when dealing with Novel foods containing *H. illucens* even if the stability of the allergens seems to be seriously affected by the digestive process.

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Acknowledgments

This research has financially been supported by "Bando per la Ricerca 2020" of University of Parma (Parma, Italy).

Keywords : Edible insects, Allergens, Arginine Kinase, Simulated gastrointestinal digestion, Epitopes

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21502) - IMPACT OF THE SIMULATED OROGASTRIC DIGESTION ON THE BIOACTIVITY OF N.GADITANA MICROALGAE

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Abstract

Helicobacter pylori is one of the most common human pathogens worldwide and is considered a risk factor for gastritis, ulcers and gastric cancer, whose current antibiotics-based treatment has been compromised by their multiple side-effects and increase of resistant bacterial strains [1]. This situation has led to increased interest in natural derived compounds with antibacterial, anti-inflammatory and antioxidant properties for the treatment of *H. pylori* infection [2]. Microalgae *Nannochloropsis gaditana* has attracted increasing attention because of its adaptability, sustainability, and high nutritional value [3]. Despite multiple bioactivities have been reported for compounds present in this microalga, their behaviour during the transit through gastrointestinal tract has not been elucidated. Thus, deciphering the impact of digestive enzymes on the release of compounds with protective effects at the gastrointestinal level would be of great interest for the development of new functional foods. The aim of this work was to evaluate the impact of simulated orogastric digestion on the protein profile of *N. gaditana*, and on its antioxidant, anti-inflammatory, and antimicrobial effects as basis of its potential as ingredient of natural and sustainable protective alternatives at gastric level. Both intact and pre-treated (ultrasound and freeze-thaw cycles) *N. gaditana* biomasses were subjected to simulated orogastric digestion following the INFOGEST protocol. Protein behavior during digestion was evaluated by the measurement of the degree of hydrolysis and the electrophoretic profile. The antioxidant activity of orogastric digests was evaluated by biochemical assays. The protective effects against oxidative stress and inflammation was analyzed in human gastric AGS cells through the measurement of cell viability, intracellular ROS generation, and IL-8 production at both basal and *H. pylori* infected conditions. Microalgae proteins were susceptible to the action of digestive enzymes, releasing small and medium size peptides. The pretreatment resulted in a loss of the integrity of the cell wall and modification of the protein profile and susceptibility to the digestion process. The gastric digests showed antioxidant effects, thus suggesting the contribution of released peptides on the ability of these digests to scavenge free radicals. The gastric digests also modulated biomarkers related with the AGS infection by *H. pylori*, which encourages further research to study its protective role at the gastric level. The results show that microalgae *N. gaditana* is a potential source of bioactive peptides, that can be released under gastric digestion conditions. These compounds, along with others of different nature, could contribute to the modulating effects exerted by the digests over the inflammatory response associated to *H. pylori* infection.

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Acknowledgments

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Keywords : microalgae biomass, in vitro digestion, gastric protective effects, antioxidant activity, anti-inflammatory effects

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21507) - EFFECT OF EXCIPIENT NANOEMULSIONS ADDITION TO SPINACH ON VITAMINS' BIOACCESSIBILITY

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Abstract

Excipient foods have been gaining a lot of interest in recent years for their ease of use and projected benefits. Instead of fortifying or enriching food products, excipient foods are meant to be co-ingested with nutrient-rich foods aiming to improve the absorption of available nutrients. Excipient nanoemulsions (EXC) may be developed similarly to O/W nanoemulsions, albeit maintaining an empty core (without bioactive compounds). When co-ingested with nutrient-rich foods, EXC are meant to protect sensible nutraceuticals and facilitate their absorption. However, their behaviour will be affected by food processing, that also modulates how foods are digested and absorbed. Understanding EXC behaviour under digestion and absorption is fundamental to develop tailored food products for specific populations, such as older adults that sustain age-related changes that greatly affect their ability to eat, digest and absorb nutrients.

In this sense, the objectives of this work were to assess the effectiveness of EXC in preserving/increasing the bioaccessibility of spinach's liposoluble vitamins (Vit) when cooked and processed differently. O/W EXC were produced with 3 % lecithin and 5 % corn oil. Spinach leaves were cooked either by boiling for 5 min or sautéing for 10 min. Afterwards, boiled spinach was minced (C), minced with EXC (M), or puréed with EXC (P); sautéed spinach was minced with EXC (S).

Textural analyses showed that EXC addition contributed to a reduction of hardness, chewiness, gumminess and resilience of C sample, whereas S sample led to higher values for all textural parameters. Also, P samples demonstrated appropriate rheological attributes for dysphagic consumption. Cooked and processed samples were submitted to INFOGEST older adult protocol. After *in vitro* digestion, Vit A, E and K bioaccessibility was evaluated by UHPLC. EXC addition resulted in increased Vit K bioaccessibility (i.e., 49.1, 35.3, 38.3 and 34.1 %, for M, P, S and C, respectively). A similar trend was observed for Vit E bioaccessibility (i.e., 21.2, 15.4 and 15.1 %, for P, S and C, respectively). Conversely, samples without EXC had higher Vit A bioaccessibility. Thus, EXC addition increased 1.5 to 2-fold the Vit concentration for both the digesta and micellar phases, with higher concentrations being obtain in boiled samples. S samples yield was comparable to C in the digesta phase, although it was 1.5-fold higher in micellar samples, demonstrating that EXC improved Vit solubilization.

Cell viability studies proved that EXC addition did not cause significant effect on Caco-2 and HT-29 cells viability, since cell viability values of C, M and P micellar phases samples were identical. However, S samples decrease cell viability at lower concentrations tested, possibly indicating that sautéing was not effective in reducing potential anti-nutrients present in spinach and in releasing the available nutraceuticals.

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Acknowledgments

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The authors are thankful to Lipoid for kindly donating a sample of LIPOID S 75 lecithin.

Keywords : Excipient nanoemulsions, Spinach nutraceuticals, Bioaccessibility, Older adult in vitro digestion, Citotoxicity

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21510) - ANTHOCYANIN BIOAVAILABILITY FROM EDIBLE FLOWERS: GASTROTECHNIC AND IN VITRO APPROACHES

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Abstract

There has been a noticeable global shift in dietary patterns driven by increased awareness and understanding of the impact of food choices on overall health and well-being. As a result, there is a growing demand for functional foods that go beyond basic nutrition and offer additional health benefits. Edible flowers (EFs), known for their medicinal properties since ancient times, are now making a comeback in contemporary cuisine.

EFs are rich in natural phytochemicals, some of which with a high level of anthocyanins that give flowers their red, purple, and blue colors. Due to their structural characteristics, these compounds have been implied in several health benefits, that highly depend on the metabolism and bioavailability, as well as bioaccessibility, of the EFs anthocyanins. Moreover, food processing methods greatly influence the food bioactive content, their stability, and consequently their absorption efficiency, which in turn affects the health benefits they provide [1]. Hence, acquiring a deeper comprehension of the alterations in bioaccessibility and bioavailability after the ingestion of EFs can illuminate the intricate mechanisms entwined with their health-promoting attributes.

The aim of this work was to explore the anthocyanin content in some EFs (*Viola tricolor, Cosmos bipinnatus, Centaurea cyanus* and *Clitoria ternatea*), as well as their bioaccessibility and intestinal absorption through a range of different approaches. All the species presented mono- and polyglycosylated anthocyanins with different degrees of complexity and substitution patterns. Anthocyanin stability assays were performed by varying factors such as temperature, pH, and time. The results showed that depending on the species, different factors have a specific impact, prompting for the effects of different cooking techniques on the content of such bioactives. Considering the effects of food matrices, such as protein and starch, the presence of both affected the anthocyanin content of the EFs. Simulated digestions were performed according to INFOGEST and revealed that overall, a pronounced decrease in the anthocyanin content was observed after the intestinal phase. Furthermore, the cytotoxicity of the extracts was evaluated to determine the ideal conditions to perform *in vitro* transepithelial absorption studies using gastrointestinal cell models. In all cellular models, anthocyanin absorption exhibited a time-dependent pattern, whilst in some the anthocyanin structure seemed to have a greater impact on absorption, emphasizing the influence of anthocyanin structural arrangement on their intestinal transport.

These results suggest that the structural differences in the anthocyanins present in different EFs, may have a great impact on their stability and behavior towards the cooking and gastrointestinal processes prior to their absorption as bioactive compounds.

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Acknowledgments

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Keywords : Edible flowers, Anthocyanins, Food processing, Bioaccessibility, Gastrointestinal tract

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21513) - STEERING PROTEIN AND CARBOHYDRATE DIGESTIBILITY IN BREAD BY PEA PROTEIN ENRICHMENT

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Abstract

The elderly account for one-fifth of the EU population and this ratio is expected to increase further due to the higher life expectancy. Ageing brings about diet-related chronic diseases like sarcopenia and type 2 diabetes. Their prevention requires concomitantly increasing protein intake, improving their digestibility, and reducing the glycaemic response in carbohydrate-rich foods. The need to increase protein intake results in the demand for a wider array of protein sources, with growing interest towards plant proteins, due to the urgency for a global transition from animal to plant-based diets. Limited research on plant protein digestibility in elderly conditions exists, with reported lower digestibility than animal proteins. Increasing protein intake while restraining carbohydrate digestibility is a crucial challenge to be addressed to face concomitantly sarcopenia and type 2 diabetes.

In the present research, bread was chosen as a representative case study of a real food extensively consumed by the elderly. A pea protein-rich bread for the elderly was developed by replacing wheat flour with 50 and 165 g/kg of pea protein concentrate in bread dough. These percentages were chosen to bear the claims “source of protein” and “high protein”, respectively (Reg. EU No 1924/2006). The digestibility of proteins and carbohydrates was evaluated *in vitro* by mimicking adult and elderly physiological conditions. Protein digestibility was measured by the *o*-phthalaldehyde spectrophotometric assay (OPA). Carbohydrate digestibility was assessed by measuring glucose during the intestinal phase of the *in vitro* digestion to estimate the glycaemic index (GI_e).

Although pea proteins negatively affected some key features accounting for the elderly acceptability of bread, reformulation allowed to steer carbohydrate and protein digestibility.

Carbohydrate digestibility was almost unaffected by reformulation, while significant differences were observed as a function of the physiological setting. The GI_e values under elderly conditions were lower as compared to those found in adults, reasonably due to the reduced efficacy of amylolytic activity in the elderly intestinal digestive phase compared to adults. These results could be regarded as positive considering that type 2 diabetes typically onsets during ageing.

The number of free amino acids potentially available for absorption increased significantly from 70 mmol free NH_2/g_{dw} in the case of soft wheat bread to 100 mmol free NH_2/g_{dw} in the “high protein” bread. The same proteolysis was also found in elderly settings, indicating that bread enrichment with pea proteins could be a viable option to tackle sarcopenia.

Still, adding pea proteins reduced the digestion efficiency, with a high ratio of undigested proteins, indicating that further research is required to maximise the efficiency of food design.

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Keywords : Bakery products, Protein enrichment, Elderly, Protein digestibility, Predicted glycaemic index

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21518) - DUAL-TRACER APPROACH TO STUDY AMINO ACID DIGESTIBILITY IN OLD AND YOUNG ADULTS

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Abstract

For healthy ageing and prevention of sarcopenia, the amount and quality of dietary protein intake is important. The quality is determined by amino acid (AA) composition and digestibility of the protein source. Older adults are suspected to have reduced AA digestibility, due to a series of physiological changes in the ageing GI tract as recently reviewed for an older adult INFOGEST in vitro digestion model. However, there is limited *in vivo* evidence. This study aimed to determine the difference in AA digestibility of milk, sorghum and black beans between older (65-80 years) and younger (20-35 years) adults using the dual-tracer method.

The study was executed in 10 young and 10 older adults, with - up till now - data of 7 young and 7 old participants reported. Participants ingested 20g intrinsically ^{2}H-labelled protein (from milk, sorghum or black beans) with 400mg ^{13}C-labelled amino acid mixture in a plateau feeding protocol on three separate test days. The ratio between ^{2}H and ^{13}C enrichment of the indispensable AA in blood plasma was analysed and numerically compared between the two age groups, as a proxy of digestibility.

With data from 14 participants and combined for the three protein sources, the $^{2}H/^{13}C$ ratio was numerically lower in older compared to young adults for some measured indispensable AA. Plasma ratio of leucine was 0.24±0.21 (mean ± SD) in young and 0.22±0.24 in older adults (9% lower in older adults). Numerical reduction of 14% for histidine and 6% for methionine were observed in older compared to young adults. This reduction aligns with the hypothesized lower digestibility in older adults, but proper evaluation using the principles of the dual-tracer approach should be executed.

In conclusion, numerical differences in plasma $^{2}H/^{13}C$ ratio between young and older adults were observed in our preliminary dataset of 14 participants. With an indication of lower values in the older adults for some indispensable AA, that should be confirmed by fully analysing our data according to the principles of the dual-tracer approach. These data indicate at least that the dual-tracer approach can be applied to investigate differences in AA digestibility between young and older adults. Quantifying these differences in digestibility upon ageing provides essential information for product formulation and dietary advice to an older population.

Keywords : ageing, dual tracer approach, protein digestion, human study

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21520) - INQUIRING THE ROLE OF FOOD TECHNOLOGY ON NUTRIENT DIGESTIBILITY AND BIOACCESSIBILITY

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Abstract

In the last decade, food functionality, which usually is defined based on nutrient bioaccessibility, was demonstrated to be affected by the food matrix, the technological interventions, as well as by the interaction among co-ingested food and food components. Several technological approaches have been developed to steer nutrient bioaccessibility, such as the mechanical, physical and enzymatic disaggregation of the matrix to favour their release during digestion, the encapsulation into delivery systems, such as micelles, emulsions and liposomes, to protect them from the harsh gastrointestinal environmental, and the enrichment with bioactive ingredients often obtained from the valorisation of industrial food waste.

Some interventions could deplete nutrients, but improve their bioaccessibility, resulting in a higher concentration after digestion. Conversely, other interventions that increase nutrient concentration, may worsen the bioaccessibility, with no differences (or even a decrease) in the actual amount of the nutrients available for intestinal absorption.

These figures suggest that bioaccessibility, intended as the percentage ratio between the concentration of nutrients in the digested food and that in the undigested food, is not informative about the actual impact of technological interventions on food functionality. Conversely, the concentration of the nutrient found at the intestinal level (*i.e.*, "absolute" bioaccessibility) should be used to compare technological interventions.

Another aspect to be considered is that most studies aim at increasing nutrient bioaccessibility to maximize its absorption, raising two issues: on the one hand, a lower amount of nutrients is available for the intestinal microbiota; on the other, an excessive bioactive uptake could be deleterious or even toxic for the organism.

Moreover, the actual efficiency of the technological intervention on functionality must be considered. For instance, even if nutrient enrichment could increase the absolute bioaccessibility, a considerable amount of the added compound could remain undigested and unfermented, being wasted.

Rather than providing answers, this contribution fosters *a reflection*: "Is an increase in bioaccessibility always desired?"; "Are there safety boundaries?"; "How do technological interventions affect the digestion efficiency?". We will address these questions by describing some study cases showing that opposite effects on bioaccessibility and digestion efficiency can be obtained depending on food features, nutrient nature, and technological intervention type and intensity.

Merging all the knowledge already available on technological interventions as affecting food functionality, would enable a smart design of functional food concomitantly optimizing bioaccessibility and digestion efficiency.

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Acknowledgments

Keywords : technological interventions, bioaccesibility, digestibility, food

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21523) - BROCCOLI SPROUT: PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY AFTER SIMULATED DIGESTION

Lopes, Vívian (Brazil)[1]; Brito, Flavia (Brazil)[1]; Vidal, Juliana (Brazil)[1]; Lopes, Jean (Brazil)[2]; Souza, Yasmin (Brazil)[1]; Zago, Lilia (Brazil)[1]; Citelli, Marta (Brazil)[1]; Bezerra, Rosangela (Brazil)[2]; Antunes, Adriane (Brazil)[2]; Miyahira, Roberta (Brazil)[1]

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Abstract

Seed germination is a process capable of increasing the content of phenolic compounds in seeds. Broccoli sprouts have a high content of phenolic compounds and a high antioxidant capacity that act to protect cells against reactive oxygen species, responsible for oxidative stress and inflammation. However, it is necessary for these bioactive compounds in the food to be bioaccessible after the gastrointestinal digestion process for absorption and subsequent biological activity. *In vitro* digestion techniques can be used to understand the bioaccessibility of compounds present in food. The aim of this study was to evaluate the content of phenolic compounds and antioxidant activity of broccoli sprout extract before and during simulated gastrointestinal digestion *in vitro*. Broccoli sprout extract was supplied by a Swedish company. Digestion was carried out with 6 g of powdered extract according to protocol established by INFOGEST 2.0 (Brodkorb et al, 2019). Total phenolic content was determined by Folin-Ciocalteau method and antioxidant activity by FRAP and ABTS assays in sprouted broccoli extract samples before simulated digestion and after the gastric and intestinal phases. Analyses were carried out in duplicates and the results presented as means and standard deviations. Total phenolic content and antioxidant activity before and during digestion phases were statistically analyzed by analysis of variance (ANOVA) and means were compared using Tukey test ($p<0.05$). Mean and standard deviation of total phenolic compounds before and after gastric and intestinal phases of digestion were 1740 ± 62.2, 536.5 ± 2.1, 224 ± 14, respectively. It was observed that after simulated digestion, the total phenolic content of broccoli sprout extracts decreased significantly during the gastric and intestinal phases compared to the extracts before digestion. With regard to antioxidant activity, there was a reduction after gastric digestion in all methods analyzed. After the intestinal phase, there was a reduction in antioxidant capacity for FRAP method, while ABTS method showed increased antioxidant activity in enteric phase when compared to gastric phase, but still lower than the extract before digestion. Bioaccessibility of phenolic compounds can be affected by different concentrations in plant tissues, variations in cell wall structure and the binding of phenolic compounds in food matrix. Gastric and intestinal fluids interfere with antioxidant activity of phenolics due to deprotonation of hydroxyl portions of aromatic rings. Increase in antioxidant activity by ABTS method after intestinal phase of digestion may be related to the activity of a compound other than phenolics.

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Keywords : Germination, Bioaccessibility, Phytochemicals, Plant extracts, In vitro digestion

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21526) - DESIGNING PROTEIN-RICH SNACK BASED ON FERMENTED FAVA BEANS (VICIA FABA)

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Abstract

The global trend in the increasing snacks market makes this type of product a perspective object for improving the quality of consumers' diets. At the same time, there is still a lack of enriched snacks with superior protein ingredients on the market. According to the existing data, the usage of fava beans, which are characterized by high nutritional value, seems to be a promising way for food products to fortify with plant protein and reduce fast carbohydrates and saturated fats in their content. This study aimed to evaluate the effect of fava bean treatment on the functional characteristics of bars and the digestibility of their nutrients.

For snack development, unfermented (UFB), processed without fermentation (PB), and fermented with Pleurotus ostreatus strain (FB), fava beans were used. Production of bars followed the factory's process. The results showed that the FB was characterized by higher protein content compared with UFB and PB samples. At the same time, adding fermented fava beans led to the reduction of the tannin content of the bars by 18,6 % compared with UFB. It is known that these compounds can form complexes with proteins, which, as a result, decrease protein digestibility and availability of amino acids. Based on the *in vitro* studies, it was found that the type of fava beans processing didn`t significantly affect the proteolysis extent between bars. At the same time, digested samples of bars based on fermented fava beans were characterized by the higher release of some essential amino acids, such as valine and isoleucine, among others. Also, it was found that fermentation with *Pleurotus ostreatus* positively affected the ACE-inhibitory properties of the digested samples of bars (14,43 % for UFB and 29,27 % for FB).

It was concluded that fermented fava beans might be a beneficial rich protein ingredient with improved functional characteristics for snack production.

Acknowledgments

This study formed part of the AGROALNEXT programme and was supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana. Also, authors are grateful for a grant called "Initiative: reception of Ukrainian research personal in the Valencian R+D+I system" from Generalitat Valenciana, Spain and financial support from post-doctoral grant from the Universitat Politècnica de València (PAID-10-21).

Keywords : protein, bars, fermentation, fava beans

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21542) - FLAVONOIDS - ESSENTIAL PLAYERS IN NUTRITION

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1 - Unitre

Abstract

Polyphenols, particularly flavonoids like quercetin, fisetin, and kaempferol, have received significant attention in nutrition due to their antioxidant, senolytic and anti-inflammatory properties. These compounds are commonly found in various plant-based foods and include diverse subclasses, each with unique benefits for health. Understanding their absorption, metabolism, and bioactivity within the human body is crucial for uncovering their full potential. Quercetin, for instance, exists in multiple forms differing in solubility and absorption capacity in the intestine. Often derived from sources like apples, iIts intake is affected by cooking methods, with heat treatment retaining its potency. Fisetin, also present in fruits and vegetables, demonstrates neuroprotective qualities and a higher stability under varied conditions compared to quercetin. Similarly, kaempferol, found in fruits and vegetables, displays antioxidant effects but is influenced by cooking techniques, with certain methods better preserving its polyphenolic content. Overall, these polyphenols offer promising health benefits, yet their optimal dosage and specific dietary recommendations warrant further research to harness their full nutritional potential.

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Keywords : flavonoids, polyphenols, senolytics, Food processing techniques, Bioavailability factors

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21548) - BIOACCESSIBILITY OF MINERALS AND PROTEINS FROM SOY, PEA, AND FABA BEAN

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Abstract

Shifting towards a more plant-based diet has positive effects on the environment[1], and human health as it can reduce the risk of chronic diseases[2] such as cardiovascular diseases[3]. However, due to lower digestibility and the presence of antinutrients, concerns have been raised about the nutritional adequacy of vegetarian and vegan diets[3;4]. In this study, the mineral bioaccessibility and protein digestibility of 11 commercial soy, pea, and faba bean products were assessed using the INFOGEST *in vitro* digestion protocol. Mineral bioaccessibility data were compared with estimations of iron and zinc bioavailability based on the calculation of molar ratios of phytate to mineral, which is often used as an indicator for theoretical bioavailability. Analysis of phytate concentrations and calculation of molar ratios of phytate to iron/zinc to estimate bioavailability showed that all 11 raw materials had a high content of phytate and low estimated bioavailability of both minerals. The results obtained after *in vitro* digestion showed a similar trend of very low mineral bioaccessibility, although low amounts of accessible iron were found in faba bean (concentrate and isolate), and pea (isolate and texturized pea protein). Analysis of the amino acid composition showed that the recommendations for isoleucine and valine were not met by all raw materials, with fava bean products containing the lowest amounts. No major differences were found in the overall degree of hydrolysis (DH), suggesting a good digestibility for pea (DH~60.7 - 98.1 %), faba bean (DH~87.0 - 68.7%), and soy (DH~73.7 - 63.5%). Therefore all raw materials tested can be considered a valuable source of protein when integrated into a balanced diet. However, to improve the bioavailability of minerals in plant-based raw materials and foods, the development and implementation of processing methods to reduce the amount of phytate is essential.

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Keywords : antinutrients, phytate, bioavailability, in vitro digestion, INFOGEST

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21550) - HESPERETIN INCREASES DIFFERENTIATION AND CELL FUSION OF OXIDATIVE STRESS TREATED MYOBLASTS

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Abstract

Introduction: One of the factors contributing to impaired muscle regeneration in elderly is a decreased differentiation capacity of muscle satellite cells. Oxidative stress plays a central role in this process. The aim of this study is to investigate if hesperetin and ellagic acid (bioavailable metabolites from orange polyphenol hesperidin, and pomegranate ellagitannins) enhance myoblasts differentiation in oxidative and non-oxidative conditions, and to explore underlying mechanisms.

Methods: Myoblasts (C2C12 cells) were exposed to hesperetin (5, 20, 50μM), ellagic acid (0.05, 0.1μM) or a combination of these (20μM hesperetin, 0.05μM ellagic acid) during 120h of differentiation in the absence and presence of oxidative stress-inducing compound menadione (9μM) in the first 5h. Proliferation was assessed after 24h using 5-ethynyl-2'-deoxyuridine (EdU) staining. Myosin heavy chain (MyHC) expression, as marker of myoblast differentiation, was assessed by fluorescence microscopy and muscle cell fusion index was calculated as percentage of nuclei within MyHC positive cells. Furthermore, protein expression of (phosphorylated) p38 and myomixer were assessed using Western blot.

Results: None of the compounds induced an effect on cell proliferation. Menadione treatment did not change MyHC expression nor the fusion index. Treatment with 20μM hesperetin resulted in a 15.3% increase in MyHC expression ($p=0.0093$) and 45.4% increase in fusion index ($p=0.043$) in menadione treated cells. In the absence of menadione, 20μM hesperetin increased fusion index with 50.4% compared to control ($p=0.032$). Furthermore, the combination of hesperetin and ellagic acid increased MyHC expression with 14.8% in cells exposed to menadione ($p=0.0203$). Treatment with menadione increased p38 phosphorylation ($p=0.0059$) after 5h and decreased myomixer expression ($p=0.0348$) after 72h of differentiation, while in hesperetin and ellagic acid conditions, p38 phosphorylation and myomixer expression was not significantly different from control.

Conclusion and discussion: Hesperetin increased myoblast differentiation in the presence of oxidative stress induced by menadione, and increased cell fusion both in the presence and absence of menadione. Therefore, hesperetin should be considered as nutritional prevention or treatment strategy to maintain muscle function in age-related diseases such as sarcopenia. Future research should focus on underlying mechanisms as well as translation of these results to clinical practice.

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Acknowledgments

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Keywords : Hesperetin, Ellagic acid, Myoblasts, Differentiation, Oxidative stress

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21560) - EFFECT OF FOOD MATRIX ON THE DIGESTIBILITY OF PAPRIKA AND CINNAMON OLEORESINS-LOADED PARTICLES

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Abstract

Recently, paprika and cinnamon oleoresins (PCOs) have drawn the attention of the food industry due to their functional capabilities. These oleoresins are rich in carotenoids, cinnamaldehyde, eugenol, and other distinctive compounds that impart in their antioxidant and antimicrobial potential, as well as contributing to their color and flavor properties. However, their low stability and low aqueous solubility limit their efficacy and beneficial effects when incorporated into food products. In this way, co-encapsulation is a promising alternative to overcome these challenges. In a previous study [1], we also identified a synergistic effect of PCO mixtures by incorporating them into spray-dried particles. However, the adequate choice of the food matrix to which the particles will be added is essential to allow their diffusion and absorption in the gastrointestinal tract. Thus, in this study, we co-encapsulated PCO mixtures using spray drying. Next, the particles were incorporated (0.5% w/w) into water or commercial mayonnaise and their digestibility and carotenoid bioaccessibility were evaluated. After *in vitro* digestion, particles dispersed in water presented higher lipolysis (37.40 ± 2.58%) as the low viscosity of the medium facilitates the diffusion of bile salts and lipase into the particles. Furthermore, the use of mayonnaise as a delivery system significantly increased bioaccessibility (22.7%), which suggests that the high lipid composition of this matrix improves the delivery of the active compounds from PCOs. Therefore, in this study we observed that the composition of the food matrix affects both lipid digestion behavior and bioaccessibility of PCO-loaded spray-dried particles. We hope that this study can be insightful for the future application of PCO as an ingredient in food products.

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Keywords : co-encapsulation, bioaccessibility, spray drying, mayonnaise, in vitro digestion

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21561) - METABOLIZATION OF BIOACTIVE COMPOUNDS FROM JABOTICABA PEEL THROUGHOUT SHIME IN VITRO DIGESTION

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Abstract

Jaboticaba, also known as the 'Brazilian berry', is a small round purple fruit, rich in nutrients and bioactive compounds. Despite jaboticaba's peel (JP) valuable composition, it is usually discarded. Given bioaccessibility importance for JP valorization, and the issues with *in vivo* experiments, *in vitro* simulations of the physicochemical and microbiological characteristics of the human gastrointestinal tract (GIT) offer unique advantages. The aim of this project was to analyze the phenolic profile of JP bioactive compounds, along with the metabolites generated throughout *in vitro* digestion in a Simulator of Human Intestinal Microbial Ecosystem (SHIME). JP extract and the samples collected during the 76 h SHIME digestion were analyzed by liquid chromatography (LC), after extraction (resuspension, centrifugation, and filtration). LC systems operated at negative polarity with a Poroshell 120 EC-C18 column, and water:formic acid and acetonitrile as mobile phases (0.5 mL/min). The first system (HPLC-ESI) was applied as a first screening with DAD for UV at 280, 305, 320, 360 and 520 nm, and Ion Trap detector for MS/MS^2 (*m/z* 100−1500). The second system was a UPLC-ESI-QTOF in scan MS mode (*m/z* 100–1100), from which the area under the curve was used for intensities comparison. GC-FID was used to determine short-chain fatty acids in colonic samples with a capillary column of fused silica Nukol. JP extract was mostly constituted of hydrolysable tannins, cyanidin and delphinidin based-anthocyanins, quercetin and derivatives, ellagic and gallic acids and derivatives. Neither the appearance of new metabolites, nor the disappearance of any original phenolic compounds, were detected in the first digestion phases. Intensity decay could not be observed in all anthocyanins in the gastric and intestinal phases as was expected, probably due to the cleavage of bigger compounds, which may also have happened in other compound classes. This scenario changed when the colonic fermentation was reached, with drastic reduction of anthocyanins intensity, being probably metabolized to phloroglucinol, gallic, protocatechuic and 3-hydroxybenzoic acids. Data indicates that quercetin derivatives and hydrolysable tannins were degraded to smaller molecules (quercetin, gallic acid and ellagic acid), which may have been further metabolized to 4-hydroxybenzoic acid, protocatechuic acid and urolithin D[FTB1] . Partitional clustering with K-means analysis evidenced samples division in (1) extract, stomach and duodenum; (2) colonic fermentation before 25 h; (3) colonic fermentation after 25 h. No significant differences could be detected in SCFA concentrations, despite the slight increase in butyric and acetic acids until 16 h of fermentation. A clear shift in phenolic profile has been observed during SHIME digestion of jaboticaba peel, which should be considered in further studies about biological activities.

Acknowledgments

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Keywords : anthocyanins, hydrolysable tannins, metabolomics, berry, dynamic digestion

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21562) - IMPACT OF SOLID-STATE FERMENTATION WITH PLEUROTUS OSTREATUS ON IN VITRO DIGESTIBILITY OF FAVA BEANS

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Abstract

Proteins constitute one of the most important macronutrients in the diet. Over the last 50 years, protein consumption has grown in developed countries, with a strong increase in the intake of animal-origin products. Therefore, alternatives to sources of animal origin must be sought. Legumes have been declared as valuable alternative plant sources of dietary protein. Their consumption has been associated with a lower prevalence of diseases like obesity or type 2 diabetes; nonetheless, the presence of anti-nutritional factors can affect their nutritional quality. Solid-state fermentation appears as a sustainable bioprocess available to modify the nutritional and functional profiles of plant-based materials positively. Thus, this study aims to explore the impact of Solid-State Fermentation (SSF) with *Pleurotus ostreatus* in fava beans (Vicia faba) on its nutritional value and its digestibility and functionality (amino acid profile and Angiotensin Converting Enzyme (ACE) inhibitory activity). Fermentation increased total protein content, up to 16%, in beans, due to fungal biomass generation; meanwhile, this process reduced total carbohydrates by 10%. Moving onto digestibility, fermented beans exhibited reduced glycolysis (up to 20%) but low proteolysis (25%) compared to the values found in unfermented ones. However, the bioaccessibility fraction of the fermented beans was characterized by a higher release of certain amino acids, such as isoleucine and threonine, which are essential amino acids related to hemoglobin and collagen synthesis, respectively. Lastly, fermentation considerably increased both the ACE inhibition percentage in the fava bean protein and the bioaccessibility fraction of the digest (up to 60% inhibition). In conclusion, fava beans fermented with *Pleurotus ostreatus* could be a valuable new ingredient for designing rich protein-based products.

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Keywords : Fava beans, fermentation, Pleurotus Ostreatus, protein, in vitro digestion

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21565) - ANTI-INFLAMMATORY PROPERTIES OF PIGMENTS EXTRACTED FROM ARTHROSPIRA PLATENSIS (SPIRULINA)

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Abstract

Spirulina is a photosynthetic cyanobacterium with significant nutritional properties and a minimal environmental footprint. It is a great source of protein (63 g/100 g) and antioxidant compounds such as b-carotene and phycocyanin. Phycocyanin, the most abundant pigment, has been found to have numerous health benefits. It may, however, be essential to investigate other bioactive molecules in Spirulina that can help alleviate intestinal inflammation. The aim of this study was to assess the potential anti-inflammatory properties of three pigments previously extracted from Spirulina: phycocyanin, pheophytin a, and pheophorbide a. To this end, Caco-2 cells, stimulated with interleukin-1b (IL-1b; 50 ng/ml) were used as a model of intestinal inflammation. The neutral red assay was used to evaluate the cytotoxicity of the compounds in the presence of IL-1b. The cells were supplemented with increasing concentrations of phycocyanin, pheophytin a and pheophorbide a (1, 5, 10, 25 and 50 mg/ml), for 1 hour and then exposed to IL-1b for 24 hours. Then media were collected and used to evaluate inteleukin-8 (IL-8) secretion via sandwich ELISA. All three pigments reduced IL-8 secretion compared to the IL-1b-stimulated positive control (no supplementation) starting from the lowest dose tested (1 mg/ml). Most potent was pheophorbide with 35-85% inhibition at the lowest-highest dose, compared with phycocyanin and pheophytin that reduced IL-8 secretion at lowest dose by 25% and 16%, respectively. Overall, under the experimental conditions tested, all pigments showed anti-inflammatory properties although the magnitude differed. Further analyses are underway to better understand their mechanism(s) of action.

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Keywords : inflammation, Spirulina, Caco-2, interleukin-1b, interleukin-8

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21566) - NUTRITION DECLARATION. IS IT TIME TO CONSIDER NUTRIENT BIOACCESSIBILITY?

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Abstract

In Western countries, the intake of lipids exceeds the daily dietary reference intake (DRI). This constitutes a health risk, particularly when excessive intake is related to saturated fats. For this reason, the nutrition declaration must contain indications on the fat and saturated fat content per 100 g of food. This causes two problems: the first is related to the extraction method to be used, the second to the reliability of the chemical composition as a parameter for discrimination between different foods. In fact, to be absorbed at the intestinal level and therefore exert their effects in the organism, lipids must become bioaccessible during digestion, i.e., be released from the food matrix. Since the food matrix has a great impact on the release of nutrients, we evaluated the bioaccessibility of lipids in four commercial foods: biscuits, walnuts, spreadable cheese and canned pickled mackerel. Foods were digested in vitro using the INFOGEST protocol and lipids were extracted from not digested and from the soluble (SF) and not soluble fraction (NSF) of digested foods using two different methods [1, 2] to test their extractive capacity. Regardless of the type of extraction used, the percentage amount of lipids detected in the SF, i.e., released from the matrix, appeared significantly different, and the relative release (RR) ranged from ~ 92% in the spreadable cheese to ~ 20% in walnuts and canned mackerel. After derivatization and gas chromatographic quantification of fatty acid methyl esters in SF and NFS, a similar relative release was observed (spreadable cheese ~ 90%; biscuits ~ 70%; canned mackerel ~ 27%; nuts ~ 20%). This study confirms that the bioaccessibility of lipids strongly depends on the food matrix in which they are incorporated. Considering the role of lipids in health and well-being, including their energy value, our findings suggest a step beyond simply classifying foods based on their chemical composition.

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Keywords : bioaccessibility, lipids, extraction

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21567) - BIOACCESSIBLE PROFILE OF POLYPHENOLS FROM UP4HEALTH'S OLIVE POMACE-BASED INGREDIENT: SUSTAINABILITY AND HEALTHINESS CONVERGING IN THE AGRIFOOD SECTOR

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Abstract

The UP4HEALTH initiative addresses challenges related to agrifood industry residual streams, focusing on transforming biowastes such as olive pomace into healthy and sustainable ingredients rich in bioactive phytochemicals, all within the framework of a pre-industrial scale integrated biorefinery. However, the knowledge of the chemical composition and digestive behaviour of these phytochemicals is essential to assessing the functional potential of the ingredients, as only the compounds in their accessible form upon the completion of digestion will ultimately be absorbed and reach the target tissues to exert health-promoting activities. This study aimed to assess the polyphenol composition and bioaccessibility of UP4HEALTH's olive pomace-based ingredient, derived from the supercritical fluid extraction of olive oil (PBF). The residual fibrous matter was ground and subjected to *in vitro* digestion using INFOGEST static model at a concentration of 0.08 g/mL (PBF:water, *w/v*), a ratio established for its incorporation into food prototypes. The polyphenols and related compounds in the ingredient and the bioaccessible fraction obtained upon dialysis of the chyme were analysed by LC-DAD-ESI-HRMS/MS. Twenty-five compounds were identified and quantified in PBF, with the caffeoyl phenylethanoid glycoside verbascoside and hydroxytyrosol as the major compounds (5.79±0.04 and 2.46±0.11 mg/g ingredient, respectively). Phenylethanoids and their derivatives was the primary compound class in the non-digested sample, followed by characteristic secoiridoids of olives such as oleuropein aglycone (1.51±0.06 mg/g). On average, 55% of the total extractable polyphenols in the ingredient were bioaccessible after simulated digestion. Notably, caffeic acid (271%), tyrosol (226%), isoverbascoside (136%), hydroxytyrosol (114%) and secologanoside (110%) were the most bioaccessible compounds, and those presenting relative bioaccessibilities exceeding 100% among all the detected molecules. These substantial transfer rates to the aqueous bioaccessible fraction suggest that these compounds likely underwent further generation during digestion, potentially via hydrolysis or isomerization from related or conjugated compounds with more complex structures. Correspondingly, the lowest bioaccessibility values were observed in acylated secologanosides and phenolic glycosides. The high bioaccessibility of key polyphenols in PBF underlines the possibility of employing it as a functional ingredient to develop bioactive-enriched food products, an approach that also aligns well with global initiatives for sustainable development and circular economy in the agri-food sector. Investigations on the bioaccessibility of PBF compounds in prototypes of cereal bars are ongoing and will offer valuable insights into the digestive behaviour of these compounds in actual food matrices.

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Keywords : Olive pomace, Bioaccessibility, Polyphenols, In vitro digestion, Functional ingredient

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(21569) - DEVELOPMENT OF MACHINE LEARNING PREDICTIVE MODELS FOR CURCUMINOID BIOACCESSIBILITY ACROSS VARIOUS CARRIER FOOD FORMULATIONS

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Abstract

Background: Evaluating the efficiency of absorption of bioactive components remains integral to gauging the nutritional quality of diverse food products. However, a notable research gap exists in comprehensively examining how food matrix composition influences this absorption as the task is ominous in scope. To delve deeper into this research question, curcuminoids were chosen as subject compound. Extracted from Curcuma longa, these compounds are renowned for their health benefits but notorious for exhibiting poor bioavailability.
Project objective: This research project systematically explores the effect of food matrix composition on the bioaccessibility of curcuminoids, as it is arguably the limiting factor of their absorption. Additionally, it aims to construct predictive models that offer insights for optimizing formulations of functional foods and provide more detailed information to consumers about the outcome of a food's consumption.
Methods: Static in vitro digestion simulations, following the precepts of the consensus protocol proposed by INFOGEST, were employed on curcuminoid-enriched food formulations, and bioaccessibility was characterized using chromatographic (HPLC) and spectrophotometric (UV-Vis) techniques to quantify curcuminoids. Dietary fibre supplements containing soluble and insoluble fibres as well as other nutrients were used to generate a variety of, chiefly, custard and biscuit formulations. The resulting dataset served as the basis for constructing predictive models that estimate bioaccessibility based on specific physicochemical properties (compositional content, texture, ingredient density or liquid retention, and others) of the food matrices.
Results: Macronutrient content (sum of protein, lipid and carbohydrate content) emerged as a highly informative explanatory variable for curcuminoid bioaccessibility and was integrated as its main differential factor in a Bayesian hierarchical model. This model accurately predicted curcuminoid bioaccessibility (optimization performance of 0.97 R2) for the majority of cross-validated test formulations (LOOCV of 0.93 R2). These initial results pave the way for further exploration, suggesting researchers to employ stochastic modelling as it can aid characterizing a broader range of food matrices that influence absorption of bioactive compounds.
Conclusions: This research is a proof-of-concept study that indicates the intricate interplay between food matrix composition or structure and bioaccessibility could be mathematically modelled. It establishes a platform for future investigations that could deliver advancements in understanding bioactive compound absorption, with possible implications for the food industry and consumer choices.

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Keywords : food matrix, bioaccessibility, systematic screening, probabilistic modelling, Machine Learning

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22595) - PLANT-BASED BURGERS: PROTOTYPES DEVELOPMENT, STUDY OF PROTEIN DIGESTIBILITY, AND SAFETY ASSESSMENT

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Abstract

The study aimed to develop different new formulations of plant-based meat analogues trying to achieve a good meat-like structure, taste, nutritional composition, protein quality, and also ensure the safety of products.

Prototypes were developed at the German Institute for Food Technology (DIL). Recipes were developed using different protein sources (pea and soy), binders (methylcellulose and transglutaminase), and protein textures (low- and high-moisture). Following the development and refinement of recipes, prototypes (N=12) were studied investigating their overall attributes, such as composition (macronutrients) and protein quality (profile and integrity). Protein digestibility was evaluated by estimating proteins solubilised and peptides released after digestion. Furthermore, preliminary studies on risk assessment were performed evaluating the presence of anti-nutritional factors (anti-trypsin) and mycotoxins in both protein isolates and protein textures – used in the recipes – and final products. On some of these products, a preliminary assessment of methodologies for determining the allergenic potential after digestion is also underway.

All prototypes showed good similarity with meat concerning appearance (colour and shape), taste, and texture (bite and mouthfeel). Looking at these attributes, the pea-burger, made with a mixture of high- and low-moisture textures (ratio 1:1) and methylcellulose, appears to be the best sample. Results highlighted a comparable nutritional profile of products with the meat control, showing also a lower amount of saturated fatty acids. Nevertheless, new formulations achieved a good amino acidic profile with a very good content of essential amino acids. The products showed good protein digestibility, with some differences according to the ingredients used. The investigation of the anti-nutritional factors in soy proteins and products showed the presence of anti-trypsin activity. Some differences in the anti-trypsin activity were observed in samples, also consistent with what observed for digestibility, related to the textured and binder used. The analysis, performed on the occurrence of 11 mycotoxins in these burgers, showed the presence of 1 mycotoxin (Tentoxin, produced by *Alternaria alternata*) in soy protein isolate, textures, and, in lower amounts, soy-based burgers. Concerning allergenicity, a digestibility method optimised for immunoblotting analysis was assessed on the most representative samples.

Results highlighted that plant-based burgers can be a good replacer for meat. Indeed, using good quality protein to produce textures, low amounts of fat, and the right binder can lead to obtaining products with a comparable nutritional profile of meat, achieving good protein digestibility and biological value. Furthermore, this is one of the first studies with a safety assessment approach to these kinds of new products.

Keywords : meat analogues, protein quality, protein digestibility, risk assessment, anti-nutritional factors

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22596) - ASSESSMENT OF PROTEIN QUALITY, DIGESTIBILITY AND POTENTIAL BIOACTIVITY OF SOY FLOUR EXTRACTS

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Abstract

Soybean is widely used to produce food and feed livestock due to its high nutritional value, cost-effectiveness, and technological properties. Different protein extraction techniques are applied to promote the valorisation of plant proteins, including the reuse of food waste and by-products. Traditionally, soy proteins are wet extracted from soybean meal using heat and basic pH to produce high-yield protein concentrates and isolates. However, some aspects related to the quality of proteins extracted under different conditions and their possible impact on biological properties are still overlooked.

Therefore, in this work the effect of two soybean varieties (i.e., Energy and Namaste) on protein digestibility after processing was evaluated. Soybean meal was characterised and subjected to two commonly applied procedures to extract plant proteins (pH 7.5, 1 hour, RT and pH 11, 3 hours, 60 °C), to evaluate their impact on gastrointestinal digestion *in vitro*, characterising protein solubilisation. Furthermore, in recent years soy has gained more and more interest due to its beneficial effect on health. Therefore, the bioactivity of the digested mixtures was studied by characterising the effects on total GLP-1 secretion and DPP-IV enzyme inhibition.

The results showed that in all conditions, soy flours and extracts have a high capacity to stimulate the release of GLP-1 and to inhibit the activity of the DPP-IV enzyme at intestinal level. However, some changes in protein solubilisation were observed across different varieties and extraction conditions.

Keywords : soy, protein extraction, protein solubilisation, GLP-1, DPP-IV

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22598) - BIOACCESIBILITY AND BIOAVAILABILITY OF POLYPHENOLS FROM THE TIGER NUT BY-PRODUCT AND IMPLICATION IN THE OCHRATOXIN A BIOAVAILABILITY

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Abstract

Tiger nut (*Cyperus esculentus L.*), is a small, sweet tuber rich in fiber, antioxidants, and minerals. The Valencian variant in Spain is especially notable, holding a Protected Designation of Origin (PDO) status. These tubers are the key ingredient in the traditional Spanish beverage, "Horchata de chufa". Tiger nut beverage production yields a substantial solid by-product (TNBP), which constitutes roughly 60% of the total weight.

This study aims to analyze the polyphenolic content of TNBP digest and its effect on the bioavailability of Ochratoxin A (OTA).

TNBP was subjected to *in vitro* digestion according to the INFOGEST protocol (Brodkorb *et al.,* 2019). Polyphenols were determined in the bioaccessible fraction (BF) and their bioavailability was assessed in Caco-2 cells in conjunction with the mycotoxin OTA (40 µM) for 4 hours. Polyphenols were analysed by HPLC-UV/VIS, and OTA by LC-MS/MS.

A total of 12 polyphenols were determined in the BF. The caffeic acid hexoside and ferulic acid hexoside were the most abundant and showed bioaccessibility values of 122% and 78% and a bioavailability of 73% and 62%, respectively. When OTA was present, bioavailability was 63% and 71%, respectively. On the other hand, OTA bioavailability was increased up to 79±2% when combined with TNBP digest. During the 4h assay, the bioavailability of OTA was constant in the solvent while in the presence of the digest a decrease is observed over the 4h, ranging from 21 to 34%.

While the presence of TNBP digest significantly increased the bioavailability of OTA, it also showed a decreasing trend in OTA bioavailability over time in the presence of the digest. These results highlight the importance of further research to fully understand the implications of TNBP consumption, particularly in relation to OTA exposure, and to explore the potential of TNBP as a functional food ingredient given its rich polyphenolic content.

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Keywords : Bioavailability, tiger nut by-product, Caco-2, Ochratoxin A

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22604) - CALCIUM AND PHOSPHORUS IN ORGANIC AND A2 MILK AND IN VITRO DIGESTION ASSAY: PRODUCTION SYSTEM AND NUTRITIONAL IMPLICATIONS

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Abstract

The consumption of different types of bovine milk, such as A2 and organic milk, is increasingly popular among consumers, due to the perceived health benefits and their associated digestibility properties. Even though their richness in essential minerals is widespread in different cultures, studies comparing the bioaccessibility of minerals in organic and non-organic milks are still scarce. The study aimed to determine the content of calcium and phosphorus in A2 and organic milk and to estimate their bioaccessibility by the INFOGEST 2.0 *in vitro* digestion protocol. Three brands of pasteurized organic milk (ORG 1, ORG 2 and ORG 3) and two brands of pasteurized non-organic A2 milk (AT 1 and AT 2) were purchased from local markets of São Paulo, Brazil. Total and bioaccessible Ca were determined by FAAS with diluted acid in wet mineralization. Total and bioaccessible P were quantified by colorimetric method in the visible region. The overall Ca concentrations evidenced that most organic samples presented higher values compared to A2 samples. ORG B showed the highest Ca content (130.34±0.47 mg $100g^{-1}$), followed by ORG C (115.26±4.23 mg $100g^{-1}$), AT 2 (114.58±3.45 mg $100g^{-1}$), ORG A (105.88±6.59 mg $100g^{-1}$), and AT 1 (95.70±6.05 mg $100g^{-1}$). In contrast, AT 2 evidenced high content of total P (83.55±2.24 mg $100g^{-1}$) similar to ORG A (81.74±1.11 mg $100g^{-1}$), whilst remaining samples presented equivalent values, varying between 74.19 and 75.41 mg $100g^{-1}$. *In vitro* digestion procedure enabled the observation of high bioaccessible proportions of Ca for ORG A (28.1%) and ORG C (20.7%), meanwhile ORG C and both A2 milk samples showed percentages below 15%. The same behavior was not observed for bioaccessible P, which presented values between 58.2 and 77.8% for all samples. Different aspects could interfere in the solubilization of Ca and P, during the gastrointestinal digestion, such as the precipitation of casein micelles in gastric curds, the aggregation of long chain fatty acids with Ca salts, and interactions between the electrolytes of the harmonized procedure and the samples' elements. The findings demonstrated that the production system might have substantial impact for the total and bioaccessible contents of calcium, in milk, as observed for the results of organic and non-organic samples. In addition, both products evidenced similar results for total and bioaccessible phosphorus, evidencing that non-conventional milks are suitable sources of essential minerals.

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Keywords : bioaccessibility, essential elements, avalialbility, production system

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22606) - EFFECT OF A PRE-HYDROLYSIS ON THE IN VITRO DIGESTIBILITY OF ARTHROSPIRA PLATENSIS

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Abstract

Spirulina, a cyanobacteria that is 60% protein, could provide an alternative to animal proteins, the production of which is a major contributor to greenhouse gas emissions. Protein from this organism, which is also rich in vitamins and minerals, has good amino acid profile and contains bioactive activities. However, its digestibility has been poorly assessed and varies greatly from one study to another. Moreover, spirulina protein digestibility remains lower than that of animal proteins. One possibility to improve spirulina digestibility is to pre-hydrolyze the proteins into peptides using various proteases. Additionally, pre-hydrolysis of Spirulina by various enzymes could significantly increase spirulina protein digestibility.

In this study, alcalase and bromelain were used at different concentrations (0.25% and 1%) to pre-hydrolyze dry or frozen spirulina. Samples were analyzed by size exclusion chromatography and mass spectrometry before digestion. Then, the INFOGEST *in vitro* static digestion model was used to assess spirulina digestibility and free amino acids were assayed after digestion. Pre-hydrolysing spirulina did not improve significantly protein digestibility, which stands at around 81%. However, qualitative differences were observed in the pre-hydrolyzed solutions before and after digestion. In particular, peptide size varied according to the enzyme used (alcalase vs bromelain), its concentration (0.25% vs 1%), and the storage method (dry or frozen spirulina). Additionally, at the end of the gastric phase, bioactive peptides could be identified although they were still encrypted in longer sequences.

Keywords : spirulina, protein digestibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22607) - METHOD TO CHARACTERIZE AMYLASE TRYPSIN INHIBITORS BINDING TO TOLL-LIKE 4 RECEPTOR USING HUMAN TLR4 REPORTER HEK293 CELLS LINE

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Abstract

Amylase Trypsin Inhibitors (ATIs) have attracted scientific interest for their potential role in irritable bowel syndrome (IBS) and non-celiac wheat sensitivity (NCWS). These inhibitors survive the gastrointestinal digestion, are suspected to bind to the TLR4-MD2-CD14 complex and trigger intestinal pro-inflammatory responses. The potential binding capacity of ATIs to the TLR4-MD12-CD4 complex was preliminary shown using the immunoprecipitation method, but no cell studies validated these results. For cell assays, the use of ATIs-enriched fractions requires taking into account contaminants such as Lipopolysaccharides (LPS). These major components of the membrane of Gram-negative bacteria can also interact with Toll-like receptor 4 and therefore mask the response induced by the proteins. The complexation between LPS and proteins makes the purification of the proteins difficult, resulting in a high or complete loss of the protein fraction. In this study, we provide a methodology to study the binding potential of ATIs enriched fraction to TLR4-MD2-CD14 using Human TLR4 reporter HEK293 cells line. The method takes into account Lipopolysaccharide (LPS) contamination in the protein extract and any remaining contaminants. In our approach, several treatments were used in the TLR4 binding assay to estimate the response of the different constituents. For instance, Polymyxin B (PMB) was used to block the binding of LPS. Proteinase K was used to hydrolyze the proteins present in the fraction and eliminate the response induced by the proteins. Finally, a double treatment of PMB and Proteinase K was performed to estimate the contribution of the remaining contaminants. The results show that ATIs-enriched fractions treated with PMB and with Proteinase K and PMB resulted in, respectively, 94% and 95% of the binding signal. Complete hydrolysis of the protein by Proteinase K showed a reduction of 42% in the binding capacity of the fraction. This suggests that LPS present in ATIs enriched fractions are the main molecules responsible for the activation of TLR4-MD2-CD14. The partial loss of binding capacity after Proteinase K may highlight a potential interaction between ATIs and LPS that would intensify the LPS-induced responses. Altogether, these findings point to an indirect activation of TLR4-MD2-CD14 by ATIs. We hypothesize that the complexation with ATIs might allow the LPS to cross the intestinal epithelium and access the gut immune compartment, where it stimulates the TLR4-MD2-CD12 receptor. This suggests an indirect role of ATIs in intestinal inflammation reactions.

Keywords : Amylase Trypsin Inhibitors, Celiac disease, Toll-like receptor 4, Lipopolysaccharide, Wheat

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22615) - ALTERNATIVE DIETARY PROTEINS AND IN VITRO GUT BARRIER RESPONSES

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Abstract

There is a need to identify sustainable alternative dietary protein sources to reduce the global overreliance on animal proteins. The transition from an animal-based protein diet to more sustainable protein sources must be assessed in terms of environmental impact but also in terms of human health. This study aimed to track the digestibility of four alternative protein ingredients (Honey *Chlorella vulgaris*, Fava bean isolate, Cricket powder and Microbial protein biomass) and assess their impact on the gut barrier. A static *in vitro* gastrointestinal digestion using the INFOGEST method for the adult human was performed. *In vitro* digestibility was determined by OPA method, SDS-PAGE and free amino acid analysis. Polarised coculture of Caco-2/HT29-MTX cell monolayers were differentiated in transwells for 21 days prior to digesta treatment. Measurement of Transepitheial electrical resistance (TEER) indicated gut barrier integrity. Amino acid bioaccessibility and bioavailability data was determined by free amino acid analysis of the digesta, the Caco-2/HT29-MTX apical chamber and the basolateral chamber respectively. At the end of the intestinal digestion, Fava bean isolate had a 100 ± 0 % digestibility by OPA method, followed by Cricket powder (87 ± 5 %), Honey *Chlorella vulgaris* (74 ± 3 %) and Microbial biomass (73.5 ± 0.8 %). Total free amino acids differed with protein ingredients with Cricket powder having the highest total free amino acid amount in the digesta sample (5355 ± 784.5µg/mL) and in the apical chamber (551.2 ± 35.5µg/mL). Although Honey *Chlorella vulgaris* had the highest absolute value of amino acid bioavailability in the Caco2/HT29-MTX basolateral chamber (2.7 ± 1.7µg/mL), there were no significant differences between protein samples tested ($p>0.05$). TEER values were similar for Caco-2/HT29-MTX cell monolayers treated with different digesta samples ($p>0.05$ and $>700\ \Omega/cm^2$).

This study delivers *in vitro* digestibility and bioavailability data for four sustainable food protein ingredients. Our *in vitro* evidence shows these ingredients do not damage gut barrier integrity. Further additional evaluations for digestibility, gut health and allergenicity are necessary to assess the nutritional profile of these alternative protein sources.

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Keywords : alternative proteins, gut barrier, digestibility, health

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22618) - INDIGESTIBLE PROTEINS AND PEPTIDE-BOUND AGES IN ULTRA-PROCESSED FOODS: DULCE DE LECHE AS A MODEL OF SEVERELY TRANSFORMED FOOD MATRICES

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Abstract

Free and peptide-bounds dietary advanced glycated end products (AGEs) can trigger oxidative and inflammatory cascades in several target organs, at least partly *via* stimulation of specific receptors (RAGE, receptors for AGEs). Rising consumption of AGEs through ultra-processed foods has been associated with the increased prevalence of noncommunicable diseases, including metabolic syndrome, obesity, diabetes, kidney damage, and food allergies [1]. Caramel-like confectionery used for candy bars, toffees, flavoring toppings, puddings, milk jam, and desserts is produced by mixing milk cream with glucose syrup, molasses, or inverted sucrose at 115 -130 °C. The traditional Latin American delicacy *dulce de leche* is produced by simmering whole or reconstituted milk with sugar and baking soda for a long time until it achieves a creamy consistency, which results in extensive Maillard reaction-induced modifications of milk proteins [2].

Our study aimed to unravel the impact of extreme processing on milk proteins and their digestion products. *Dulce de leche* was subjected to simulated gastroduodenal digestion using adult or infant static models. SDS-PAGE analysis revealed such profound alterations in *dulce de leche* that even the most abundant milk proteins were undetectable. Covalent interchain cross-links or sugar-derived bridging moieties generate macro-aggregates of milk proteins, which were resistant to pepsin and only partially susceptible to duodenal proteases. Gastroduodenal digests contained a complex multitude of variously sized polypeptides. The LC-MS/MS analysis enabled the identification of numerous peptides exhibiting the hallmark modifications from Maillard reactions alongside hundreds of unmodified small protein fragments, while many others remained unidentified due to unknown structural changes [3]. The abundance of peptide-bound AGEs in caramel-like products and other ultra-processed foods raises health concerns, particularly regarding the interaction with gut-resident immune cells and systemic distribution, which relate to their bioaccessibility and bioavailability, respectively, as well as their potential effects on the gut microbiota. Assessing the health implications of indigestible protein aggregates and peptide-bound AGEs in ultra-processed foods is an urgent need, especially since they are primarily targeted at children who have a developing immune system, increased intestinal permeability, and high intake-to-body weight ratio.

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Keywords : Ultra-processed milk proteins, Peptide-bound AGEs, Maillard-reactions, LC-MS/MS, Protein macro-aggregates

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22630) - ALMOND (PRUNUS DULCIS) BY-PRODUCTS FROM SUPERCRITICAL FLUID EXTRACTION EXHIBIT PROMISING ANTIOXIDANT CAPACITY AFTER SIMULATED DIGESTION

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Abstract

Supercritical fluid extraction (SFE) has been rising in popularity for boosting extraction yields and enhancing industrial efficiency without the need for hazardous solvents, thus earning recognition as an eco-friendly technology. Therefore, almond bioresidues were subjected to SFE with CO_2 by ISANATUR (Navarra, Spain), a company specialized in extractions from by-products. More specifically, the industrial residue from almond oil extraction (referred to as APBF+) was subjected to SFE to further extract oil at operational pressures of either 20 MPa or 24 MPa, thereby generating two sub-residues (APBF20 and ABPF24 samples, respectively). In view of the possible use of these bioresidues as ingredients in food or food supplement formulations, this work aimed at verifying and comparing the bioactivity (antioxidant activity) of the three residues after being subjected to a digestion process. For that purpose, *in vitro* digestion experiments employing the INFOGEST protocol were separately carried out with the almond by-product (APBF+) and their SFE residues (APBF20 and APBF24). A blank assay with 5 mL of water instead of the sample was also performed. The samples underwent oral, gastric, and small intestinal phases of digestion, with the chyme being subjected to dialysis using a 3.5 kDa membrane to obtain the bioaccessible fraction of each sample. Finally, the antioxidant potential of the bioaccessible fractions was evaluated by two different assays, namely TBARS (Thiobarbituric Acid Reactive Substances) and $DPPH^{\cdot}$ (2,2-diphenyl-1-picrylhydrazyl radical scavenging). In the $DPPH^{\cdot}$ assay, the bioaccessible fractions from APBF20 and APBF24 exhibited EC_{50} values of 3.8 mg/mL and 4.4 mg/mL, respectively, both presenting an antioxidant potential higher than that found for the bioaccessible fraction of the initial almond residue (APBF+, EC_{50} of 5.3 mg/mL). When comparing the EC_{50} values obtained for the bioaccessible fractions of all three residues with that of the blank (20.7 mg/mL), it became apparent that following digestion, the by-products still exhibit valuable antioxidant activity. Regarding the TBARS assay, SFE samples did not differ from the APBF+ between themselves, since all showed an EC_{50} of 2.9 mg/mL. Compared to the blank (EC_{50} of 4.16 mg/mL), the by-products presented satisfactory antioxidant capabilities post-digestion. In summary, when subjected to digestion, the almond by-products retain antioxidant potential, with the SFE samples presenting similar or even superior activity than the residue from a single oil extraction that originated them. Therefore, almond by-products resulting from SFE are corroborated as an interesting source of functional compounds with antioxidant properties to be incorporated into food and nutraceutical products. Further studies are underway to identify the phenolic compounds in the residues and quantify them along the INFOGEST phases.

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Keywords : Prunus dulcis, Antioxidant capacity, Simulated digestion

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22633) - THE INFLUENCE OF FOOD MATRIX ON THE IN VITRO DIGESTIBILITY OF WHOLEGRAIN CEREALS AND THEIR ANTIOXIDANT PROPERTIES

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Abstract

Wholegrain flours have drawn particular attention lately because of their potential to enhance life quality by preventing diseases linked to poor nutrition and by displaying numerous health benefits. The food matrix, which results in intricate relationships between specific chemical constituents and the food product's digestibility, is crucial to this subject. The objective of this study was to evaluate the nutritional potential of different oat and maize genotypes with varying grain colors as well as the antioxidant and digestive properties of microencapsulates obtained from blue maize. A modified in vitro multi-step digestion process comprising oral, gastric, duodenal, and colon phases was used to assess the digestibility of the investigated samples. The procedure developed by Hamzalıoğlu and Gökmen and proposed by Papillo et al. was carried out without any attempt to mimic the intricacies of gastrointestinal digestion. Oat hulls contained higher levels of total phenolic compounds and phenolic acids than flour. The majority of ferulic acid was found in the hulls and whole-grain flour. The oat hulls exhibited a greater antioxidant capacity. Conversely, the β-glucan level in the hulls was just 0.03–0.06%, whereas in the whole-grain oat flour samples it ranged from 4.07% to 5.33%. Brown whole-grain flour had the best *in vitro* digestibility (48.24%), followed by black (44.72%) and yellow oat flour (44.54%). Considering that the *in vitro* digestibility varied from 12.02% in the black genotype to 16.69% in the brown genotype, the powdered oat hulls' degradability was noticeably lower. Significant variations were found in the *in vitro* digestibility of all the studied maize flours. The highest digestibility was found in the flour of sweet maize hybrid (57.36%), while the lowest level was found in the flour of blue popping maize (19.67%). The pericarp was least affected by the digestive processes, while the germ showed the highest degree of degradation when it came to the digestibility of the various kernel sections. The microencapsulates had an average total free phenolic compound content of 31380 mg CE/kg. The initial raw material had an anthocyanin content of 1426 mg CGE/kg, whereas the average content in microencapsulates was 10677 mg CGE/kg. The microencapsulate digestion fluids containing 30% hydroxypropyl-β-cyclodextrin (HPCD) showed the highest anthocyanin residues (54-69%) after each *in vitro* phase. The microencapsulation system with 15% maltodextrin and 15% HPCD, on the other hand, demonstrated the least stability. As a result, these microencapsulates had the highest digestibility of 73.63%. The results indicate that the intricate processes of food degradation by digesting enzymes are significantly influenced by variations in chemical composition and inherent kernel structure. Nonetheless, more research on this specific topic is required in the near future.

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Acknowledgments

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Keywords : maize, oats, in vitro digestibility, food matrix, antioxidant properties

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22635) - COMBINING IN VITRO DIGESTION AND ABSORPTION STUDIES; THE IMPACT OF THE GUT BARRIER-A MODEL STUDY WITH CURCUMIN

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Abstract

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Colloidal delivery systems improve the oral bioavailability of lipid-soluble bioactives, providing protection throughout the gastrointestinal tract. To evaluate the efficacy of such delivery systems, a comprehensive approach combining *in vitro* digestion with absorption/transport studies is required.

In this study, we used curcumin loaded delivery systems as models to evaluate curcumin bioaccessibility, simply measured after INFOGEST static digestion, and compared with its bioavailability once the digesta were subjected to an *in vitro* co-culture absorption/transport model, Caco-2/HT29-MTX, consisting of both absorptive and mucus producing cells. Ultra-high performance quadrupole time-of-flight mass spectrometry was utilized to quantify the recovered curcumin post digestion (analyzing both the whole digest and micellar phase) and absorption/transport (analyzing the apical, cellular, and basolateral fractions).

Protein nanoparticles made of lupin proteins protected curcumin from degradation better than oil in water emulsions stabilized with lupin proteins. A 70% recovery of the initial curcumin content was identified in the whole digesta of protein nanoparticles, whereas emulsions exhibited a 35% recovery. The analysis of curcumin recovery in the micellar phase yielded comparable results with the whole digest in the case of emulsions however, a significantly lower recovery was noted in the micellar phase of protein nanoparticles compared to the whole digest. These observations raise questions regarding the validity of widespread application of the micellar phase for investigating bioaccessibility and its utilization on the apical side in absorption experiments, as this fraction may not accurately reflect the actual amount of the recovered compound, depending on the initial matrix of the delivery system as well as digestion-induced structural changes.

Following the absorption and transport test through the co-culture absorption model, no curcumin was detected in the basolateral compartment, regardless of the treatment. However, a notable proportion of curcumin, 54% for protein nanoparticles and 24% for emulsions, was retrieved within the cells. These results pointed to the complexity of the mucus–nutrient interactions and the significant role that the mucus layer may play in the absorption and transport process. Our results also highlight the importance of the structures following *in vitro* digestion in imparting differences not only in bioaccessibility, but also in the cellular uptake of nutrients.

Keywords : In vitro digestion, Co-culture absorption model, bioaccessibility, Bioavailability, Food structure

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22642) - BIOACCESSIBILITY OF CLNAS PRESENT IN PLANT OIL BY IN VITRO APPROACH

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Abstract

Conjugated linolenic acids (CLnAs) consist in a group of isomers of α-linolenic acid with conjugated double bonds. They have drawn growing attention due to their biological properties including antidiabetic, antiobesity and anticarcinogenic activities. CLnAs are especially found in plant seed oils such as pomegranate seed oil, which contains high levels of punicic acid (PunA, C18:3 c9t11c13) (Dhar Dubey et al., 2019; Vermonden et al., 2021). However, little is known about the bioaccessibility of these CLnAs. Indeed, like most lipids, CLnAs are mainly present in the form of triglycerides that need to be hydrolyzed by digestive lipases to cross the intestinal barrier.

The aim of this study is to assess the bioaccessibility of CLnAs *in vitro*, using the INFOGEST digestion protocol. The bioaccessibility of PunA in pomegranate seed oil was compared to the one of a more conventional fatty acid, oleic acid (OA, C18:1 c9) in olive oil. The influence of the presence of proteins on fatty acid bioaccessibility was also assessed. Lipids were extracted from digestive fluids using the Bligh and Dyer method. Solid-phase extraction (SPE) was then carried out to separate lipids into absorbable (free fatty acids and monoglycerides) and non-absorbable (di- and triglycerides) fractions. Fatty acid methyl esters were analyzed by GC-FID. Malondialdehyde (MDA) assay was carried out to assess if CLnAs were oxidized during the process.

In vitro digestion showed that the bioaccessibility of PunA in pomegranate seed oil is good (around 75%) and similar to that of OA in olive oil. Oxidation monitoring showed lower MDA levels when tubes were flushed with Argon and protected from light. However, this difference was not observable when considering PunA losses, indicating that (i) MDA production is anecdotal in relation to fatty acid concentration and (ii) even though slight losses of PunA might be due to oxidation, they were not lowered with Argon flush and protection from light. We therefore decided to remove oxygen for further investigations to better mimic physiological conditions.

These results suggest that (i) pomegranate seed oil is efficiently digested *in vitro* using INFOGEST protocol and (ii) the oral route might be appropriate for administration of PunA. Further examinations are being conducted to examine the impact of the food matrix (such as the presence of proteins) on PunA bioaccessibility and to evaluate this bioaccessibility using *in vivo* models

References

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Vermonden *et al*., 2021 (Nutrients)

Keywords : punicic acid, bioaccessibility, Conjugated linolenic acids

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22646) - WHEY PROTEINS AND PEA NUTRALYS® S85 PLUS PROTEINS ISOLATE ENHANCE POSTPRANDIAL AMINOACIDEMIA IN YOUNG AND OLDER HEALTHY INDIVIDUALS

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Abstract

When developing new plant-based solutions for food or medical nutrition, it is crucial to consider the quantity and the quality of constitutive ingredients. Regarding proteins, measuring digestibility first is mandatory when considering bioavailability. Then, during the journey from digestion in the intestinal lumen to the utilization in peripheral tissues such as muscle, amino acids can be sequestered by the splanchnic area, which affects the postprandial amino acid availability. Furthermore, other factors such as increased amino acid splanchnic extraction and increased insulin and anabolic resistances with aging can also result in a lower bioavailability of the amino acids[1]. The aim of this study was to measure, in both young and older healthy individuals, the post-prandial amino acid availability following the consumption of a pea protein isolate, i.e. NUTRALYS® S85 Plus (PP), as compared to a whey protein isolate (WP).

In a randomized, controlled, cross-over study, fifteen young (<30 years old) and fifteen older (>65 years old) subjects consumed 2 different sources of proteins (WP or PP) in water at a level of 0,41g/kg of body weight. Blood sampling was performed before and throughout the 6 hours after protein consumption to measure venous concentration of feeding-related aminoacidemia. Concentrations were normalized with the initial aminoacidemia and the ingested amino acid quantities. The area under the curve (AUC) were calculated from the aminoacidemia measured over time.

For young volunteers, the AUC of plasmatic concentrations of leucine, branched chain amino acids (BCAA) and non-essential amino acids (NEAA) were not significantly different whatever the original protein isolate. The AUC of plasmatic concentration of the 9 essential amino acids (EAA) and the sum of sulfured amino acids (SAA) were significantly higher after WP ingestion than with PP. For older volunteers, the AUC of leucine, of BCAA and of NEAA were not significantly different between both groups. The AUC of EAA and SAA were higher after ingestion of WP than after ingestion of PP. However, the blood concentrations of the 11 non-essential amino acids were higher in the PP group. Interestingly, older subjects showed higher AUCs for all amino acids compared to young subjects.

WP is considered as the gold standard dietary protein to reach blood concentrations of leucine and BCAA required for optimal stimulation of the whole body anabolism, especially at muscle level in older subjects[2]. Consumption of PP or WP enhances postprandial aminoacidemia in young and older healthy volunteers. Despite the lower aminoacidemia for EAA observed with PP compared to WP, several studies demonstrate comparable effect between these two sources in regard to muscle protein metabolism[3,4]. Further studies are needed to better understand the role of non-essential amino acids, e.g. arginine, beyond the BCAA in muscle health.

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(4) Babault et al. **2015**

Keywords : bioavailability, amino acid, pea protein, elderly, anabolic resistance

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22653) - TRANSGLUTAMINASE MODIFICATION AFFECTS IN VITRO GASTRIC DIGESTION OF PEA PROTEIN AND DUODENAL LIPOLYSIS OF EMULSIONS

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Abstract

Transglutaminase (TG) is often used as a food additive to improve the texture, structure, and other functional properties of protein-based products. TG catalyzes the formation of covalent bonds between amino acid residues, such as lysine and glutamine in proteins, leading to the creation of protein networks with improved binding and structure. However this structural modification may alter the digestive fate of food proteins as these changes may affect the accessibility of certain protein regions to digestive enzymes or how proteins interact with bile salts in the digestive environment. Bile salts aid in the digestion of lipids, but they also play a role in the digestion of proteins.

In the present work the effect of TG modification of pea proteins on in vitro gastric digestion and duodenal lipolysis was studied using the harmonized INFOGEST protocol (Brodkorb et al., 2019).

During gastric digestion no significant differences were found between the kinetics of hydrolysis of the native (PPI) and the TG modified (TG-PPI) pea protein isolates (6% protein concentration), being de degree of hydrolysis 6% after 60 min. However, a strong difference in the solubilisation in the gastric environment was observed; after 60 min of gastric digestion the soluble fractions were 87.6% and 63.5 % for PPI and TG-PPI respectively.

Regarding the kinetics of fatty acids release from olive oil- in water emulsions stabilized by PPI and TG-PPI, a strong decrease was observed for TG-PPI. While PPI emulsions released 86 % of the fatty acids after 2 h of duodenal digestion, TG-PPI emulsion only released 65 %. The micellar structures at the end of the lipolysis where similar (13 nm).

The marked decrease in lipolysis is correlated with the fraction of protein solubilized in the gastric phase since it has been shown that this contributes to forming mixed micelles with bile salts that have a greater solubilization capacity of the lipolysis products (fatty acids and monoglycerides mainly). Furthermore, an increase in the insoluble fraction in the case of TG-PPI could contribute to a partial precipitation of bile salts which would affect its role in the removal of lipolysis products from the interface of emulsion droplets.

In conclusion, the crosslinking of pea proteins with TG, by reducing the rate and extent of lipolysis, could be a way to control the uptake of lipids that are linked to obesity and cardiovascular health.

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Universidad de Buenos Aires, Agencia Nacional de Promoción Científica y Tecnológica, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

Keywords : lipolysis,, pea protein emulsions, transglutaminase, gastric digestion

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22655) - GASTRIC EMPTYING OF VEGETABLE PROTEINS AFFECT THE MICELLAR SOLUBILIZATION CAPACITY OF BILE SALTS

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Abstract

Proteins undergo partial digestion by pepsin in the acidic environment of the stomach. Gastric emptying rate may affect the degree of protein breakdown before entering the small intestine, thus can impact the subsequent interaction with bile salts in the duodenum. The primary function of BS is to emulsify dietary fats and aid in the absorption of lipids, and any hydrophobic substances in the small intestine. Bile salts have been shown to interact with the products of protein hydrolysis, being this interaction a key point to modulate several physiological processes (Bellesi and Pilosof 2021).

In the present study we focused on understanding how soy and pea proteins entering the duodenum after different gastric static residence times G10 and G60 (10 and 60 min respectively), simulating a dynamic gastric emptying, affect the BS micellar solubilization of poorly soluble molecules as oleic acid (OA). Ten minutes can be considered to represent the shortest residence time of food in the stomach before entering the intestine (Wang, Crevel, & Mills, 2023) and 60 min can be considered a large residence time as the half-emptying times reported for soy protein solutions is 36–38 min (Calbet & Holst, 2004).

The solubilization of OA in BS or BS/peptides micelles taking place in the duodenal phase of digestion when the hydrolyzed proteins enter the duodenum after gastric residence times G10 and G60 was evaluated: (1) just at the moment when the hydrolyzed proteins enter the duodenum, G10-D0 and G60-D0, (duodenal peptidases were removed in the simulation of duodenal conditions) and (2) after 60 min of duodenal digestion G10-D60 and G60-D60 (in the presence of duodenal peptidases).

All the proteins studied presented a strong synergy with BS in the micellar solubilization of oleic acid, doubling and even tripling their capacity indicating that mixed micelles provide a more efficient means of solubilizing and transporting hydrophobic molecules.

Increasing the residence time of proteins in the stomach increased the moles of oleic acid solubilized per mole of BS at the beginning of the duodenal stage, but towards the end of the duodenal stage (G10-D60 and G60-D60) the differences were smaller.

When the efficiency of the peptides was evaluated in terms of volume of solubilized oleic acid per gram of soluble protein, commercial pea protein was the most efficient, presenting maximum synergy with BS at the beginning of the duodenal stage.

In conclusion, the type and size of peptides, released at different times of gastric digestion, could have different implications in physiological processes modulated by the BS, contributing to the overall effectiveness of lipid digestion and nutrient absorption in the gastrointestinal tract.

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Acknowledgments

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Keywords : Digestion, Bile salts, Plant proteins, Gastric Residence

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22660) - SELENIUM BIOACCESSIBILITY IN PLANT-BASED AND BEEF-BASED BURGERS AND NUTRITIONAL POTENTIAL

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Abstract

The popularity and consumption of plant-based foods, particularly analogs mimicking animal products, has grown among vegetarians, vegans, flexitarians, and consumers in general. This trend is relate with concerns about sustainability, ethics, animal welfare, and health and the food industry focuses on developing plant-based products, such as plant-based burgers (PBBs). However, few information about the nutritional value of these products compared to animal-based ones are available, making the assessment of nutrients such as selenium (Se) are important. Se is present in organic and inorganic forms in nature and plays role as antioxidant, acts in thyroid metabolism, immune system support, and reducing the risk of non-communicable chronic diseases. In this context, this study aimed to evaluate the bioaccessibility of Se in commercial PBBs formulated with legumes (chickpea, pea, and soy) and beef-based burger (BBB) available in the Brazilian market. Samples of different brands were acquired and *in vitro* digestion assays following the INFOGEST 2.0 protocol were employed. Determination of total Se content and bioaccessible fraction involved sample preparation by incineration with acid and detection by hydride generation atomic absorption spectrometry (HG-AAS). Total Se content (µg/100 g) varied to 8.72, 8.83, 10.6, and 28.5 for soy PBB, BBB, chickpea, and pea PBBs, respectively. Only pea PBB significantly differed ($p < 0.05$). Regarding the bioaccessible Se fraction, pea PBB had the highest value (8.78 µg/100 g), while BBB had the lowest (3.22 µg/100 g), differing statistically from soy PBB (4.33 µg/100 g) and chickpea PBB (4.73 µg/100 g). Bioaccessibility percentage was higher for soy PBB (50%) and lower for pea PBB (31%), with chickpea PBB and BBB having 44% and 36% bioaccessibility, respectively. These results underscore the importance of assessing bioaccessibility concerning the total Se content in food matrices. They also reveal that the majority of PBBs evaluated are an alternative to BBB for the intake of Se available for absorption. The chemical form of the mineral in the products evaluated and the composition of the matrixes must influence the release of Se for absorption into the digestion tract.

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Keywords : food analogues; animal-based; micronutrient; in vitro digestion; HG-AAS

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22661) - EXPLORING THE IMPACT OF DIETARY FIBERS ON CAROTENOID BIOACCESSIBILITY AND BIOAVAILABILITY

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Abstract

Background

Carotenoids have been associated with a reduced risk of several chronic diseases, including cardiovascular diseases, type-2 diabetes, obesity, brain-related diseases and some types of cancer. Due to their potential health benefits and acting in part as pro-vitamin-A compounds, factors affecting bioavailability and bioaccessibility of carotenoids have always been of interest. Several dietary factors can affect the release of carotenoids from the food matrix and their incorporation into mixed micelles during digestion, as for example the content of dietary fiber, type and amount of dietary fat. A factor that so far has received little attention is different types of dietary fibers.

Objectives

The main objective for the current phase of this project is to study in a systematic approach the interactions between different types of dietary fibers (DF) and carotenoids by simulated gastro-intestinal (GI) digestion experiments. The primary research hypothesis is that DF would negatively modulate carotenoid bioaccessibility, cellular uptake and bioavailability, but that prebiotic fibers would exert less if any negative effects on the above parameters. The secondary hypothesis would be that carotenoids continue to be potentially available for absorption in the colon, but that this availability would likewise be a function of the type of DF, with soluble and highly fermentable DF having a higher potential colonic bioaccessibility vs. poorly fermentable DF, which may obstruct carotenoid release, and thus bioaccessibility. Research has shown that soluble fibers such as pectin may have an impeding effect on the bioaccessibility of carotenoids. The underlying mechanisms concerning this phenomenon are not clear, but could include perturbation of lipid-droplet to mixed micelle processing, occlusion of carotenoids, binding of bile salts and digestive enzymes, altering viscosity of digesta, among other. Through this study, we aim to shed light on the potential interactions between DF and carotenoids.

Methodology

In the first step, prior to investigating interactions between various types of dietery fibers and carotenoids, we will adapt and optimize the Infogest (2.0) protocol to assure complete digestive conditions that allow differentiating positive or negative effects of dietary fibers on carotenoid bioaccessibility. Beta-carotene will be gastro-intestinally digested together with different concentrations of pectin (soluble dietary fiber) to study its impact on bioaccessibility and recovery of carotenoids. The study seeks to alter parameters such as such as differential concentration of bile and pancreatin in simulated intestinal fluid (SIF), Increased rounds per minute (RPM) for the water bath to estimate the impact of high peristaltic movement and altered ratio of beta-carotene to oil in digested samples.

Outcomes

The expected outcomes for his study include a better understanding of carotenoid-fiber interactions. Since beta-carotene a hydrophilic molecule, for an increase in the amount of pectin it may be assumed that the quantity of pectin added to the digesta may impinge the bioaccessibility of carotenoids. Additionally, an increase in the RPM for the gastric and intestinal phases may improve the micellarisation making the carotenoids more bioaccessible. Eventually a varied ratio of pancreatin and bile is also likely to have an impact on micellarisation and thus the final bioaccessibility.

References

Acknowledgments

Keywords : Bioaccessibility, Bioavailability, Carotenoids, Dietary Fibers, Infogest

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22668) - POLYCYCLIC AROMATIC HYDROCARBONS AND THEIR BIOACCESSIBILITY IN OYSTER: A TOOL FOR CONSUMPTION RISK EVALUATION

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Abstract

Seafood is an important source of proteins, healthy lipids, vitamins and minerals in the human's diet (Food and Agriculture Organization, 2022). Despite these nutritional benefits, seafood can accumulate organic lipophilic nonpolar pollutants, such as polycyclic aromatic hydrocarbons (PAHs), from the aquatic environment (Nasher et al., 2016), posing potential risks to consumers. In 2019, there was the biggest oil spill accident on the Brazilian coast, leading to the contamination of marine environments and their species. Therefore, authorities and researchers collaborated to assess the contamination level and associated risks in seafood from the affected area. This research aimed to determine the contamination level and bioaccessibility of polycyclic aromatic hydrocarbons in oyster from natural environmental and marine farms, located in Delta of Parnaiba River, Piaui state. PAH extraction followed the EPA-3545A protocol (EPA, 2007a), and bioaccessible portions were extracted using the liquid-liquid extraction technique according to the EPA-3510C protocol (EPA, 1996). The identification and quantification of PAHs followed the EPA-8270D protocol (EPA, 2007b). The in vitro digestion simulation was performed according to Brodkorb et al. (2019). The mean concentration of the 39 evaluated PAHs did not differ between the samples. The mean concentrations of PAHs varied between 0.26 and 47.22 μg kg−1, with the highest level quantified in oyster from the natural environmental. Naphthalene, acenaphthene, anthracene, fluorene, chrysene, benzo(b)fluoranthene, and benzo(a)pyrene were detected in the bioaccessible fraction, and the bioaccessibility of these compounds was 11.53, 19.33, 7.78, 48.2, 11.95, and 14.41%, respectively. Among the PAHs measured, naphthalene was the predominant one. The bioaccessible fraction of the farmed oyster samples showed the lowest amount of potency equivalent concentrations (BaP) (1.39 μg kg-1) compared to oyster from natural environment (4.0 μg kg-1). According to the BaP, the samples did not exceeded the maximum value of 18.0 μg kg-1 established by the National Agency for Sanitary Vigilance and Safety (ANVISA, 2019) for mollusks. Thus, the consumption of oyster from the areas affected by the oil slick is considered safe for consumers.

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Keywords : Seafood, oil spill, potency equivalent concentrations, consumption

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22673) - AGE-RELATED GASTROINTESTINAL ALTERATIONS AFFECT ADIPOLYSIS INDUCED BY DAIRY AND HYBRID HIGH-PROTEIN YOGURTS IN DIFFERENTIATED 3T3-L1 ADIPOCYTES

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Abstract

The partial replacement of animal-derived ingredients with plant-based ingredients can represent interesting alternatives in the process of transitioning to a more sustainable diet. This replacement can impact the fatty acid profile of products, nutritional quality indexes and adipolysis. This study compared adipolysis in *in vitro* cellular models of high-protein dairy-based and hybrid yogurts after *in vitro* gastrointestinal digestion under conditions that simulate digestion in adults and elderly people. Dairy and hybrid mixtures, with 50% replacement of dairy protein by almond protein, were used to obtain control and hybrid yogurts. The yogurts were evaluated for their fatty acid profile and the atherogenic index (AI), thrombogenic index (TI), hypo/hypercholesterolemic ratio (HH) and health-promoting index (HPI) were calculated. *In vitro* simulation of gastrointestinal digestion was performed according to the INFOGEST protocol in conditions that simulate adult and elderly digestion. Adipolysis was evaluated in differentiated 3T3-L1 adipocytes. For this purpose, the cells were exposed to digested samples, and the release of glycerol was measured, which is directly related to the degradation of triglycerides. The effect of treatments on the fatty acid profile and nutritional quality indexes was evaluated by one-way ANOVA. The effect of treatments, digestive conditions, and the interactions of these factors on adipolysis was evaluated by factorial ANOVA. Hybrid yogurts had lower AI (0.87 versus 2.36) and TI (1.10 versus 3.45) and higher HH (1.72 versus 0.63) and HPI (1.15 versus 0.42), suggesting that the partial replacement of dairy protein with almond protein affected nutritional quality indexes. However, no significant difference was observed in the release of glycerol between the control and hybrid yogurts, which indicates that the yogurts did not show a significant difference in triglyceride accumulation in differentiated 3T3-L1 adipocytes after digestion. On the other hand, lower glycerol release was observed in conditions that simulate the digestion of elderly people and, consequently, greater accumulation of triglycerides than in conditions that simulate the digestion of adults. These results suggest that nutritional quality indexes obtained from fatty acid profiles are not directly associated with the prevention or modulation of obesity. Furthermore, digestive conditions must be considered when designing healthier products for specific age groups.

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Keywords : plant-based, gastrointestinal digestion, adipolysis

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22676) - BIOACCESSIBILITY OF MINERALS FOLLOWING IN VITRO GASTROINTESTINAL DIGESTION OF GLUTEN-FREE BREAD ENRICHED WITH FLAXSEED OIL CAKE

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Abstract

Growing interest in gluten-free diets, driven by the prevalence of conditions such as celiac disease, has raised concerns about the nutritional quality of gluten-free products, particularly regarding mineral content [1]. Flaxseed Oil Cake (FOC), a by-product of flaxseed oil extraction, emerges as a promising source of minerals, characterised by elevated levels of calcium, magnesium, and potassium.

This study aimed to explore the potential of incorporating FOC as a novel ingredient in gluten-free bread (GFB) formulations at different levels (5%, 15%, and 30%), with a specific focus on mineral content and their bioaccessibility, assessed through *in vitro* simulation of human digestion.

Our findings showed that incorporation of FOC into GFB result in a significant increase in the total mineral content, including calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), iron (Fe), copper (Cu), cobalt (Co), manganese (Mn), and molybdenum (Mo). However, the bioaccessibility of these minerals exhibited complex interactions, with enhanced bioaccessibility observed for P and K, while reduced bioaccessibility was observed for Fe, Mo, and Mn in FOC-enriched GFB compared to the control. It is worth noting that despite variations in bioaccessibility, the total mineral content remained higher in all experimental GFBs.

These results provide valuable information about the potential of FOC-enriched GFB as a source of essential minerals and highlight the need for further research to optimise the nutritional value and bioaccessibility of these minerals in gluten-free products. This approach not only benefits gluten-free consumers but also aligns with the circular economy concept, contributing to the reduction of food waste.

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Keywords : Minerals, Gluten-free bread, Flaxseed Oil Cake, ICP-MS, In vitro bioaccessibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22679) - ACIDIC HYDROTHERMAL PROCESSING OF WHEAT MAY ENHANCE IRON AND ZINC BIO-ACCESSIBILITY AND UPTAKE IN CACO-2 CELLS

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Abstract

Wheat is a staple food crop which contains significant concentrations of iron (Fe) and zinc (Zn) (20-35 mg/kg). It hence is an important source of these minerals for the human body. However, due to their chelation with phytic acid, only 5-10% of Fe and Zn is bio-accessible for human uptake. To increase its bio-accessibility, one can apply hydrothermal processing under conditions allowing optimal endogenous phytase action. A multifactorial response surface model was here constructed to find optimal conditions (45 – 60 °C, pH 4.0 – 7.0, 8 h – 24 h, and incubation media: water and sodium acetate, lactate or citrate buffers) for hydrothermal processing for maximal phytic acid reduction in wheat. The model showed that the use of acetate buffer during hydrothermal processing resulted in more phytic acid reduction (64%) compared to the use of citrate buffer (49%). Surprisingly, no significant increase in Fe and Zn bio-accessibility was found when wheat was processed using the acetate buffer, while wheat processed using the citrate buffer had increased Fe and Zn bio-accessibility readings of 64% and 53%, respectively. These findings can be attributed to the chelating ability of citrate. Unlike phytates, citrate chelates are soluble and therefore contribute to Fe and Zn bio-accessibility. Next, the Fe and Zn bio-availabilities were estimated by submitting *in vitro* digests to human colon carcinoma cells (*i.e.* Caco-2 cells), thereby using ^{70}Zn and ^{57}Fe stable isotopes to trace mineral absorption. The Fe and Zn uptake by Caco-2 cells treated with *in vitro* digests of wheat processed using citrate buffer were 53% and 75% higher compared to untreated wheat, respectively. In contrast, no significant differences in mineral uptake were found when cells were treated with *in vitro* digests of wheat processed using the other incubation media. These results imply that at least a fraction of Fe and Zn chelated by citrate is taken up by the cells, either as complete chelates or by donating minerals to the transporters on the intestinal enterocytes. In conclusion, hydrothermal processing of wheat using citrate buffer holds promise for developing whole grain-based products with an increased Fe and Zn bio-availability.

References

Keywords : mineral bio-accessibility, Caco-2 cell lines, wheat, phytate

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22684) - THE FATE OF NEWLY FORMED COMPOUNDS FROM PROCESSED FOOD TO DIGESTION: CASE STUDY OF A PEA-BASED SPONGE CAKE

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Abstract

Nowadays we are living the beginning of a transition to a more sustainable way of living; this process also involves nutrition and food. In this context, the use of emerging new ingredients and the widespread tendency to use vegetable sources of proteins as an alternative to the animal ones, brought to substitute traditional ingredients, as milk, meat or even cereals, with legumes.[1]

Most of the time legume-based products are regarded as healthier than their conventional counterparts. However, it has been recently shown that legume-based new ingredients could contain higher concentrations of Maillard reactions and caramelization precursors that could bring to a strong reactivity during processing. The consequence is the generation of newly formed compounds (NFCs) that have been identified as possible contributors to health problems.[1]

The reactivity, the bio-accessibility and, therefore, the health effects of these NFCs could be strongly influenced both by food matrix composition and structure, and by the digestion process.[2]

To investigate the NFCs reactivity and bio-accessibility, a sponge cake based on pea protein isolate is studied as a model for legume-based processed products and it is digested through the *in vitro* INFOGEST static digestion. A correspondent sponge cake traditionally formulated with wheat flour is considered as a term of comparison.

Among all the NFCs, some furanic and dicarbonyl ones are selected as target molecules.

Furanic compounds seem to be more abundant in legume-based products than in wheat ones[1], they are less studied thanother thermal process contaminants, and they are attracting increasing interest due to the recent suspect of health concerns. Dicarbonyl compounds are reactive precursors of a large variety of thermal process contaminants, including furanic compounds.[2]

The selected NFCs are quantified in the sponge cake products after baking and after the gastric and the intestinal phases of the *in vitro* digestion. Starch and protein hydrolysis is also followed during the digestion.

The goal is to understand the selected NFCs release and reactivity during the digestion process and the NFCs interactions with the products of the macronutrients hydrolysis. In addition, there is the interest in evaluating how the structure of the sponge cake matrix influences these phenomena.

This line of research could, in the future, contribute to the optimisation of industrial processes in order to reduce the generation of NFCs during the industrial production of legume-based processed food.

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Acknowledgments

Keywords : baking, thermal reactions products, furanic compounds, dicarbonyl compounds, reactivity and bio-accessibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22686) - INFLUENCE OF IN VITRO DIGESTION ON THE PHENOLIC BIOACCESSIBILITY OF APPLE DERIVATIVES

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Abstract

Background. Apples and their derivative products are widely consumed worldwide, due to their health-promoting properties which are mainly attributed to polyphenols. Processing applied to raw apples to obtain derivates is known to alter the phenolic content. In this regard, the production of apple puree and homogenate led to a 20% decrease in phenolic content due to mechanical and thermal treatments regardless of the intensity of matrix disruption (Alongi et al., 2023). Such operations are mainly responsible for phenolic removal and/or oxidation and polymerization. However, limited information is available on the influence of food processing on phenolic bioaccessibility after gastrointestinal events.

Aim. This study investigated the joint effects of processing and gastrointestinal digestion on apple phenolic compounds.

Methods. Whole apple pulp (A) was pasteurised and subjected to high-speed and high-pressure homogenisation, obtaining apple puree (P) and apple homogenate (H). A, P, and H were *in vitro* digested based on the INFOGEST protocol. Polyphenols were identified and quantified in undigested and digested samples and their bioaccessibility (BAC) was computed. A model system containing quercetin-3-glucoside as a representative phenolic compound of apple was also digested to understand the role of the apple matrix on phenolic BAC.

Results. Upon digestion the lowest phenolic BAC, < 1%, was found in A. Conversely, P and H showed higher retention of phenolic compounds, with BAC ~ 15%. The phenolic depletion observed in P and H can be reasonably attributed to the chemical oxidation phenomena occurring during the gastrointestinal transit. The lower BAC found in A could be mainly referred to the action of polyphenol oxidase (PPO) which retains its activity even under gastrointestinal conditions (T and pH). Conversely, in P and H, PPO was reasonably inactivated upon pasteurisation, resulting in a higher phenolic BAC. This hypothesis was confirmed by the 20% BAC of quercetin-3-glucoside digested in the absence of PPO.

Conclusion. Depending on the technological intervention, after digestion, different phenolic BAC can be obtained from the same matrix. Results indicate that when processing allows the inactivation of oxidative enzymes present in the food matrix, phenolic BAC is ~10-fold higher than that of fresh apple pulp. Conversely, when no enzyme inactivation occurs, digestion acts as an incubator for endogenous enzymes of the matrix.

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Keywords : Apple destructuring, high-speed homogenization, high-pressure homogenization, in vitro digestion, phenolic bioaccessibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22689) - AQUEOUS EXTRACTS OF CORNFLOWERS (CENTAUREA CYANUS) AND THEIR BIOLOGICAL EFFECTS

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Abstract

The cornflower is a flowering weed and an ornamental plant. The petals are the edible part of the plant, known in folk medicine for centuries for their bioactive properties. Nowadays they are also used as food, decoration and for dyeing.

In this work, the bioavailability of cornflower (*Centaurea cyanus*) petals before and after gastrointestinal digestion *in vitro* and their effects on toxicity and bioactive potential were investigated. The selected extraction was performed with distilled water at 90°C for 30 minutes. Temperature affects the TPC and DPPH values, while temperature and duration of extraction influence the flavonoid and FRAP values.

The extracts obtained were subjected to the harmonized INFOGEST protocol for in vitro digestion. The chemical composition was analyzed before and after the digestion process in terms of phenols (TPC) and flavonoids (TFC); in addition, the profile of phenolic compounds was determined by UPLC-MS and flavonoids by TLC-MS. The antioxidant activity was determined using the FRAP, DPPH and ABTS methods. A comparison of the extracts before and after digestion shows an increase in TPC, but a significant decrease in TFC. Chlorogenic acid, caffeic acid, ferulic acid and p-coumaric acid, isoquercitrin and coumarin were identified as the major compounds in the selected extract. The in vivo test with the different cell lines, showed a dose-dependent effect, with a low mortality rate at all tested concentrations.

The promising results of the chemical and biological evaluation of the extracts indicate that the natural compounds isolated from the petals of *Centaurea cyanus* can be used as potential ingredients for functional food formulations, and/or as biotherapeutic agents.

Acknowledgments

This study was financially supported by the Slovenian Research Agency (research program P1-0005 “Functional foods and food supplements”).

Keywords : phenolic acids, flavonoids, bioavailability

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22691) - QUANTIFICATION OF ALLYL -, BENZYL -, BUTEN -, PENTEN-, AND PHENYL – ISOTHIOCYANATE IN BIOACCESSIBLE FRACTION OF CRUCIFEROUS VEGETABLES OBTAINED THROUGH INFOGEST STATIC IN VITRO DIGESTION MODEL

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Abstract

Isothiocyanates (ITCs) are phytochemicals with a functional group – N = C = S, naturally ocurring in a wide variety of vegetables such as broccoli, Brussel sprouts, cabbage, kale, mustard and rocket [1]. They are of great interest for human nutrition due to their antioxidant, anti-inflammatory and anti-cancer properties [1 - 2]. The high volatility and low polarity of many of these compounds make their analysis difficult. The target of this research was the optimization and validation of GC – MS methodology for the determination of five ITCs in bioaccessible fraction. Some experimental conditions of the bioaccessibility protocol (centrifugation Temperature and time; bile salts amount) were also studied

The assayed conditions of the INFOGEST digestion method were used to estimate the ITCs bioaccessibility in cruciferous. The analyses were performed on a GC-MS system consisting of a Bruker GC Mod. 456 with a Bruker mass detector Mod Scion TQ.

Extraction of ITCs from samples for their subsequent chromatographic analysis was carried out by weighing 5 g of sample, to which 3 g of anhydrous sodium sulfate and 5 mL of ethyl acetate were added. The mixture was shaken in a vortex for 10 minutes, centrifuged at 5000 rpm for 5 minutes, taking an aliquot of the extract for direct analysis by GC/MS.

One parameter to take into account is the temperature at which the extraction process is carried out. Extraction with ethyl acetate at room temperature (T = 21°C) does not imply significant losses for any of the ITCs studied, obtaining recovery percentages of above 90%. However, heating to just 70 °C for 30 min in the extraction process represents losses of around 50% for the five ITCs studied, which increase up to 75% if the heating is maintained for 60 min.The developed method allows detecting concentrations of ITCs lower than 0.05 µg/g. Besides, the relative standard deviation of the regression (RSD%) was lower than 15% in all cases.

Ethiopian mustard (*Brassica carinata*) showed a concentration of 4.95 µg/g for allyl – ITC, which is in agreement with a previous study [3]. Benzyl – ITC was the only ITC analysed in the bioaccessible fraction of green tissues of white mustard (*Sinapis alba*) (2.32 µg/g). 4-penten-1-yl – ITC concentration of 8.81 µg/g was found in bioaccessible fraction of turnip tops (*Brassica rapa*). Since ITCs bioaccessibility was low this implies the need to develop strategies to improve the solubility of these compounds in the intestinal lumen.

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Keywords : isothiocyanate, GC - MS, cruciferous

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22697) - MICROENCAPSULATION OF BENZYL - ISOTHIOCYANATE BY SPRAY-DRYING AS A TOOL TO IMPROVE BIOACCESSIBILITY IN WHITE MUSTARD (SINAPIS ALBA)

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Abstract

Isothiocyanates (ITCs) are phytochemicals obtained by the hydrolysis of glucosinolates, a group of compounds that are present in the cruciferous vegetables. The enzyme myrosinase present in the idoblasts of cruciferous plant cells degrades glucosinolates to ITCs [1].

White mustard (*Sinapis alba*) has been used as a condiment for over 5000 years. It is rich in glutropaeolin, the glucosinolate precursor of benzyl – ITCs. This later is of great interest for human nutrition due to its antioxidant, anti-inflammatory and anti-cancer properties [2]. Nevertheless, benzyl-ITC has shown low bioaccessibility due to its hydrophobicity. Microencapsulation could be used as a strategy to mitigate this limitation. Among microencapsulation techniques, spray-drying has proved to be a cost-effective method for producing good quality microparticles in a reproducible and fast way [3].

Spray-dried microparticles were developed at different inlet temperatures (105 – 170 °C) using two different encapsulating materials (mannitol and maltodextrin). Three doses of benzyl – ITC standard (1.5; 3.5 and 8.5 mg) were tested. The obtained microparticles were then incorporated into a mustard sauce for further testing. The assay conditions of the INFOGEST digestion method [4] were used to estimate the benzyl - ITC bioaccessibility. The microparticles were analysed by SEM, FT-IR and TGA. Experiments were also conducted with benzyl – ITC supplemented as a free standard, without microencapsulation (used as control).

The use of different materials impacted the ability of microparticles to associate benzyl – ITC, the association efficiency ranging within 2 - 21% (mannitol microparticles) and 35 - 76% (maltodextrin microparticles). The supplementation of mustard sauce with benzyl – ITC in the free form (without microencapsulation) showed very low bioaccessibility values (3 - 5%). Microencapsulation with mannitol and maltodextrin improved the bioaccessibility of benzyl – ITC, reaching values around 50% (without statistically significant differences between both encapsulating agents). However, this improvement was only effective for the first two doses studied (1.5 and 3.5 mg). For the 8.5 mg dose, microencapsulation had no effect on improving the bioaccessibility (5%).

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Keywords : microencapsulation, benzyl - isothiocyanate, cruciferous, mannitol, maltodextrin

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22699) - DO FOODS DERIVED FROM LEGUMES PRESERVE THE GENUINE NUTRITIONAL PROPERTIES OF THESE SEEDS?

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Abstract

The consumption of legumes has decreased in recent years, often being replaced by new, more convenient legume-derived foods that are easier to cook and consume.[1] However, little is known about the preservation of nutritional and bioactive profiles of legume-derived foods compared with legume seeds. Degree of protein hydrolysis (DH; OPA method), amino acid bioaccessibility, and antioxidant properties after *in vitro* gastrointestinal digestion (INFOGEST protocol) were investigated in cooked pasta and snacks from chickpeas, lentils, and peas, as well as in cooked textured soy and tofu, comparing them with the traditional boiled seeds. Pastas derived from green peas maintained or increased DH after gastrointestinal digestion compared to the original seeds. The presentation in snack form showed decreased DH, probably due to the detrimental effects of the intense thermal treatment on seed proteins.[2] Both textured soy and tofu kept similar DH than soybean. The amino acid composition exhibited wide variations among foods, with notably high lysine content and bioaccessibility, particularly in soybeans and their derivatives. Total phenolic content (TPC) and antioxidant activity before and after digestion of foods were measured using the Folin-Ciocalteu, ABTS, and FRAP methods, respectively. Cooking of dried seeds resulted in a decrease in their TPC and antioxidant actions ($p < 0.05$); however, the gastrointestinal process consistently increased all three parameters, especially in the case of TPC in the bioaccessible fraction of legume-derived foods. When comparing the TPC and antioxidant profiles of the bioaccessible fractions of cooked legumes with their corresponding derivatives, a net increase in TPC was observed in all derivatives ($p < 0.05$). Antioxidant actions, measured with both the ABTS and FRAP methods, were also enhanced in legume-derived foods, especially in those derived from green pea and soybean. The only exception was chickpea derivatives, which exhibited similar or lower antioxidant ability compared to cooked chickpeas. In general, these findings demonstrate that new legume-derived foods provide a convenient and effective way to increase legume consumption in the population, while maintaining the nutritional benefits that legumes offer in terms of amino acid bioavailability and antioxidant content.

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Keywords : legume, legume-derived foods, degree of hydrolysis, amino acids bioaccessibility, antioxidant actions

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22700) - FUNCTIONALITY OF TIGER NUT BEVERAGE AND ITS BY-PRODUCT: IN VITRO BIOACCESSIBILITY AND BIOAVAILABILITY OF POLYPHENOLS

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Abstract

Tiger nut is the key ingredient of a Spanish plant-based beverage (TNB), whose production generates tons of by-products (TNBP).

This study aimed at characterizing the polyphenols in TNB and TNBP, and evaluating their bioaccessibility, antioxidant, digestive enzyme inhibitory activities as well as bioavailability and antioxidant activity after absorption, through an *in vitro* model.

TNB and TNBP underwent an *in vitro* digestion (INFOGEST method) (Brodkorb *et al.,* 2019), and the assessment of polyphenols in the supernatants after each stage by HPLC was carried out. Total antioxidant capacity (TAC) of the undigested (ND), bioaccessible fraction (BF), and residue fraction (RF) obtained from the digestions were determined by ABTS and QUENCHER assays. BF was also tested for ability to inhibit α-amylase, α-glucosidase, and lipase. The bioavailability of the polyphenols in a Caco-2 cell model and TAC of the basolateral permeates were also assessed over a 4h period.

The results revealed that total polyphenol content in TNB was 3-fold higher than in TNBP, being ferulic acid hexoside, epicatechin derivative, and caffeic acid hexoside accounting for approximately 60% of the total polyphenols. The average bioaccessibility of polyphenols from TNBP was 56% compared to 24% from TNB; caffeic and ferulic acid hexosides were the most bioaccessible polyphenols with 124% and 118% for TNBP-BF and TNB-BF, respectively. Consistently, TAC increased 8- and 5-fold for TNBP-BF and TNB-BF, respectively, upon digestion. Regarding to the fractions reaching the colon, TNBP-RF showed a TAC of 2- and 4-fold higher than TNBP-ND and TNBP-BF, respectively. TNBP-BF reduced α-glucosidase activity by 80%, whereas TNB-BF inhibited lipase by 77%. Five polyphenols were absorbed through the Caco-2 cells and showed a mean bioavailability of 69% and 90% for TNBP and TNB, respectively. Caffeic and ferulic acid hexosides from TNBP had absorption peaks at 2h and 4 h, respectively, and a maximum TAC after 3h whereas polyphenols from TNB peaked after 2h coinciding with the highest TAC.

Altogether, the results demonstrate that TNB and TNBP are valuable sources of polyphenols potentially exerting metabolic activities through inhibition of digestive enzymes in the gastrointestinal tract and antioxidant activity after absorption. Moreover, data suggest that TNBP may be a better carrier of polyphenols in the colon thus potentially impacting health through the gut microbiome.

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Keywords : Food waste, Antioxidant activity, α-glucosidase activity, Lipase activity, Caco-2

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22707) - DEHYDRATED KIWIFRUITS AS SOURCES OF BIOACCESSIBLE VITAMIN C

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Abstract

Currently, dehydrated foods are of great interest because they have longer shelf life and are rich in nutrients and bioactive compounds. Prior research showed high vitamin C levels in fruits (strawberry, raspberries) and other vegetables (sweet potato). Kiwi is an Asian fruit that spread to Europe and America. It is a fruit of the *Actinidia* genus, which includes more than 70 species. Previous studies have reported the importance of kiwi as a source of nutrients and bioactive compounds such as fiber, vitamin C or other antioxidants, and its antioxidant potential in cell culture. Fewer studies have focused on the bioactivity produced by the antioxidant compounds remaining in the soluble fraction of the intestinal lumen after digestion.

This work aims to determine vitamin C content of dried kiwi, and changes due to digestion. Five kiwifruit varieties (*A. deliciosa, A. deliciosa* var Hayward, *A. deliciosa* var Hayward organic production, *A. arguta* and *A. chinensis*) were compared. Fruits were freeze-dried (in darkness), ground, homogenized, and stored at -20 °C in a dark, dry environment. Vitamin C analysis was performed through extraction with metaphosphoric acid and HPLC-UV, determining either ascorbic and dehydroascorbic forms (Sánchez Mata et al., 2012). Samples were digested in vitro following the standardized INFOGEST protocol. Vitamin C analysis was also performed in the final digesta and the bioaccessible fraction obtained after centrifugation (5000 rpm for 20 min at 4ºC).

The vitamin C content in freeze-dried fruits ranged between 269-747 mg/100 g dw, in agreement with the Food Composition Database BEDCA (2023), which reports values of 418 mg/100 g dw for kiwi fruits. “Gold” kiwi fruits (*A. chinensis*) samples showed the highest vitamin C content, even higher than values reported in the BEDCA database. After in vitro digestion, the vitamin C content was reduced to 20-103 mg/100 g dw. This is expected, as prior studies have suggested that vitamin C degrades with time, temperature, and pH, as shown by Kaya et al. (2010) who reported the loss of 80% of vitamin C at a temperature of 35-65ºC.

In the digested samples 64-95% of ascorbic acid was presented in the soluble fraction, which corresponded to 9.8-73.3 mg bioaccessible ascorbic acid per 100 dried kiwi, thus ready to be absorbed through passive diffusion or active transport. While about 2.6 to 15 mg of ascorbic acid/100 g dried kiwi, was present in the insoluble digested fraction and would pass to distal parts of the gut; this fraction would exert potential *in situ* antioxidant properties and may be reabsorbed in distal colon to produce effects in the organism. These results show that dehydrated kiwi fruits could be a good source of bioaccesible vitamin C and an antioxidant capacity. These findings contribute to valorizing the nutritional and functional value of dehydrated fruits as a part of a healthy diet.

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Keywords : Actinidia, antioxidants, digestion, ascorbic acid, INFOGEST

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22708) - SALIVA AS A PROMISING MATRIX FOR BIOMONITORIZATION OF FIREFIGHTERS' OCCUPATIONAL EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS

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Abstract

Occupational activity as a firefighter was classified as carcinogenic to humans (Group 1), since they are exposed to different occupational risks (e.g., chemical, biological, physical, and psychological) [1]. Moreover, firefighters are exposed to a wide variety of chemical hazardous compounds (e.g., particulate matter, carbon monoxide, heavy metals, and many volatile organic compounds, including polycyclic aromatic hydrocarbons (PAH)) through inhalation, dermal contact, and ingestion [1]. Due to the raising concern with impact on firefighters' health, human biomonitoring studies have emerged in the last years [1]. However, data characterizing European firefighters remains limited. Saliva has the potential to be applied as an alternative matrix for the biomonitoring of exposure to chemical hazards and have been poorly characterized.

The in vitro toxicity studies are important assays to evaluate the cellular mechanisms that are in the basis of the adverse health effects resulting from firefighting occupational exposure. This work aims to establish the concentrations of PAHs in the saliva of firefighters who participated in fire combat activities and evaluate the in vitro toxicity in human buccal and intestinal two-dimensional and three-dimensional (3D) epithelial culture models, representative of the gastrointestinal tract of the collected extracts.

Saliva samples were collected from participants without exposure to fire events as well as from firefighters who conducted firefighting activities (before, and immediately after). The levels of 18 PAHs were extracted according to the adapted method of Santos et al. [2] and quantified by chromatographic analysis. After the extract's exposure to in vitro models, the cell viability was assessed using the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay and the permeability in culture models was evaluated being the concentrations also quantified.

The concentrations of total PAHs immediately after participation in fire combat were slightly increased comparing to pre-fire levels (1.96 – 2.36 µg/L versus 2.12 – 3.62 µg/L). The obtained results for buccal (TR146 and HSC-3) and intestinal (Caco-2 and HT29 MTX) models tested were similar, with cell viabilities equal or above 70%. Concerning 3D models, the viability of the intestinal bi-culture model appeared to be more affected than the buccal model.

These preliminary results demonstrated the importance of firefighters wore the respiratory protection during fire events, preventing the ingestion of health hazards compounds. Further studies should address a superior number of participants and different classes of compounds.

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Keywords : Firefighters; Saliva; PAHs; in vitro toxicity

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22709) - COMPOSITION AND BIOACCESSIBILITY OF CAROTENOIDS FROM A PUFFED EXTRUDED MIXTURE OF CASSAVA AND PEACH PALM (BACTRIS GASIPAES KUNTH.) FLOURS

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Abstract

Puffed extruded products are convenience food products frequently consumed as snacks by different audiences. Traditionally, they are made from starchy flour, such as corn and rice, but there is a tendency to add value to underutilized starchy crops. Cassava flour has been used as an excellent starchy source to produce puffed extruded products, and the increased search for healthy foods has stimulated our study for supplementing extruded cassava flour products with relevant bioactive compounds, such as carotenoids. From this perspective, we investigated the supplementation of cassava flour with the addition of peels of peach palm fruits (*Bactris gasipaes* Kunth.), which is a by-product with a very high carotenoid content with provitamin A activity and antioxidant properties. In this study, the puffed extruded product was prepared with a mixture of 92.5% cassava flour and 7.5% peach palm peels. The *in vitro* simulated digestion was carried out according to the INFOGEST 2.0 protocol adapted to carotenoids. Changes in the microstructure during oral (OP), gastric (GP), and intestinal (IP) phases were observed by scanning electron microscopy (SEM). The carotenoids composition and *in vitro* bioaccessibility were determined by HPLC-DAD. Twelve carotenoids were identified. (all-*E*)-Lutein showed the highest bioaccessibility (66%), probably due to the presence of oxygenated groups in the molecular structure that allows better micellarization, while (all-*E*)-β-carotene showed 60% bioaccessibility, which did not differ ($p < 0.05$) from the isomers 13*Z*-β-carotene (59.9%), 9*Z*, 13*Z*-β-carotene (61.4%), and 9*Z*-β-carotene (59%). The other carotenoids presented about 59-61% *in vitro* bioaccessibility. The SEM images showed that the microstructures of the produced puffed extruded product were degraded during the digestive phases. Therefore, supplementation of this type of food product with peach palm peels proved to be an excellent alternative to improve the carotenoid composition of extruded cassava products, bearing good bioaccessibility.

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Keywords : carotenoids, extrusion, vitamin A, cassava, fruit

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22713) - GERMINATION AND ENZYMATIC HYDROLYSIS OF MUSTARD GRAINS PROVIDE DIFFERENT POLYPHENOL BIOACCESSIBILITY AND ANTIOXIDANT POTENTIAL AFTER SIMULATED IN VITRO DIGESTION

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Abstract

Germination and enzymatic hydrolysis have been reported as excellent processes for increasing the bioactivities of grains from different species. This study investigates whether simulated gastrointestinal digestion (INFOGEST protocol) affects the bioaccessibility and antioxidant potential of white mustard (*Sinapsis alba*) grains. The samples were obtained under different conditions, previously determined as the most appropriate to enhance the properties of interest, namely: i) non-germinated, ii) germinated at 25 °C for 72 h in the dark, and iii) non-germinated and enzymatically hydrolyzed with a ternary mixture of proteases, cellulases and pectinases. Total phenolic content (TPC) and antioxidant potential (ABTS, DPPH and FRAP assays) were investigated. Considering the isolated effects of processes applied without digestion, germination caused a greater increase in TPC and antioxidant potential compared to enzymatic hydrolysis. Simulated digestion resulted in an increase in TPC and antioxidant potential for all samples analyzed, with the best results varying according to sample preparation. The maximum increase (59%) in TPC was detected for the non-germinated sample, followed by the non-germinated and enzymatically hydrolyzed (43%) and germinated (39%) samples. The non-germinated and enzymatically hydrolyzed sample reached 249 μmol Trolox equivalents per gram (μmol TE g^{-1}) after digestion, compared to 196 μmol TE g^{-1} before digestion, representing a 27% increase in ABTS-radical scavenging. For the DPPH and FRAP methods, the germinated sample showed the most prominent results, reaching 14 and 85 μmol TE g^{-1} after digestion, compared to 9 and 36 μmol TE g^{-1} before digestion, representing increases of 56% and 133% in antioxidant potential, respectively. These findings demonstrated that the bioactive compounds from white mustard, whether present naturally or transformed by previous processes, exhibited different behaviors during digestion. Germination was the most interesting process as it positively modifies the bioaccessibility of phenolic compounds and the antioxidant potential of white mustard.

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Acknowledgments

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Keywords : germination, enzymatic hydrolysis, mustard grains, bioactive compounds

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22715) - EVALUATION OF FUNCTIONAL FRUIT BARS: BIOACTIVE POTENTIAL AND IN VITRO GASTROINTESTINAL DIGESTION OF POLYPHENOLS

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Abstract

The goal of this research was to produce functional blackberry fruit bar in order to have an alternative shelf life prolonged snack. With this aim, bioactive potential of this product was analysed in the light of health beneficial effects while revealing nutritional and bioaccessible properties of phenolics and antioxidants. In this context, fruit bars were analyzed for total phenolic content (TPC) and total antioxidant capacity (TAC) with CUPRAC (cupric ion reducing antioxidant capacity), DPPH (2,2-diphenyl-1-picryl-hydrazyl) and FRAP (ferric reducing antioxidant power) assays as well as their bioaccessibility during *in vitro* gastrointestinal digestion. Total dry matter, titratable acidity and ascorbic acid content of the bars were determined as 70.05±0.24 g/100g, 1.05±0.10 g/100g citric acid equivalent and 9.34±0.40 mg/100g respectively. L*, a* and b* values were analysed as 28.72±0.02, 18.54±0.04 and 7.58±0.37 resulting with a purplish brown color. In pre-digestion stage, TPC and TAC of bars with DPPH, CUPRAC and FRAP assays were determined as 172.23±0.52 g gallic acid/100g dry matter (dm) and 4.27±0.05, 14.68±0.05 and 23.46±0.08 µmol trolox/g dm respectively ($p < 0.05$). TPC and TAC with CUPRAC results from post *in vitro* gastrointestinal digestion steps showed an increasing trend while exhibiting an increment with 7.58% and 36.02% respectively. On the other hand, a significant decrease of TAC in digested samples was observed from FRAP (10.15%) and DPPH (77.75%) assays ($p < 0.05$). Bars were also preferred by the panelists in terms of color, odor, appearance, taste and chewibility properties. Consequently, blackberry was succesfully evaluated into sensorially accepted, non-seasonal fruit bars. Moreover, it was stated that formulations could produce an output in terms of commercialization and industrial added value while acting as an optimum design for further products.

Keywords : Fruit bar, phenolics, antioxidant capacity, in vitro gastrointestinal digestion

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22718) - BIOACCESSIBILITY OF ACRYLAMIDE IN INSTANT SOLUBLE COFFEE AND COFFEE SUBSTITUTES FROM CEREALS AND CHICORY. STUDY ON ISOLATED SAMPLES AND COMBINED WITH MILK

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Abstract

Acrylamide is a chemical process contaminant generated in foods when exposed to elevated temperatures and low moisture conditions, through the interaction between free amino acid asparagine and reducing sugars. Previous studies focused on potato and cereal-based products have demonstrated the impact of the food matrix and potential interactions between food components on acrylamide bioaccessibility.[1] However, limited information exits for other foods. The objective of this study was to assess the bioaccessibility of acrylamide in different instant soluble coffees as well as coffee substitutes made from cereals and chicory. Additionally, the study aimed to investigate the possible influence of the mixture of coffee or substitutes with milk in the bioaccessibility of the contaminant. Using the INFOGEST protocol, samples were *in vitro* digested both independently and combined with milk. Acrylamide was determined by LC-ESI-MS/MS in initial samples and in the bioaccesible and non-bioaccesible fractions obtained after the digestion, according to the protocol described by González-Mulero et al.[1] Bioaccessibility ranged between 73-90% in soluble coffees and 78-99% in coffee substitutes, with recoveries of 74-99%. Simultaneous digestion of samples with milk exclusively increased acrylamide bioaccessibility and recovery in instant chicory. The high electrophilic property of acrylamide makes it prone to reacting with amino and sulfhydryl groups of different nucleophiles present in the food matrix through Michael addition reactions. Particularly, in coffee samples the interaction between acrylamide and melanoidins has been described, giving place to melanoidin-bound-acrylamide complexes.[2] The occurrence of this phenomenon during the digestive process might account for the observed decreases in acrylamide bioaccessibility after the *in vitro* digestion. This circumstance did not occur in coffee substitutes containing higher cereal content, as almost all of the acrylamide remained soluble after digestion. In contrast to prior findings with biscuits-milk combinations, where a decline in acrylamide bioaccessibility occurred, the addition of milk had no impact on acrylamide bioaccessibility in instant coffee. Moreover, it even enhanced its bioaccessibility in soluble chicory. This underscores the significance of the food matrix composition in influencing the availability of the contaminant.

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Keywords : acrylamide bioaccessibility, instant soluble coffee, instant soluble cereals, instant soluble chicory

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22721) - BIOACCESSIBILITY AND IN VITRO GUT HEALTH EFFECTS OF PHENOLIC COMPOUNDS FROM STONE FRUIT CULTIVARS

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Abstract

Background. Noncommunicable chronic diseases have been related to proinflammatory processes due to increased oxidative stress. Stone fruit crops (cherries, plums, peaches, nectarines, apricots, etc.) have a high diversity in phenolic composition. These compounds are known because of their high antioxidant activity and modulation of cellular processes related to redox homeostasis. This work aimed to study the mechanisms of action of these compounds, starting with estimating their bioaccesibility (defined as the fraction of compounds released from a matrix potentially available for intestinal absorption) and exploring the effect of the digested fruits in the permeability of the gut barrier, as well as the inflammatory and tight junction-associated protein expression in an *in-vitro* model. **Methods.** Six fruits underwent static gastrointestinal digestion using the INFOGEST method. Analyses of polyphenol-enriched extracts from pre- and post-digestion fruit samples through HPLC-MS are underway. Twenty-one-day-old differentiated Caco2-HT29MTX monolayers were chosen as a gut barrier model because of their polarization ability, tight junctions and mucus layer secretion. These monolayers were exposed to the fruit digesta for two hours, and their effect on gut barrier health was tracked by measuring Caco2-HT29MTX tight junction integrity via transepithelial electrical resistance (TEER). To estimate the ability of our test foods to exhibit an inflammatory effect in the gut, cytokines TNF-α, IL-6, and IL-8 were quantified by ELISA from Caco2-HT29MTX co-culture cells exposed to digested samples. Western blot analysis of ZO-1 tight-junction protein was performed on monolayers exposed to crude extracts of the fruit digesta. **Results.** 1) Neochlorogenic acid and isoquercetin have been identified in all the digested samples. 2) monolayers maintained their TEER values above 700 Ω/cm^2 after treating the cells with the fruit digesta for two hours, possibly keeping the integrity of tight junctions from the cell monolayer. 3) Cytokine concentrations obtained from the fruit digesta were higher than controls, but significant effects among the tested fruits were not observed; the low protein expression detected indicates that digested fruits did not affect the gut health status. 4) No difference in ZO-1 protein abundance was observed in monolayers exposed to digested samples compared with control. **Conclusion.** This work lets us know about the interaction between digested fruit compounds and the gut barrier health status. In particular, we observed that stone fruit digesta does not alter the permeability and inflammatory status of the gut barrier. This data can be helpful in breeding programs, particularly in selecting new accessions with higher health-promoting abilities based not only on their initial composition but also on their compounds' bioaccessibility, permeability, and bioactivity.

Acknowledgments

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Keywords : Gut health, inflammation and tight junction proteins, INFOGEST, fruit polyphenols, bioaccessibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22722) - NUTRITIONAL CHARACTERISTICS OF COMMERCIAL SOYMILK AFTER IN VITRO DIGESTION

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Abstract

The worldwide rise of plant-based drinks (PBDs), substituting cow's milk, is driven by a shift in consumer preferences towards sustainable diets. Soy-based drinks are a notable nutritional option, providing high-quality protein, vitamin B, unsaturated fatty acids, phytosterols, soy lecithins, and isoflavones. Prepared by boiling ground soybeans in water, soy drink forms a colloidal suspension with soybeans, water, and occasional additives[1]. In this study, we analyze the nutritional components of five Spanish soymilk options before and after *in vitro* gastrointestinal digestion by using the INFOGEST method, addressing an existing information gap. Three of them belonged to the same brand but varied in composition: one was labeled as low fat (B1a), another as low sugar (B1b), and the third one as high in protein (B1c). The remaining two products (B2 and B3) claimed to consist solely of soybean and water in their composition. Regarding their mineral composition, it should be noted that all B1 drinks are supplemented with calcium. Except for the one labeled as high in protein (B1c), the others exhibited a comparable protein content, although their protein profiles varied slightly. These beverages also showed different inhibitory activity against the digestive enzymes trypsin and chymotrypsin potentially affecting protein digestibility. Given the potential impact of food processing on digestibility, we evaluated thermal damage in these PBDs by measuring furosine and carboxymethyllysine. Total polyphenols and antioxidant activity (ABTS and FRAP methods) were evaluated before and after *in vitro* digestion. Total polyphenols exhibited minimal differences, with a lower content in B1a. All drinks shared similar fatty acid profile, being rich in polyunsaturated fatty acids. Differences in the chemical composition of soymilk observed could impact both the digestive process and the bioactivity of the bioaccessible fraction. Protein profile study during digestion using SDS-PAGE showed altered patterns in the gastric phase. The protein hydrolysis by OPA method revealed significant gastric-level digestibility variations, with distinctions at the intestinal level in one sample. Amino acid analysis in the bioaccesible fraction showed slight differences in the profile obtained after digestion between brands. Confocal microscopy studies on soy milk microstructure during *in vitro* digestion showed increased particle size and aggregate formation. Protein aggregates exceeding 20 µm and smaller lipid structures were observed, with different structures for each milk type.

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Acknowledgments

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Keywords : : in vitro digestion, soymilk, nutritional value, microstructure

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22723) - FORMULATION OF HAM WITH POLYPHENOLS WITHOUT NITRITE COULD PREVENT THE ADVERSE EFFECTS OF OXIDATION

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Abstract

Nitrite and nitrate additives are used in processed meat for their antioxidant and antimicrobial properties, and also to meet consumer expectations for pink color and taste. However, they may react during digestion with heminic iron and secondary amines to form nitroso-compounds (N-nitrosamines [NNO], nitroso-thiols [SNO]), some of which are suspected to increase the risk of cancer (Chazelas et al 2022). Conversely, the absence of nitrites during digestion leads to increased formation of alkenals by lipid peroxidation, which is detrimental (Gueraud et al 2023). Therefore, alternatives should be studied and natural antioxidants such as polyphenols show promising anti-nitrosating capacity (Keuleyan et al, 2021).

Cooked hams were produced by a food manufacturer using fruit extract, green tea or polyphenol-rich olive/grape extracts combined or not with 90 ppm nitrite sodium, to shed light on possible synergistic action. Lipid oxidation and nitroso-compounds were assessed in the ham and during its dynamic digestion using the DIDGI system.

In the absence of nitrites, formulation with polyphenols yielded ham free of oxidation, with no residual nitrites and almost no residual nitrates. Addition of polyphenols to nitrites did not provide additional protection against oxidation. Interestingly, in the ham processed with polyphenols, less oxidation was observed in the gastric compartment after 40-min digestion, but subsequently, in the intestinal compartment , oxidation intensified whatever the formulation. This intensification goes hand in hand with the formation of nitrites (which are not present in the products), underlining a particularly oxidizing environment.

No nonvolatile nitrosamines or nitroso-thiols were found in the ham formulated with nitrites. However, for ham formulated with nitrites, the gastric digestive conditions led to generation of NNO and SNO, which were unstable and disappeared completely in the intestinal compartment. The polyphenols tested showed no anti-nitrosating capacity.

In ham formulated with polyphenols alone, no nitroso-thiols appeared during digestion. Green tea extracts appeared to minimize the production of NNO more effectively.

In conclusion, formulation of ham with natural polyphenols may reduce oxidation. However, to investigate possible synergistic action in greater depth, lower nitrite levels should be studied.

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Keywords : nitroso-compounds, dynamic in vitro digestion, oxidation, polyphenols

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22725) - EFFECTS OF POLYPHENOLS ON PROTEIN DIGESTIBILITY OF DURUM WHEAT PASTA ADDED WITH RED AND WHITE GRAPE POMACE OF ITALIAN VARIETIES.

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Abstract

Durum wheat is commonly used throughout the world to make pasta products. Pasta products are usually rich in proteins and carbohydrates but with a low content of phytochemicals, micronutrients, and fiber. One of the most applied strategies to increase their nutritional properties is the incorporation of functional ingredients during formulation. The grape pomace, a winemaking by-product, has a promising application as functional ingredient of food products due the content of polyphenols and fiber, with potential benefits against oxidative stress- and inflammation-related pathologies. During digestion, durum wheat proteins are broken down by various proteases of the digestive system in different peptides with positive (health-promoting activity) or adverse (allergy, intolerance, toxicity) effects on human health. However, the presence of polyphenols can influence the activity of digestive enzymes altering the release and absorption of durum wheat peptides, after digestion.

This study aims to evaluate the impact of grape pomace polyphenols on durum wheat proteins and peptides released during the digestion of pasta. Fortified pasta was prepared through the replacement of durum wheat semolina with 5% pomace flour from a white grape (cv Fiano) and a red grape (cv Lambrusco) cultivar. The fate of pasta protein digestion was monitored by the degree of hydrolysis and SDS-PAGE profile, followed by the HPLC-HRMS analysis. The final data were software treated to enable protein/peptide identification.

The degree of protein hydrolysis at the end of digestion was around 1.5-fold lower for pasta made with white grape pomace, compared to pasta produced by red grape pomace and control pasta, as assessed by free N-terminal group analysis. The SDS-Page profiles showed slight differences in protein profiles between 31-14.4 kDa, of pasta made by white and red grapes pomace compared to control pasta. The mass spectrometry analysis showed that, for the control pasta, the peptides released during the digestion process originated from the low-molecular-weight glutenin subunit and alpha-gliadin. While the pasta samples added with grape pomaces showed a higher number of durum wheat proteins involved in the digestion process with peptides originated from two glutenin subunits (low and high molecular weight), alpha-gliadin, gamma-gliadin and gamma-secalin.

In conclusion, the obtained results suggest that the addition of grape pomace such as an ingredient of pasta is able to affect the durum wheat protein digestibility, potentially increasing the release of peptides with possible bioactivity. However, further *in vitro* experiments are required to assess the real effect of these digested products.

Acknowledgments

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Keywords : polyphenols, protein digestibility, grape pomace, bioactive peptides

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22726) - AFLATOXIN B1 AND ENNIATIN B1 IN SPARUS AURATA MUSCLE TISSUE – IN VITRO STATIC DIGESTION PROTOCOL AND INTESTINAL TRANSPORT (CACO-2/HT-29 CELLS) ASSESSMENT

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Abstract

Rising global population demands sustainable food solutions, driving aquaculture's rapid growth. Despite fish being a very nutritious protein source, production conditions may expose these animals to different contaminants with implications to human health. Plant-based proteins are very commonly used in fishfeed, due to economic and environmental reasons, which can result in higher exposure to mycotoxins, naturally occurring plant contaminants. The exposure of fish to these contaminants can cause impair fish health, stunted growth or even mortality, causing economic losses for the producers. Sparus aurata was selected as a model organism for this study, for its mass production in aquaculture and high consumption in Europe.

Some studies have reported the presence of both aflatoxin B1 (AFB1) (1) and enniatin B1 (ENNB1) (2) in fishfeed, suggesting their potential to bioaccumulate in fish. Considering these findings, this study sought to evaluate the human bioaccessibility of both these compounds (independently and in combination) in Sparus aurata muscle using an in vitro static digestion model, based on the INFOGEST protocol (3). Different conditions were investigated, encompassing cooking procedures (raw, grilled, and fried) and seasonings (none, thyme, and ginger), to elucidate their potential effect on mycotoxin bioavailability. In addition, the intestinal transport of both mycotoxins was assessed employing an in vitro cell model comprising Caco-2/HT-29 co-culture monolayers grown in a cell insert, mimicking the human intestinal epithelium. The results obtained have demonstrated the effect of the different cooking procedures and seasonings on the target compounds bioavailability.

The growing adoption of novel feed ingredients, including plant-based proteins, underscores the imperative to assess the potential health implications of previously disregarded fish contaminants. In vitro approaches stand as powerful strategy for evaluating bioavailability, presenting advantages such as reduced ethical considerations, expedited and reliable results, and the ability to establish meaningful connections to in vivo scenarios. The simultaneous evaluation of multiple mycotoxins is crucial given their common occurrence in nature.

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Keywords : AFB1, aquaculture, bioccessibility, ENNB1, intestinal transport

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22727) - ASSESSING THE COMBINED BIOACCESSIBILITY AND IN VITRO TRANSPORT ACROSS A CACO-2/HT-29 MODEL, OF AFLATOXIN B1, ENNIATIN B, AND STERIGMATOCYSTIN IN CHILDREN'S BREAKFAST CEREALS

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Abstract

Breakfast cereals play a vital role in supporting the nutritional needs of children, supplying essential nutrients crucial for their growth and development. Recognizing that children are more susceptible than adults to harmful food contaminants, particularly mycotoxins prevalent in cereals, raises concerns. Aflatoxin B1 (AFB1), enniatin B (ENNB), and sterigmatocystin (STG) are well-known mycotoxins found in cereals, and have the potential to be absorbed in the gastrointestinal epithelium. Despite the known vulnerability of children to mycotoxins, research on the bioaccessibility of these toxins in breakfast cereals is limited.

This study aimed to evaluate the bioaccessibility of AFB1, ENNB, and STG, individually and in combination, using an in vitro static digestion model (1). While existing research has explored the intestinal absorption of single mycotoxins, this study takes a different approach by investigating the in vitro transport of single and combined AFB1, ENNB, and STG across intestinal Caco-2/HT-29 co-culture monolayers.

In terms of intestinal bioaccessibility, AFB1 exhibited values between 3.1% and 86.2%, STG between 1.5% and 59.3%, and ENNB between 0.6% and 98.2%. Overall, lower bioaccessibilities were found in digested breakfast cereals with different types of milk compared to breakfast cereals ingested alone. Concerning intestinal transport, ENNB and STG were the most absorbed in the mixture, in both matrices. When isolated, they exhibit higher absorption only in the cereal matrix with milk, with AFB1 showing the highest value in the cereal matrix.

The study underscores the significance of examining the combined ingestion and transport of these toxins to comprehensively understand their absorption and evaluate human exposure with higher precison. This is particularly important due to the frequent co-occurrence and simultaneous exposure to these mycotoxins in cereals.

The findings of this study are important because they provide new information about the absorption of mycotoxins in breakfast cereals. This information can be used to develop strategies to reduce the risk of mycotoxin exposure in children.

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Keywords : breakfast cereals, in vitro digestion, bioaccessibility, bioavailability, mycotoxins

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22728) - DEVELOPMENT OF PLANT-BASED DELIVERY SYSTEMS: FORMULATION OPTIMIZATION AND BEHAVIOR UNDER IN VITRO DIGESTION

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Abstract

The interest of food industry in the development of plant-based foods is increasing due to the growing number of vegetarian or vegan consumers. However, there is a concern that people with plant-based diets may be deficient in some nutrients that are normally obtained from animal-based foods. Since the direct incorporation of micronutrients into food products is challenging due to their chemical instability and low bioavailability, fortification of plant-based foods with nanostructures encapsulating some of these nutrients can be an effective strategy to combat potential nutritional deficiencies. This work focused on reformulate existing nanostructures to remove animal-based ingredients and replace them with plant-based alternatives. Therefore, the emulsifying properties of two plant-based proteins, pea and potato proteins, were investigated to formulate nanoemulsions encapsulating curcumin and vitamin D_3. An experimental design was carried out to assess the concentration of protein (pea and potato) and oil (corn oil) that led to nanoemulsions with smaller Z-average diameter and PDI. The results showed that 10 % potato protein and 1 % corn oil led to nanostructures with smaller Z-average diameter (271.7 ± 12.0 nm) and lower PDI (0.37 ± 0.01). The stability of the nanoemulsions was evaluated by measuring Z-average diameter, PDI, and zeta potential during one month. To evaluate if these nanostructures could be incorporated into food products to enrich plant-based foods with micronutrients (i.e. vitamin D3 and curcumin) and protein, nanoemulsions were submitted to the harmonized static *in vitro* digestion and their cytotoxicity were evaluated using Caco-2 cell line. The prepared nanoemulsions can be used to develop novel functional plant-based foods with enhanced bioactive compounds' bioavailability.

References

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Keywords : plant-based, nanoemulsions, curcumin, vitamin D3, in vitro digestion

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22732) - EFFECT OF GUM ACACIA ON THE INTESTINAL BIOAVAILABILITY OF N-3 POLY UNSATURATED FATTY ACIDS IN RATS

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1 - ITERG; 2 - NEXIRA

Abstract

The emulsification of lipids is a technique being explored because of its interest for improving the bioavailability of lipids of interest, such as omega 3(n-3) long chain (LC) fatty acid (FA).

The choice of emulsifiers is of importance as, depending on their nature, they can impact differently the bioavailability of lipids generally due to a modification of lipolysis levels in the gastro intestinal tract. Among natural emulsifiers, gum Acacia (GA), an indigestible polysaccharide, provides protective encapsulation of n-3. It has the particularity of forming a specifically gangue around the lipid drops which could also impacts lipid digestion. Despite preliminary data supporting its interest on lipolysis the specific impact of GA on lipid bioavailability has never been explored in a complete physiological context.

Thus, we followed in a kinetics study, the n-3 bioavailability in rat lymph, orally submitted to a microalgae oil rich in DHA, formulated GA-based compared to the non-formulated bulk phase form of the same oil. The bioavailability of n-3 was significantly improved in lymph of rat provided with the GA -based emulsion. More precisely, the AUC was improved by +121% for total TG and by 321% for n-3 PUFAs. In this case, this increase follows the improvement of AUC for EPA (+244%) and for DHA (+345%). On the other hand, the benefits attributed to GA have also been related to the Tmax obtained for the transport of FA in lymph, which was 2h earlier (Tmax=4h) compared to the Tmax (6h) obtained with the miroalgae in bulk phase.

All the data showed that GA is one of the most favorable candidates in the choice of natural emulsifiers to improve the lipid bioavailability and their rate of absorption, and more specifically that of n-3, for health targets.

Keywords : gum acacia, DHA, Lymphatic absorption, emulsion, omega-3 bioavailability

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22736) - INTERFACIAL FILM COMPOSITION DETERMINES COCONUT OIL LIPOLYSIS IN NANOSTRUCTURED LIPID CARRIERS

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Abstract

The fabrication of nano structured lipid carriers (NLC) is one of the most effective ways to enhance the oral bioavailability of lipophilic nutraceuticals, as these are usually solubilized in the interior of the liquid lipid and the surrounding solid matrix acts as a physical barrier, thereby contributing to the overall chemical stability.The digestion behavior of NLC has a great impact on their oral delivery properties. The interfacial film composition, the crystalline structure of the lipid droplets as well as their size might influence the accessibility of lipase enzymes to the lipid substrate, affecting the rate and extent of lipolysis. The objective of this work was to assess the influence of the physicochemical characteristics of coconut oil (CO) NLC on the rate and extent of CO lipolysis. Different ratios of glycerol monostearate (GM) to soy lecithin (SL) (from 75/25 to 25/75) were assessed for stabilization of CO emulsions. Emulsions (10/90 O/W) were prepared by homogenization at high temperature to ensure melting of components (high speed homogenizer for 2 min and high intensity ultrasound for 10 min). Then emulsions were cooled to allow formation of NLC and digested by the INFOGEST 2.0 gastrointestinal digestion protocol (Brodkorb et al., 2019) and kinetics of free fatty acids (FFA) release was determined. Droplet size and microstructure were monitored on freshly prepared emulsions and during the digestion process by static light scattering and optical microscopy (bright field and polarized light).

Droplet size and crystallinity of freshly prepared emulsions showed a tendency to decrease as the ratio of GM/SL decreased. However, no significant correlation was observed between the initial droplet size/degree of crystallinity and the rate/extent of digestion. Although, the surfactants ratio had a strong impact on the release of FFA as the extent and rate of lipolysis increased with the ratio of GM/SL.Emulsions with higher amount of SL exhibited increased droplet size when exposed to simulated gastric fluids, that further conditioned the rate and extent of lipolysis. In contrast, nonionic GM could better resist pH changes.

Thus,NLC obtained from CO emulsions with a high GM/SL ratio would better resist the gastric transit, which could provide protection to an encapsulated lipophilic nutraceutical and also allow a higher release at the duodenal phase.

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Universidad de Buenos Aires,Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción científica y tecnológica,–Argentina.

Keywords : nanostructured lipid carriers, lipolysis, coconut oil

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22737) - IN-VITRO DIGESTION OF RADISH MICROGREENS: FATE OF ORGANOSULFUR AND PHENOLIC COMPOUNDS

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Abstract

Brassicacea microgreens are increasingly popular as convenient and sustainable fresh vegetables that are packed with phytochemicals. Different light conditions can be used in their growth and possibly impact phytochemical composition, whose subsequent fate after digestion is underexplored. Both bioaccessible and non-bioaccessible fractions are of paramount importance to predict health effects. This work aimed to assess the organosulfur and phenolic compounds of radish microgreens grown under different light conditions, before and after digestion.

Green (GR) and red radishes (RR) were grown vertically under two types of artificial LED lights (white (W) or a blue/red light combination (B+R)), with supplemental UV-C radiation. Gastrointestinal digestion was simulated using the INFOGEST in-vitro static protocol. The undigested (plant matrix), bioaccessible and non-bioaccessible fractions were evaluated for glucosinolates (GSLs) and phenolics by HPLC-DAD-ESI-MSn (identification) and HPLC-DAD (quantification); and for isothiocyanates and indoles (ITCs) by UHPLC-QqQ-MS/MS.

The qualitative profile of organosulfur compounds was similar in both GR and RR, with aliphatic GSLs representing 98% and 95% of all GSLs, respectively. GSLs presented extensive transformation during digestion, being that only their breakdown products, ITCs, were identified in both digesta fractions. The bioaccessible fraction presented significantly higher amounts of ITCs than the non-bioaccessible fraction, with sulforaphene (SFE) representing >99% of all ITCs. RR presented more ITCs than GR in both bioaccessible and non-bioaccessible fractions. Regarding phenolics, RR presented higher amounts than GR, and while both GR and RR were rich in hydroxycinnamic acids, especially sinapic acid, RR additionally presented flavonoids in the form of anthocyanins (ACs), principally acylated forms of cyanidin. In the bioaccessible fraction, radishes presented a feruloyl-sinapoyl derivative and RR additionally presented p-coumaric acid, both in higher amounts than GR. ACs were also present in the non-bioaccessible fraction of RR.

In general, better composition was achieved with R+B LEDs, with little to no effect of UV-C radiation. This was generally reflected after digestion as well, despite a high variability of compounds. RR appear as a better matrix to achieve higher amounts of compounds after digestion. These results represent a groundwork to optimize resources and growing conditions of radish microgreens as a source of bioactive compounds, and to comprehensively assess their potential benefits, not only through the assessment of bioaccessibility of specific compounds, but also to further explore their potential effects through the microbiota. It also reinforces the need to explore the highly bioaccessible SFE, whose health effects are promising but still relatively unknown.

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Keywords : Radish microgreens, Glucosinolates, Isothiocyanates, Phenolic compounds, In-vitro digestion

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22741) - FERMENTATION OF CHICKPEA-BASED PUREE ENHANCES BIOACTIVE PEPTIDE AND POLYPHENOL BIOACCESSIBILITY

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Abstract

The transition toward a plant-based diet and sustainable food production is necessary to overcome the global nutritional and environmental issues. Legumes may be a feasible alternative to meat for the content of proteins, dietary fiber, and micronutrients even though some concerns still exist for the phytate content. Fermentation may improve the nutritional properties of food.

This study, aimed at developing innovative fermented chickpea-based purees with enhanced functional properties compared to unfermented counterparts.

To this purpose, fermented purees (FP) containing 10% and 20% w: v of chickpea with 16 strains of lactic acid bacteria (LAB), previously isolated from different ecosystems, along with unfermented purees (CP) were produced and analyzed for the content of polyphenols (by HPLC/UV-VIS), bioactive peptides (BAPs, by LC-HMRS) and the total antioxidant capacity (TAC, by the DPPH method). The FP showing the highest TAC were subjected to *in vitro* digestion (INFOGEST method) to assess the bioaccessibility of polyphenols and the release of TAC over digestion.

The concentration of polyphenols in the 20% CP and FP was 1.7- and 1.1- fold higher than in the 10% CP and FP, respectively. Specifically, five polyphenols were identified in the 20% purees, among which synapic acid glucoside predominated in CP and pyrogallol in FP. The soluble and direct TAC of purees increased on average by 7.8 and 2.4 times after fermentation, respectively, *Leuconostoc mesenteroides* and *Lactiplantibacillus plantarum* being the most efficient species. Eight BAPs were identified in the purees at different concentrations (that generally increased by fermentation) and possibly exerting biological activities, such as dipeptidyl peptidase IV, angiotensin-converting enzyme, and renin inhibitors.

The 20% FP with *Leuconostoc mesenteroides* after digestion exhibited a 1.2-fold increased TAC whereas an 18-fold increase was found for CP. During digestion, CP released amounts of *p*-hydroxybenzoic acid, kaempferol-3-O-glucoside, and pyrogallol, that were 3-, 4-, and 1.5- fold higher than the undigested puree; whereas FP, mainly released pyrogallol that increased by 1.4 times, accounted for 71% of the total polyphenols in the digest and showed a bioaccessibility of ~140%.

In conclusion, fermentation, improves the nutritional profile of purees by increasing the concentrations of BAPs and polyphenols as well as their bioaccessibility and antioxidant activity during the digestion.

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Keywords : legumes, fermentation, bioaccessibility, protein digestion, bioactive peptide

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22743) - EXPLORING THE BIOACCESSIBILITY OF IMMUNOGENIC PEPTIDES IN MILK AND EGGS: INFLUENCE OF DIETARY POLYPHENOLS AND FOOD MATRIX

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Abstract

Background:

The rapid increase in the prevalence of food allergies appears on the horizon as a new challenge among nutritionists, clinicians, food researchers, and the food industry. It is estimated that FA affects up to 8% of children and 3% of adults in industrialized countries. Among them, cow's milk and egg allergy are some of the most prevalent.

New therapeutic strategies to prevent these pathologies and reduce symptoms are thus necessary. The use of dietary phenolic compounds to modulate the digestion of allergens constitutes an exciting new approach for the maintenance of oral tolerance to allergens. Indeed, these compounds can bind to dietary proteins and/or gastrointestinal enzymes, altering their bioaccessibility. As such, the objective of this work was to determine the modulatory effect of dietary polyphenols on the digestion of milk and egg allergens.

Methods:
In vitro digestion models were used to simulate the ingestion and digestion of cow's milk and egg proteins, and mass spectrometry techniques were used to track the potentially immunogenic peptides released during the digestion process. Samples were digested simultaneously with extracted green tea polyphenols and blueberry polyphenols as to pinpoint the potential effects of different phenolic compounds on the digestion of milk proteins and the formation of immunogenic peptides.

Results:
Results thus highlighted the ability of dietary polyphenols to modulate the bioaccessibility of milk and/or egg-related immunogenic peptides. The monitored peptides were differently affected by the polyphenol extracts, as some had their bioaccessibility increased by the presence of phenolic compounds, while the inverse effect was observed on other peptides. The removal of the food matrix also greatly affected the modulatory effect of polyphenols, indicating its importance on the digestion of proteins. Despite further studies are required to deeply understand the implication of this modulation on food allergies, this study opens a new way to understand the impact of the design of our meal on oral tolerance.

Conclusion:
These results thus highlight the potential role of dietary polyphenols in the development of new therapeutic strategies to reduce the prevalence of food allergies and reduce associated symptoms.

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Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22746) - BIGELS OF FOOD GRADE AS POTENTIAL CARRIER MATERIALS FOR CHLOROPHYLLS: INFLUENCE OF OLEOGEL/HYDROGEL RATIOS ON DIGESTION PROPERTIES

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Abstract

The diversified composition of biomolecules from cyanobacteria can contribute to nutritious and functional diets. Among the functional compounds, chlorophylls are in focus since having properties that result in biological functions beneficial to human health, which are in turn related to their bioaccessibility. However, the knowledge about their bioaccessibility is limited and little is known about the influence of the food composition on the digestion of these biocompounds. In this sense, this study aimed to investigate the recovery of chlorophylls from *Arthrospira platensis* (Spirulina) and their efficiency of micellarization in different types of bigels with different proportions of hydrogel:oleogel. The bigels were prepared using a mixture of oleogels with carnauba wax, sunflower oil, and lecithin, and hydrogels with agar and xanthan gum in different proportions (80:20, 60:40, 40:60, and 20:80 hydrogel:oleogel) under temperature control. Functional extracts obtained from the cyanobacteria *Arthrospira platensis* were considered for the addition of natural chlorophylls at a concentration of 6mg/100g of bigel. The chlorophyll composition was determined by HPLC-*hr* ESI/APCI–MS^2, and an in vitro standardized protocol adapted for chlorophylls was applied to evaluate the bioaccessibility of the compounds. It was stablished the determining factors for the chlorophyll's stability during the simulated digestion and the efficiency of the partitioning of chlorophylls into mixed micelles. Furthermore, the results allowed to determine which fractions of oleogel favored the bioaccessibility of chlorophyll compounds. However, independently of the bigels composition, the chlorophyll profile after in vitro digestion was mainly formed by pheophytins. Finally, our findings showed that bigels can be considered potential materials to stimulate and promote the bioaccessibility of natural chlorophylls from cyanobacteria.

Acknowledgments

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Keywords : Bioaccessibility, Chlorophylls, Bigels, In vitro digestion, Micellarization.

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22748) - IN VIVO AND IN VITRO EFFECTS OF DIFFERENT DIETARY PROTEINS ON SHORT-TERM FOOD INTAKE AND INTESTINAL HORMONE REGULATION

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Abstract

Purpose: Besides their nutritional function, proteins are known to intervene in short- and long-term energy homeostasis regulation. Nevertheless, studies on the satietogenic effect of proteins regarding their quality and origin are limited and often incomparable from one study to another. Moreover, and in parallel, digested dietary proteins are known to exert a satiating effect by stimulating intestinal hormone secretion (CCK, GLP-1, and GIP). This study aims to compare the effect of proteins from different origins (bovine haemoglobin, caseins, ovalbumin, whey proteins, fish gelatin, pea proteins and gluten) on short-term food intake regulation and intestinal hormone secretion.

Methods: Two experiments involving Wistar rats were performed. In the first experiment, the effect of a protein preload administration on food intake was studied using metabolic cages. Diet consumption was measured over eight hours after orally administered protein preload. In a second experiment, plasma hormone levels were measured 30 min after protein administration. In parallel, those proteins were *in vitro* digested using the INFOGEST static protocol. The molecular mass digestome profiles were analyzed using size exclusion chromatography, and the modulation of CCK, GLP-1, and GIP secretion was investigated in STC-1 cells.

Results: The results showed that dietary proteins decreased short-term food intake and respiratory gas exchange ratio (RER) and increased plasma levels of GIP, insulin, and glucagon in rats differently. Ovalbumin, whey and gluten proteins stand out from the other protein sources because of their superior satiety effect. The *in vitro* analysis globally corroborates the *in vivo* results. Digested proteins stimulated the secretion of GLP-1, CCK and GIP hormones in STC-1 cells, with a more pronounced effect for haemoglobin.

Conclusion: This study shows the effect of proteins on energy homeostasis through their effect on short-term food intake regulation correlated with the modulation of metabolic markers. It also highlights the influence of the protein origin on these different mechanisms.

Acknowledgments
This research was funded in the framework of the CPER BiHautsEco de France research program, which is financed by the European Union, the French State, and the French Region of Hauts-de-France.

Keywords : food intake regulation, dietary proteins, static digestion, intestinal hormones

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22749) - BIOAVAILABILITY PREDICTION OF CHLORELLA VULGARIS HYDROLYSATES AFTER STANDARDIZED GASTROINTESTINAL MODEL (INFOGEST)

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Abstract

Chlorella vulgaris is a known source of bioactive compounds, with special highlight on its high protein content and, consequently, peptides and essential amino acids. Microalgae are considered sustainable, due to their relatively easy production and their ability to capture environmental CO_2 [1]. Due to *C. vulgaris* high protein content, it represents a promising source of bioactive hydrolysates with commercial potential.

Enzymatic hydrolysis was used to produce bioactive hydrolysates from Yellow and Green *Chlorella vulgaris* species, and both hydrolysates showed high protein content and bioactive properties interesting for being incorporated into food products. However, before proceeding to a final application, the bioavailability of the proteins and peptides should be guaranteed. An *in vitro* gastrointestinal (GIT) digestion model (INFOGEST) assay was used to assess the hydrolysates' bioacessibility through the GIT. For that, protein, peptide and free amino acid profile were determined and soluble protein was measured. The hydrolysates antioxidant capacity was evaluated in all GI phases by oxygen radical absorbance capacity (ORAC) and ABTS assay. A membrane dialysis was used during the intestinal digestion step to evaluate the fraction able to cross the membrane, and consequently absorbed. The hydrolysates anti-hypertensive and anti-diabetic properties were analysed by their ability to inhibit Angiotensin-I-converting enzyme (ACE) and α-glucosidase, respectively.

Yellow *C. vulgaris* showed a more pronounced effect on the antioxidant activity after the GIT digestion, showing an approximate 4 and 1.4-fold increase for ABTS and ORAC, respectively. For green *C. vulgaris*, the increase was approximately 1.6 and 1.1-fold for ABTS and ORAC values, respectively. The increased antioxidant capacity of the hydrolysates after GIT digestion may be explained by the presence of proteases in different GIT steps that can act on the hydrolysates, resulting in the release of peptides with lower molecular weight (MW) and more antioxidant potential. Furthermore, since we are determining the antioxidant activity in the fraction that can cross the dialysis membrane, we know that in bloodstream we are in the presence of lower MW peptides. This result is in accordance with several studies that show that peptides with MW lower than 3 kDa are, in general, more bioactive[2]. Regarding the anti-hypertensive activity, a decrease was observed after dialysis in both hydrolysates, with an approximate 5 and 4-fold decrease for *C. vulgaris* yellow and green, respectively. Thus, in the future, to improve the peptide resistance in the GIT, encapsulation systems may be developed, not only protecting the peptides after ingestion but also allowing a controlled release.

In conclusion, our results showed that *C. vulgaris* hydrolysates can be used as high-value ingredients for the development of functional foods.

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Acknowledgments

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Keywords : Bioactive hydrolysates, Functional food, Microalgae, Sustainability

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22752) - THE EFFECT OF CARRAGEENAN AND XANTHAN GUM ON PROTEIN DIGESTIBILITY AND PROTEIN FERMENTATION DURING IN VITRO GASTROINTESTINAL DIGESTION OF PORK

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Abstract

Hydrophilic emulsifiers, like κ-carrageenan and xanthan gum, have been reported to alter protein digestibility. Several studies also indicate a possible role of κ-carrageenan and xanthan gum in the development of intestinal inflammation and gut microbial changes. When protein digestibility is reduced, a greater fraction of amino acids and peptides may reach the colon, providing more substrate for protein fermentation. Moreover, a high heating temperature of meat is proposed to influence protein digestibility. However, little is known on the influence of hydrophilic emulsifiers in meat products on protein digestibility, and on the formation of protein fermentation metabolites in the colon.

In this experiment, pork was minced and homogenised with or without 1% κ-carrageenan or 1% xanthan gum. Later, meats were heated (until core temperature 70ºC or 100ºC) and exposed to an *in vitro* digestion model, simulating the human oral, gastric and duodenal phases. Next, a dialysis (3.5kDa) was performed to mimic the absorption of small peptides and amino acids in the small intestine. The protein digestibility was determined in digests at the duodenal stage, by comparing the protein content (Kjeldahl) of the digest samples with and without dialysis. Finally, the retentate of the dialysis was *in vitro* fermented using human fecal inocula originating from four individual volunteers, to simulate the large intestinal phase. Following 24h of fermentation, H_2S, methanethiol, dimethyl n-sulfides, phenol, cresol and indole were analysed by micro-GC and GC-MS-SPME.

The heating temperature did not influence the protein digestibility and the formation of fermentation metabolites during digestion of processed meats. Nonetheless, the pork digests with xanthan gum were less digestible compared to the control meat (74% vs. 87% resp., $p < 0.001$) and the meat with κ-carrageenan (86%, $p < 0.001$), the latter being not different from the control. Concomitantly, a higher formation of all analysed sulfur metabolites ($p < 0.01$ for all), phenol ($p < 0.001$) and indole ($p < 0.001$) was observed in ferments of meat with xanthan gum compared to the control and κ-carrageenan, whereas no significant differences were found in the ferments of control pork with κ-carrageenan.

In conclusion, in the present study, the addition of xanthan gum decreased pork protein digestibility, resulting in an increased formation of protein fermentation products, whereas the addition of κ-carrageenan had no significant effects.

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N/A

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Keywords : Processed meat, Carrageenan, Xanthan gum, Protein digestibility, Fermentation metabolites

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22755) - IMPACT OF GASTROINTESTINAL DIGESTION ON THE BIOACTIVITY AND BIOACCESSIBILITY OF CAROTENOIDS AND PHENOLIC COMPOUNDS FROM ALGAE

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Abstract

A nutrient-rich diet plays a crucial role in modulating chemical signals, including antioxidants, to enhance resistance to gut's pathogens and prevent diseases[1]. This study delves into the impact of *in vitro* simulated gastrointestinal digestion (Infogest)[2] and absorption (dialysis membrane) on the bioactivity and bioaccessibility of carotenoids and phenolic compounds derived from algae, specifically, *Osmundea pinnatifida, Codium* spp and *Chlorella vulgaris*. Different extraction methods (including enzymatic and conventional hexane-based) were used to obtain extractable bioactive compounds (BCs) from these algae.

Results revealed 4-hydroxybenzoic acid, epigallocatechin as predominant phenolic compounds in *Codium spp.*. Epigallocatechin and gallic acid were quantified only in *Codium* spp. whereas hydroxymethylfurfural, 4-hydroxybenzoic acid and syringic acid were found in both macroalgae. Gastrointestinal digestion exhibited a higher recovery index for phenolic compounds in *O. pinnatifida*, while carotenoids experienced a marked reduction after stomach conditions for both extracts.

Osmundea pinnatifida contained three times more polyphenols than *Codium* spp., mainly anthocyanins. Antioxidant activity of *O. pinnatifida* was substantial (1328.28 ± 75.32 µmol of Trolox Eq./ g DW), while *Codium* spp. displayed significant total carotenoids content (184.55 ± 2.92 µg β-carotene eq./g DW). *Codium* spp. exhibited higher polyunsaturated fatty acid (PUFA) content than *O. pinnatifida*.

Enzymatic chlorella extract demonstrated anti-inflammatory and moderate anti-hypertensive activity. Gastrointestinal digestion led to the identification of carotenoids-derived aroma compounds, showcasing the transformation of carotenoids along the digestive tract. Notably, functional properties of carotenoid solutions included antioxidant and antidiabetic activities, with lutein exhibiting the highest values in the absorbed fraction.

In conclusion, this research highlights the intricate interplay of extraction methods, gastrointestinal digestion, and microbial interactions in determining the bioaccessibility and functionality of carotenoids and phenolic compounds from algae. The findings open avenues for understanding the dynamic behaviour of these bioactive compounds in complex matrices, offering insights for developing functional foods with enhanced health benefits.

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1 doi: 10.3390/nu15102265

2 doi: 10.1038/s41596-018-0119-1

Acknowledgments

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Keywords : Bioactive compounds, Carotenoids, Phenolic compounds, algae, bioaccessibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22757) - THE ADDITION OF PLANT PROTEIN TO HIGH-PROTEIN YOGURT REDUCES THE BIOACCESSIBILITY OF ESSENTIAL ELEMENTS

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Abstract

The partial replacement of dairy proteins by plant proteins in dairy products is a promising strategy to reduce the consumption of proteins of animal origin. Additionally, this strategy can diversify the composition of essential elements in these products. This study evaluated the total content and bioaccessibility of essential elements (Ca, P, and Mg) in control and hybrid (50% dairy proteins replacement by almond proteins) high-protein yogurts. Mixtures for yogurt manufacture were standardized to 14% total solids by the dispersion of whole milk powder and caseinate, or whole milk powder, caseinate and almond protein in water. The yogurts were produced through the fermentation of dairy and hybrid mixtures and submitted to *in vitro* simulation of gastrointestinal digestion according to the INFOGEST protocol. Total and soluble mineral contents after the *in vitro* digestion were quantified by flame atomic absorption spectrometry (Ca, Mg) and by colorimetric method (P). The complete experiments were performed in triplicate and the results were evaluated by ANOVA. The partial replacement of dairy proteins by almond proteins increased the total Mg (from 8.36 to 46.52 mg $100g^{-1}$) and P (from 23.72 to 30.28 mg $100g^{-1}$) and decreased the Ca content (from 141.92 to 94.67 mg $100g^{-1}$). However, control yogurts showed higher bioaccessibility for all elements evaluated (Ca 92.46%, Mg 81.76%, P 58.22%) when compared to hybrid yogurts (Ca 27.78%, Mg 12.99%, P 42.78%), resulting in higher Ca (131.08 versus 26.22 mg $100g^{-1}$) and equivalent Mg (6.82 versus 6.04 mg $100g^{-1}$) and P (13.83 versus 12.96 mg $100g^{-1}$) soluble contents. The effect of the composition of the matrices in the solubilization of minerals throughout the digestion process might be associated with the presence of fibers and phenolic compounds in seeds, such as almonds, that can interact with Ca and Mg, complexing the elements and reducing their solubilization capacity along the digestion steps, and the higher concentrations of long-chain fatty acids, which can lead to the formation of insoluble soaps with Ca. Although the partial replacement of proteins increased the total Mg and P contents of the products, it did not increase the soluble content of these elements after digestion. Additionally, this replacement not only reduced the total Ca content but also reduced its bioaccessibility. Our results indicated that the dairy matrix favored the bioaccessibility of all elements evaluated in the present study.

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FAPESP grant #2022/00029-8

Keywords : minerals, plant protein, dairy products

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22762) - FOOD PROCESSING AND SIMULATED GASTROINTESTINAL DIGESTION AFFECT DIFFERENTLY THE IGE-BINDING CAPACITY OF SHELLFISH ALLERGENS

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Abstract

Shellfish comprise a multitude of molluscs and crustaceans, being among the most widely consumed seafood species due to their nutritional and organoleptic properties. However, molluscs and crustaceans are known to elicit severe/life-threatening allergic reactions in shellfish-sensitized/allergic individuals, even after harsh food processing conditions and gastrointestinal (GI) digestion. Therefore, it is crucial to evaluate how different processing conditions affect the subsequent GI digestion of different shellfish allergens and their potential to elicit allergic reactions [1]. Hence, this work aimed at evaluating the effect of different food treatments and *in vitro* digestibility on the IgE-binding capacity of shellfish allergens using sera from mollusk/crustacean-allergic patients.

For this purpose, several mollusc (e.g., octopus, squid, clam) and crustacean (e.g. shrimp, lobster) species were submitted to different treatments (boiling, oven-cooking, grilling, marination) and processing conditions (e.g., duration, temperature). Proteins were extracted, quantified by BCA, analyzed by SDS-PAGE in non-denaturing conditions and immunoblotting with sera from mollusc/crustacean-allergic patients. Some thermally treated shellfish species were digested following the INFOGEST 2.0 protocol [2] and further compared with their digested raw counterparts.

In general, the SDS-PAGE results showed a higher number of bands in processed than in raw samples, indicating protein fragmentation triggered by thermal processing. Immunoblotting results demonstrated that, in most cases, the IgE-binding capacity of allergens like tropomyosins, paramyosins and arginine kinase was stronger in processed (boiled, oven-cooked) than in raw shellfish. Interestingly, water from boiling molluscs and crustaceans presented high quantity of proteins with IgE-reactivity (most likely tropomyosins), meaning that part of the allergens is water-soluble and can be eliminated by discarding the boiling water. Still, the correspondent boiled sample contained a high number of reactive protein(s). Most allergens seem to preserve their IgE-binding capacity during gastric digestion, though being greatly reduced by subsequent intestinal digestion.

These findings suggest that thermal processing might contribute to increase the allergenicity of molluscs, while GI digestion can mitigate it. This is the first report on the evaluation of the IgE-binding capacity of allergens of multiple mollusc/crustacean species as affected by single/hurdle food processing and GI digestion.

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Acknowledgments

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Keywords : Shellfish allergy, Food processing, Gastrointestinal digestion, Food allergen digestion

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(22766) - RATIONAL DESIGN OF CEREALS FOR THE OLDER ADULT: FABRICATION, PALATABILITY AND IN VITRO DIGESTIBILITY OF A COMPOUND PLANT-BASED PRODUCT

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1 - Agroscope

Abstract

The challenge of addressing the aging population's nutritional needs led to initiatives like The EAT4AGE project, aimed to develop palatable, nutritious, and digestible foods for preventing undernutrition in active aging. It involved diverse partners, from research institutions to corporate entities, pooling resources to bridge the health gap observed by the National Institutes of Health. The focus was on tailored nutrition to address the complex changes associated with aging, such as mental decline, psychological changes, and compromised health.
The study highlighted nutritional gaps in older adults, emphasizing high-quality protein, dietary fibers, and specific micronutrients. Protein, crucial for addressing sarcopenia and appetite control, gained attention. Recognizing the interest in sustainable, plant-based diets, the research identified Macamides in the Maca root and Oleuropein in olive leaf extract (OLE) as functional food bioactives for seniors.

The proposed solution involved designing a shelf-stable extruded cereal tailored to older adults' palate and needs, leveraging extended shelf life, affordability, sustainability, and ease of use. Extrusion addressed challenges related to allergens, antinutritional factors, and limited digestibility in plant-based products.
This research, part of the EAT4AGE project, focused on a study examining the palatability, masticatory acceptance, and potential digestibility of a co-extruded cereal product among seniors aged over 65.

Various analytical methods comprehensively assessed the nutritional composition, physical attributes, oral breakdown, and digestive behavior of the cereal products. Physicochemical characterization confirmed nutritional content, and sensory evaluations conducted internationally demonstrated positive outcomes.
The co-extruded products were developed to enhance the intake of easily digestible, high-quality proteins, dietary fibers, unsaturated lipids, and functional supplements like Maca and OLE for their potential health benefits. Physicochemical characterization confirmed protein content above 12%, a fat content of 20%, and low sugar, effectively addressing the nutritional needs of seniors for a high-quality and calorically dense food product. Texture analysis revealed improved chewability and oral comfort, crucial aspects for the elderly population.
This contribution offers an attempt to bridge the gap between increasing lifespan and health span by developing targeted, palatable, and nutritionally rich functional foods tailored for older adults, considering their unique physiological status. Thus, this research establishes a new paradigm for tailoring food solutions for seniors, holding promise for harnessing food processing towards manufacturing of innovative, nutritionally optimized products to improve the health and quality of life of older adults.

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Keywords : healthy aging, functional foods, co-extrusion, palatability, oral comfort, plant-based cereals, EAT4AGE, In vitro protein digestibility, DIAAS, SDS-PAGE, proteomic analysis

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22769) - ON THE WAY TO STANDARDIZE CONDITIONS TO ASSAY FOOD COMPOUNDS PERMEABILITY USING AN IN VITRO INTESTINAL MODEL

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Abstract

Permeability cell-based models are widely used for all kinds of food compounds (lipids, protein, carbohydrates, phenols etc.) as well as food contaminants. Different types of intestinal epithelial cell lines are used, including enterocytes, goblet and immune cells. However, the comparability of the assays between laboratories remains unknown due to the large variety of molecules and conditions assayed. The objective of the INFOGEST WG3 on Intestinal Barrier Models is to define a common protocol for testing food compounds. The co-culture of enterocytes with goblet cells by the use of Caco-2 and the mucus producing HT29-MTX is proposed. In a first trial, to evaluate the influence of the cell line origin, the clones from 7 laboratories were cultured in monolayers in one laboratory (Hevia et al., 2023). Real time impedance and capacitance comparison showed important variations in the transepithelial electric resistance (TEER) profile during the differentiation period, only ascribable to the clone. Also the material of the cell culture inserts (polyethylene terephthalate, PET; polycarbonate, PC; or polytetrafluoroethylene, PTFE) has shown an impact on this parameter. With the use of the same clones and agreed protocols for culturing, TEER measurement and permeability markers of paracellular and transcellular transport, comparable values have been obtained although some discrepancies remain to be studied. The collaborative work within groups is expected to culminate in a consensus protocol for culturing Caco2/HT29MTX monolayers for food permeability experiments.

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Acknowledgments

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Keywords : Caco-2, HT29-MTX, interlaboratory trial, permeability, TEER

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(22773) - THE POTENTIAL OF FUCOIDAN TO PROTECT AGAINST NEURODEGENERATIVE DISEASES (GASTROINTESTINAL MODEL)

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Abstract

Fucoidan (FUC) is an algae-derived polysaccharide that shows a wide range of interesting biological properties, such as anti-inflammatory, antioxidant, antibacterial, anti-coagulant, anticancer, neuroprotective, among others. Our focus is to explore FUC's potential for neuroprotection, since brain disorders are progressing across the world, having negative consequences on health, and quality of life and being the leading cause of years lived with a disability. FUC appears as a promising agent for brain diseases due to its ability to inhibit reactive oxygen species and apoptosis, neuroinflammation protection ability, neuronal protective function [1].

Despite FUC's high potential, when developing a food product, it is necessary to guarantee that its action is maintained after passing throughout the gastrointestinal (GIT) tract and also that it is able to cross the intestinal barrier and reach the bloodstream. Thus, our main goal was to develop a film for oral administration incorporating encapsulated FUC. Patients with brain disorders (psychiatric or older) often show difficulty in swallowing conventional oral delivery systems. So, we developed an oral film with several advantages, such as convenient administration, no water required, controllable rates of disintegration and dissolution in the oral cavity, ultra-thin, and being structurally less obtrusive leading to higher acceptability. Furthermore, we have developed a delivery system to improve FUC's functionality, protecting it against GIT digestion and guaranteeing its passage to the bloodstream.

FUC was encapsulated into sodium alginate microparticles by spray dryer technique. Afterwards, FUC microparticles were included in oral films developed by solvent casting technique. To guarantee FUC's bioavailability in our product, an *in vitro* simulation of the GIT digestion was performed, using a standardized gastrointestinal model (Infogest). To understand the possible effect of the films and the microparticles on FUC's bioavailability, INFOGEST model was performed with free FUC, encapsulated FUC and film with the encapsulated FUC. After infogest, the samples were submitted to a membrane dialysis, to evaluate, in the fraction released to the bloodstream, FUC's content, anti-hypertensive, anti-diabetic, anti-inflammatory and antioxidant potential. Furthermore, cytotoxicity assays were performed to guarantee the product's safety.

The obtained results showed that FUC has interesting bioactive properties, that may be promising for application in the brain field. Furthermore, both the films and the encapsulation system showed appealing results, allowing a slower FUC release and guaranteeing that we have enough FUC content in the fraction released into the bloodstream, and also bioactive activity on that fraction. At last, the cytotoxicity assay allowed us to confirm the FUC's dosage for a safe oral intake.

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Acknowledgments

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Keywords : Bioactive compounds, Sustainability, New molecules, Brain benefits, Macroalgae

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(22778) - EFFECTS OF DIFFERENT PROCESSING TECHNIQUES ON OXIDATION DURING MEALWORM PROTEIN FRACTIONATION AND SIMULATED GASTROINTESTINAL DIGESTION

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Abstract

Alternative protein sources are increasingly in demand to replace, at least in part, meat in the human diet. Commonly, these alternative protein sources are of vegetable origin, but could also include protein from insect, such as mealworm (*Tenebrio molitor).* Several processing steps are required to extract and purify mealworm protein from its original matrix. (Little is known how the oxidative quality of mealworm is affected by the killing and drying method, protein extraction, and subsequent gastrointestinal digestion.

In this study, mealworms were subjected to two different killing methods: blast-freezing (-38°C, 4h) or blanching (100°C, 40s), and two different drying methods: oven drying (65°C, 3d) or freeze-drying (-20°C, 4d), and then ground into four different full-fat flours. These flours were then defatted with ethyl acetate (1:3) to produce four defatted flours and finally subjected to isoelectric point precipitation to produce four different protein concentrates. This way, a total of twelve samples were obtained and subjected to in vitro digestion, simulating the conditions from the mouth to the small intestine. HPLC-FLD was used to measure the levels of lipid oxidation products (hexanal, propanal, and 4-hydroxy-2-nonenal) and the glycoxidation product pentosidine. In addition, total Maillard reaction products (MRPs) were extracted using methanol and determined spectrophotometrically.

The different processing and extraction steps had a clear effect on the levels of oxidation products found in the mealworm extracts and after their in vitro digestion. Freeze-drying promoted lipid oxidation in full-fat flours and increased further during in vitro digestion. In oven-dried samples, the amount of lipid oxidation products was almost absent. All defatted flours, independent of prior treatment, showed relatively very low amounts of lipid oxidation products, which were probably lost during defatting and did not increase after digestion. In the protein concentrates, the blanched samples showed an increase in lipid oxidation products, however the blast-frozen samples did not. Compared to the other treatments, relatively high levels of pentosidine and MRPs were formed in all the blast-frozen oven-dried samples, these were also present in the protein concentrate prepared by blast-freezing and freeze-drying. *In vitro* digestion did not increase the pentosidine concentration, but increased the amount of MRPs. Of interest, all samples with high glycoxidation concomitantly contained relatively low levels of lipid oxidation products.

In summary, varying processing techniques influenced the oxidative quality of mealworm protein extracts. Also, the study revealed a potential link between elevated glyc(o)xidation and reduced lipid oxidation during both processing and digestion.

Acknowledgments

This study was financially supported by VLAIO (HBC.2021.0546).

Keywords : Tenebrio molitor, In vitro digestion, Lipid oxidation, Glyc(o)xidation, Processing techniques

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(22783) - EVALUATION OF THE BIOACCESSIBILITY OF COCOA PRODUCTS WITH ENCAPSULATED FUNCTIONAL ADDITIVES THROUGH IN-VITRO DIGESTION STUDIES

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Abstract

Currently, there is a global trend not only to consume products that provide an experience but also to offer functional benefits to human health due to the need to increase the absorption of bioactive components. This study assessed the bioaccessibility of encapsulated functional additives in cocoa products. The addition of additives occurred during the production stages of grinding and tempering in cocoa foods, where each of the additives was incorporated: encapsulated high oleic palm oil dried by spray drying (PAPSD) and encapsulated high oleic palm oil dried by refractive window (PAPVR). When generating the functional food, the lipid profile was determined (GC-FID following the AOCS Official Method CE2-66 and Ce 1-62 standard methods), natural vitamin E (tocopherols and tocotrienols), and carotenoids (HPLC/FLD/DAD following AOCS Official Method Ce 8-89), before and after simulated digestive process using a study in an *in-vitro* INFOGEST. The results showed that cocoa products with the inclusion of additives retained over 90% of bioactive compounds after processing stages, compared to theoretical concentrations, and exhibited a higher presence of these compounds than the control. The trend of gastrointestinal bioaccessibility is essentially influenced by the type of processing, with better absorption results for unsaturated fatty acids than saturated ones. This research highlights the potential use of encapsulated functional ingredients in cocoa matrices and their absorption of bioactive compounds, creating new opportunities to develop innovative products with added value to human health.

Finally, a key challenge in marketing innovative products lies in gaining acceptance from traditional consumers. Hence, an assessment of overall acceptance within a potential market niche, along with the declaration of functional claims, was conducted. The findings indicated that there were no significant differences between products incorporating encapsulates and those without.

References

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Keywords : Functional foods, Bioaccessibility, Cocoa, Bioactive compounds

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(22784) - MICROBOTS FOR COLONIC DELIVERY OF PHENETHYL ISOTHIOCYANATE (PEITC): USING INFOGEST TO OPTIMISE FORMULATION AND BIOACCESSIBILITY

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Abstract

Globally, gastrointestinal diseases impact 40% of the population and burden healthcare services. Inflammatory bowel disease (IBD) is a common disorder affecting over 6 million people worldwide, leading to significant morbidity and mortality[1]. Therefore, novel approaches are needed to control colonic diseases effectively with minimal side effects.

Using natural bioactive compounds such as isothiocyanates (ITCs) from cruciferous vegetables has shown promise in treating various diseases. Phenethyl isothiocyanate (PEITC), an ITC found in watercress, has potential bioactivities such as antioxidant, anti-inflammatory, and anticancer effects. Hence, PEITC shows potential as a natural therapeutic strategy for IBD due to its ability to alleviate inflammation and inhibit the growth of harmful intestinal bacteria without harming healthy gut microorganisms[2]. However, further research is necessary to establish PEITC's therapeutic potential for IBD and its impact on gut microbiota and overall intestinal health.

Encapsulation technology, crucial for enhancing PEITC's effectiveness and bioavailability in food or nutraceutical matrices, offers promising therapeutic and preventive applications in IBD treatment. Colon-targeted delivery systems are highly effective, especially those activated by gut microbiota. These systems use microbiota and enzymes like β-glycosidase to release drugs in the colon, a strategy ideal for PEITC delivery given its poor water solubility and instability [3].

This research aims to develop a microbot for targeted PEITC delivery to the inflamed colon. It will assess the interaction with gut microbiota and the effectiveness of target functionalities, including antioxidant and anti-inflammatory properties. The delivery system will feature a biodegradable polysaccharide coating, ensuring precise colon targeting. The INFOGEST protocol will simulate human gastrointestinal digestion *in vitro*, being a valuable tool for optimising the microbot's formulation and evaluating PEITC bioaccessibility. This preliminary stage will guide the enhancement of microbot design for subsequent *in vivo* testing.

Thus, developing microbots for oral administration will provide practical solutions for preventing and controlling gastrointestinal diseases, particularly IBD, with minimal impact on the patient's quality of life.

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Acknowledgments

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Keywords : Phenylethyl isothiocyanate (PEITC), Microbots, INFOGEST protocol, Gut microbiota interaction, Colon-targeted drug delivery systems

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(22785) - BIOACCESSIBILITY OF NOVEL BIOACTIVE PEPTIDES FROM THE BODY MUCUS OF THE LUSITANIAN TOADFISH HALOBATRACHUS DIDACTYLUS USING AN IN VITRO DIGESTION MODEL

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Abstract

The bioprospection of marine resources for drug discovery is receiving increasing attention (1). Adverse marine environmental conditions lead organisms to develop a collection of bioactive molecules for survival (1). Mucus, acting as a first line of defense against pathogens (2), is known to protect fish from the surrounding environment. Our previous studies have already demonstrated the bioactive potential of body mucus from the Lusitanian toadfish Halobatrachus didactylus. We performed LC-MS/MS to identify potential peptides within the mucus peptide fraction, selecting them based on in silico predictions of their bioactivities. It was important to assess the capacity of our bioactive peptides to resist the gastrointestinal tract and cross the intestinal epithelial barrier, thereby confirming their possible applicability as health potentiators (3). In this study, two peptides coded HdLPN (sequence PFPGPLPN) and HdVLPN (sequence VYPFPGPLPN) were submitted to an in vitro digestion model using the protocol INFOGEST 2.0. The digested content from the dialysis process after gastrointestinal simulation, both permeate and retentate using 3.5 kDa membranes, were evaluated in vitro for their antioxidant activity through ORAC assay, and the permeate for antihypertensive potential through inhibition of angiotensin-converting enzyme (iACE). The antioxidant activity of the two digested peptides, in both permeate and retentate forms, was comparable to the control (which utilized water in place of peptides); similar results were obtained for their antihypertensive activity in the permeate form. The results for both bioactivities showed no significant differences when comparing the digested peptides' retentate and permeate forms with control. This suggests that the enzymatic hydrolysis occurring during digestion degrades the bioactivity of these peptides, as they exhibited antioxidant activity prior to digestion (HdLPN 0.20±0.02 µmol Eq. Trolox/ mg peptide and HdVLPN 1.51±0.07 µmol Eq. Trolox/ mg peptide), and there was no subsequent activation or potentiation related to antihypertensive activity, which was also absent before digestion. This research primarily focused on evaluating how digestion affects bioactive peptides derived from the mucus of H. didactylus. However, comprehensive analysis, including mass spectrometry, is essential to fully understand the impact of digestion on the hydrolysis of these peptides. A forthcoming study could explore biocompatible materials for safe delivery methods of peptides, e.g. encapsulation, enabling them to withstand gastrointestinal digestion and effectively reach target organs to exert their intended bioactivity (3).

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Acknowledgments

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Development Fund (FDCT), project reference 0005/2019/APJ" and the scientific collaboration of CBQF under the FCT - Fundação para a Ciência e Tecnologia project UIDB/Multi/50016/2020.

Keywords : fish mucus, bioactive peptides, antioxidant, antihypertensive, bioaccessibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22786) - UNRAVELLING CAROTENOID DIGESTION: INSIGHTS INTO BIOACCESSIBILITY AND FUNCTIONAL PROPERTIES

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Abstract

Carotenoids, vital lipid-soluble compounds essential for human health (1), face bioaccessibility challenges during digestion due to resistance and degradation within protein complexes and plant cell walls (2). This complexity is influenced by factors like dietary sources, seasonal variations, food composition, matrix structure, lipid presence, dosage, and absorption rate (3). Understanding the delicate interplay of these elements is crucial for unlocking the full potential of carotenoids in promoting human health. To explore the impact of digestion on carotenoid stability and functionality, beta(β)-carotene, lutein, lycopene, a mixture of these three carotenoids, and *Osmundea pinnatifida* underwent simulated gastrointestinal digestion, using the INFOGEST methodology, and absorption (dialysis membrane with 3 kDa) and were evaluated in terms of functional properties. The results revealed distinct transformations during *in vitro* gastrointestinal simulation, with the generation of carotenoids different from the initial sample (e.g., β-cryptoxanthin), emphasizing the intricate changes carotenoids undergo. Recovery indexes highlighted the challenge of retrieving carotenoids during digestion (< 0.5%), emphasizing the complexity of their fate in the digestive process. The absence of detected or identified carotenoids in *O. pinnatifida* by HPLC analysis suggests that, within a complex matrix like algae, the bioaccessibility of carotenoids may be significantly compromised, requiring extraction methods to release these pigments and isolate them effectively. UPLC-qTOF MS analysis provided detailed fragment patterns, revealing variable relationships among fragments across different gastrointestinal phases. Functional property assessment showcased notable antioxidant (~17.5 µM TE) and anti-diabetic (7.6 – 97 % inhibition) activities in the tested carotenoid solutions. The Alga and the β-carotene groups displayed the highest values in absorbed fractions, revealing their effectiveness. Additionally, all carotenoid samples exhibited antimutagenic effects regardless of concentration, with no observed cytotoxicity except at higher concentrations. This study provides valuable insights for optimizing carotenoid utilization and realizing their multifaceted health benefits. Despite bioaccessibility challenges, understanding these complexities contributes to unlocking the full potential of carotenoids for human health.

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Acknowledgments

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Keywords : natural pigments, gastrointestinal digestion, bioaccessibility, metabolites

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22789) - SAPONINS AND PHYTOSTEROLS DECREASE BIOACCESSIBILITY OF LUTEIN AND Γ-TOCOPHEROL DURING IN VITRO DIGESTION

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Abstract

Introduction: The grains of the pseudocereal quinoa (*Chenopodium quinoa* Willd.) contain higher amounts of iron, calcium, magnesium and zinc, and the fat-soluble vitamin α-tocopherol and β-carotene than rice, barley, and wheat. Quinoa seeds differ in their amounts of bitter tasting saponins and are classified into sweet and bitter varieties, which furthermore differ in their content of phytosterols. The aim of the present study was to investigate if different concentrations of saponins and phytosterols impact the stability, solubility, and bioaccessibility of lutein and γ-tocopherol in quinoa during in vitro digestion.

Methods: Based on literature and unpublished data from our laboratory, a quinoa model composition of the compounds of interest was prepared to mimic the amounts present in a 90 g serving of quinoa. Lutein (1.44 µg/mL), γ-tocopherol (12.24 µg/mL), iron (64.8 µg/mL), zinc (21.6 µg/mL), oleic and linoleic acid were combined and in vitro digested in the presence of a medium concentration (720 µg/mL) of β-sitosterol and increasing amounts of saponins (no, low (72 µg/mL), medium (3816 µg/mL), and high (6336 µg/mL)). In another set of experiments, the nutrients were digested in the presence of increasing concentrations of β-sitosterol (no, low (288 µg/mL) and high (1296 µg/mL)), while saponins were kept constant at a medium concentration.

The in vitro digestion was performed according to the INFOGEST 2 protocol. Concentrations of lipid-soluble compounds were quantified by HPLC.

Results: The bioaccessibility of lutein was higher than that of γ-tocopherol, although both were low (0.006 – 3.3 %). Increasing concentrations of saponins and β-sitosterol, in the presence of constant concentrations of the respective other compound, dose-dependently reduced the bioaccessibility of lutein and γ-tocopherol. Digestive stability, solubility and bioaccessbility of lutein and γ-tocopherol were lowest at the highest concentration of β-sitosterol. The stability and solubility of both compounds were highest in the absence of saponins.

Conclusion: Phytosterols and, more strongly, saponins decrease the bioaccessibility of lutein and γ-tocopherol during in vitro digestion of a model mixture of nutrients reflecting one serving of quinoa. Hence, saponins and phytosterols in quinoa may impair the bioavailability of lipid-soluble nutrients, which warrants further investigation.

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Acknowledgments

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Keywords : saponins, phytosterols, fat-soluble vitamins

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22791) - A METAGENOMIC-DRIVEN BIOREFINERY SOLUTION IN AGRI-FOOD WASTE TO IMPROVE BIO-ACTIVE COMPOUND EXTRACTIONS AND BIO-AVAILABILITY

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Abstract

The biorefinery process consists of a suite of chemical techniques aimed at separating relevant compounds from biomass sources producing value-added products. Over the last years, these technologies have gained attention as integrated into sustainable and bioeconomy approaches to valorize agri-food residues.

The present work represents a step-forward in the biorefinery field, by incorporating a metagenomic characterization of biological fractions of agri-food waste to improve extraction and enhance bioavailability of bio-active compounds.

The structure of bacterial communities of the lignocellulosic fractions of different vegetable residues (stem, leaves, roots, etc.) was characterized by a metagenomic approach, specifically the 16S rRNA (for bacteria) and 18S rRNA (for fungi) amplicon-based sequencing methods. The metagenomes will be used to identify bacterial and/or fungal genes encoding hydrolytic enzymes associated with an improvement of the bioconversion or biodegradation processes (e.g., cellulase, xylanase, ellagitannin acyl hydrolase). Then, at laboratory scale, biodegradation processes were promoted by applying suitable environmental conditions and/or adding functional microorganisms isolated from the same vegetable biomass based on the metagenomic results. Then, different extraction processes were applied (microwave-based extraction, infusion, decoction and Soxhlet). The antimicrobial capacity (agar-diffusion method), polyphenol content (Folan Ciocalteu) and the antioxidant potential (DPPH and ABTS) were quantified for both treatment samples and controls. The bio-accessibility of the extracted compounds was evaluated following standard methods. Results evidenced that microbiology-based biorefinery processes might improve both high-value compounds extraction and bio-availability of thereof, but, likewise, the assays were subjected to relevant sources of variability, reducing the reproducibility and robustness of the method. Nevertheless, the results are promising and pave the way for novel approaches in biorefinery. They offer customized methods that not only enhance the extraction of bio-active compounds but also improve their bioavailability, thereby linking biorefinery processes directly to dietary benefits.

Acknowledgments

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Keywords : Polyphenols, Cazymes, Bioconversion, Food waste, Bioaccessibility

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22792) - COMPARATIVE STUDY OF BRAZILIAN AND SPANISH RED FRUITS BIOACTIVITY BEFORE AND AFTER IN VITRO DIGESTION

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Abstract

Red fruit consumption can bring several benefits to human health due to its bioactive content, especially phenolic compounds. Pitanga (*Eugenia uniflora*), is a Brazilian native fruit rich in phenolic compounds and is as savory and fragrant as cherries. Cherries (*Prunus avium*) are also rich in phenolic compounds and are well consumed worldwide. However, there are several challenges to growing cherries in many places due to their environmental requirements. The discovery of novel red fruits from autochthone plants is a sustainable and interesting alternative. Bioactive compounds found in red fruits have several biological benefits, especially antioxidant activity. Their effectiveness on human health remains uncertain, so *in vitro* digestion assays to evaluate their bioaccessibility and bioactivity might contribute to elucidating them. Cherries from a local supermarket in Madrid, Spain, and Pitanga from orchards in Campinas, Brazil were collected, washed, pulped (without seeds) and freeze dried. Phenolic extracts of each fruit were also obtained to test the fruit matrix effect in their bioaccessibility. The *in vitro* digestion assays were performed using the INFOGEST 2.0 protocol version. Digested samples were centrifuged to obtain the bioaccessible fraction, which was freeze dried for analysis. The QUENCHER methodology (Q) (Del Pino-Garcia et al., 2015) that allows a direct reaction, using very little sample, and generating less waste, was used to determine flavonoids, anthocyanins, and total polyphenols content. The results have shown that Pitanga presented higher concentrations of flavonoids and polyphenols, while anthocyanins were similar in both fruits. Digested samples had lower amounts of anthocyanins and polyphenols for both fruits and their extracts. Only flavonoids presented higher content in digested samples. The bioaccessibility of anthocyanins, flavonoids and polyphenols was significantly different in pulps and extracts. *In vitro* digestion coupled with QUENCHER methods is an interesting approach to evaluate the impact of the food matrix on red fruits bioactivity.

References

Del Pino-García et al., 2015.

Keywords : pitanga, cherries, QUENCHER, INFOGEST, phenolic compounds

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22793) - THE BIOAVAILABILITY OF (6S)-5-METHYLTETRAHYDROFOLATE VERSUS FOLIC ACID IN INFANTS DIFFERS ACCORDING TO STARTING BLOOD FOLATE CONCENTRATIONS

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Abstract

Background: Folates play an important role during infant development and growth. In human milk the predominant folate form includes (6S)-5-methyltetrahydrofolate (5-MTHF). It is a methyl donor in one-carbon metabolism and a source of purine and thymidylate that are required for division and differentiation of all rapidly growing cells in the body such as red blood cells. Feeding infants an infant formula with the calcium salt of 5-MTHF (5-MTHF-Ca) or folic acid, resulted in higher whole blood folate levels in the 5-MTHF-Ca group (Troesch et al. 2019). **Objective:** In an exploratory analysis, we aimed to study the comparative bioavailability of 5-MTHF-Ca and folic acid in formula fed infants. **Method:** The study was conducted in Serbia and the analysis included 167 infants, receiving infant formula with 15.8 µg 5-MTHF-Ca or 15.2 µg folic acid per 100 kcal from the age of < 1 month (baseline visit) until 16 weeks (visit 4, V4). The change in red blood cell (RBC)-folate concentrations between baseline visit and V4 was calculated. The ratio of the mean change in RBC-folate in the 5-MTHF-Ca group to that in the folic acid group (a surrogate marker of bioavailability) was studied according to quartiles of baseline RBC-folate concentrations. **Results:** In infants with low baseline RBC-folate levels (first quartile Q1 < 876 nmol/L), the bioavailability was equal for 5-MTHF-Ca und folic acid [ratio of the mean change of RBC-folate and ratio of the 5^{th} and 95^{th} confidence intervals of this mean change were = 0.98 (0.94, 1.01)]. Among the upper three quartiles of baseline RBC-folate concentrations, the mean change in RBC-folate was higher in the 5-MTHF-Ca group relative to that in the folic acid group, therefore the ratio of the mean change was 1.15 in Q2, 1.43 in Q3, and 1.96 in Q4 of baseline RBC-folate concentrations. **Conclusion:** Overall, 5-MTHF-Ca showed a 31% higher bioavailability than folic acid. In infants with low baseline folate status, the two folate forms were equally bioavailable. Thus, both forms can ensure folate uptake in infants with low folate levels. With increasing baseline RBC-folate concentrations, the bioavailability of folic acid declined progressively relative to 5-MTHF-Ca. Possible reasons for the decline in bioavailability of folic acid at higher baseline folate status are discussed, but this topic will require further investigations.

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Keywords : folate, (6S)-5-methyltetrahydrofolate, bioavailability, red blood cell folate

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22794) - SIMULATED DIGESTION OF NEW BREAKFAST CEREALS RICH IN FIBRE AND BIOACCESSIBILITY OF BIOACTIVE COMPOUNDS AND THEIR POTENTIAL HEALTH BENEFITS

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Abstract

The main goal of this work was to evaluate the digestion rate of new breakfast cereals rich in fibre, the bioaccessibility of bioactive compounds and bioactivities of these food products. The breakfast cereals were developed using only two ingredients: wheat bran and carrot or apple by-products flour. After the bran and flour mixing, and hydration the dough was let to stabilize at 4 °C overnight and then extruded using a cold extrusion equipment. The extrudates were then dried at 50 °C until moisture below 7% and then roasted at 180 °C for 4 minutes in a circulating air oven. The *in vitro* digestion simulation was proceeded following the the standardized static digestion model INFOGEST 2.0 protocol (Brodkorb et al., 2019) and a commercial control was used for comparison (All-bran Plus® from Kellog's). After digestion, the simulation of the absorption from small intestine into the bloodstream was performed using a dialysis tubing (3.5 kDa molecular wight cut-off).

For each stage of the digestion and dialysis (retentate and bloodstream fractions), it was evaluated free sugars, total phenolic content (TPC), total flavonoids content (TFC), antioxidant activity (ABTS and ORAC methods). Antidiabetic and anti-hypertensive activities were also evaluated in the intestinal stage of digestion and in bloodstream fraction after dialysis.

The results showed that free sugar had a low absorption rate into bloodstream (18-24% of the free sugars in intestinal stage were transferred into the bloodstream fraction of the dialysis). The antidiabetic activity was higher for the formulations with higher content in carrot or apple flour comparing to formulations with higher wheat bran content. Comparing carrot and apple formulations, carrot formulations presented the highest antidiabetic activity with 68% and 46% α-Glucosidase inhibition for formulation with 70:30 carrot flour/wheat bran and 40:60 carrot flour/wheat bran respectively. Antioxidant activity was maintained throughout the GIT but not in the bloodstream fraction, and the results align with the TPC and TFC. The anti-hypertensive capacity of the samples was maintained after GIT (comparing the results of the analysis between oral and bloodstream aliquots) for all samples except the sample with higher carrot flour content, where there was a loss of approximately 50% of this activity.

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Keywords : apple by-products, carrot by-products, breakfast cereals, wheat bran

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22797) - INFLUENCE OF PROTEIN FRACTIONS ON THE TRACE ELEMENT BIOACCESSIBILITY OF TURNIP TOPS (BRASSICA RAPA) GROWING UNDER MEDITERRANEAN CONDITIONS

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Abstract

Vegetable species belonging to the *Brassicaceae (*formerly *Cruciferae*) family are some of the most economically important plant groups for humans. A previous study [1] has shown that unlike other green leafy vegetables (such as spinach or Swiss chard), the trace elements bioaccessibility present in cruciferous vegetables is high, similar in some cases to that of powdered milk. This is due to a low content of some anti-nutritional compounds such as oxalates. *Brassica rapa L.* is another species belonging to the *Brassicaceae* family. In northwest Spain and Portugal, there has been a long tradition of cultivating *B. rapa subsp. rapa* to obtain turnip tops [2].

The aim of this work was to study the influence of three protein fractions (casein, lactalbumin and soy) on the trace elements bioaccessibility (Fe, Mn, Ni, Se and Zn) of turnip growing under Mediterranean conditions. The purpose was the use of this vegetable not only for direct fresh consumption but also as a main ingredient in the development of food mixtures. The assays were conducted with different protein fractions (casein, lactalbumin and soy), increasing amounts of the aforementioned compounds to represent 5, 15 and 25% in the final mixture.

The influence of casein on the bioaccessibility of Fe and Mn in turnip tops was negligible. In the case of Zn, the improvement in its bioaccessibility was only effective as from the highest dose (25%). Caseins had no effect on Mn bioaccessibility either, which is somewhat justifiable considering the low Mn content in milk. On the other hand, it was highly obvious that these proteins caused an important increase in the Se bioaccessibility. This was noted to be from 18% (control) to 96% as from the lowest dose (5%).

The effect of lactalbumin on the trace elements bioaccessibility present in turnip tops was very similar to that found for casein. There was none for Fe, Mn and Zn; a slightly decrease for Ni and a remarkable improvement in Se bioaccessibility (reaching 85%) as from the lowest protein dose (5%)

Finally, unlike the two previous protein fractions, soy protein improved the bioaccessibility of most of the trace elements studied (with the exception of Ni for which no effect was observed). The bioaccessibility percentages increased from 32% to 93% for Fe; from 50% to 88% for Mn; from 17% to 100% for Se; and from 36% to 100% for Zn.

The results obtained here can be taken into consideration when selecting different ingredients for formulating new foods developed from cruciferous vegetables.

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Acknowledgments

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Keywords : Caseins, Bio-availability, Mediterranean foods

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22802) - IMPACT OF IN VITRO GASTROINTESTINAL DIGESTION ON UPCYCLED BLACKCURRANT DRIED EXTRACT: ANTHOCYANINS PROFILE AND ANTIOXIDANT ACTIVITY BEHAVIOR

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Abstract

Pomaces, the major by-product of fruit juice processing industries, is rich in bioactive compounds. Among them, polyphenols boost the body's antioxidant capacity, improving cardiovascular health, reducing the risk of diabetes and inflammation, and promoting intestinal microbiota health. Blackcurrant (*Ribes nigrum L.*) is a highly antioxidant berry rich in anthocyanins, a polyphenols class that also gives the fruit a black-purple color. Thus, blackcurrant pomace polyphenols have the potential to be functional food ingredients that can enhance sustainability in the agri-food processing chain with health benefits. In order to have a beneficial effect on health, polyphenols must be bioaccessible. This means they must be released from the food matrix during gastrointestinal digestion (GID) and available for absorption in the gut [1].

This study aimed to assess the bioaccessibility of anthocyanins and the antioxidant activity of polyphenolic extract from blackcurrant pomace. For this purpose, an enzymatic method was used to release the polyphenols from the pomace, which were then spray-dried. The INFOGEST 2.0 protocol was used to simulate the *in vitro* GID of the powder. The anthocyanins profile (HPLC-DAD), total phenolic content (TPC, Folin-Ciocalteu method), antioxidant capacity (ABTS, DPPH), and cytotoxicity (PrestoBlue assay) were evaluated.

The blackcurrant powder extract initially contained over 900 mg/L of total anthocyanins. The major compounds are cyanidin-3-O-glucoside (54%), pelargonidin-3-O-glucoside (19%), and delphinidin-3-O-glucoside (18%). The anthocyanins remained stable during the gastric phase of GID, with a full recovery index of 20% and 12% for cyanidin-3-O-glucoside. Still, they drastically decreased in the intestinal stage due to a pH change that caused a break in the anthocyanin B-ring [2]. The TPC of the extract significantly reduced during the oral and gastric phases but increased slightly during the intestinal phase, with a 19% recovery index. The extract's antioxidant activity decreased, resulting in a bioaccessibility index of 19% and 23% for ABTS and DPPH scavenging activity, respectively. Despite a decrease in TPC along the GID, the extract still exhibited antioxidant capacity due to the existence of various phenolic compounds, specifically hydroxybenzoic and hydroxycinnamic acids, resulting from the degradation/transformation of anthocyanins [3]. A 2.5% (w/v) of antioxidant extract powder is safe for food formulations.

Thus, this work provides insights into the effects of GID on anthocyanins and the potential use of blackcurrant pomace as a source of bioactive ingredients, promoting a circular economy.

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Acknowledgments

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Keywords : Blackcurrant Pomace, Anthocyanins, Gastrointestinal Digestion, Bioaccessibility, Antioxidant Activity

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22820) - THE INFLUENCE OF TEA PREPARATION ON GANODERMA LUCIDUM'S TRITERPENE BIOACCESSIBILITY

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Abstract

Ganoderma lucidum a historically significant fungi in traditional medicine, is widely consumed for its health-promoting properties, with the benefits being attributed to its secondary metabolites. While extensive research has focused on the chemical characterization of *G. lucidum* metabolites, the fraction of these compounds bioaccessible post-ingestion of its preparations remains largely unexplored. This study aimed to assess the *in vitro* bioaccessibility of triterpenes in teas (infusions and decoctions) prepared with the outer peel of *G. lucidum* fruiting bodies. Dried powder samples of mushroom provided by Käapa Biotech (Finland) were homogenised to prepare the infusions (5 min in contact with 100°C water, 1:100 solid/liquid ratio) and decoctions (5 min boiling with water 1:50 solid/liquid ratio) that were subjected to *in vitro* digestion using the internationally recognized INFOGEST protocol. Extracts and bioaccessible fractions were cleaned and concentrated by SPE and analysed by HPLC-DAD-(ESI-)MS/MS. Results indicated that the total triterpenoid content was higher in decoctions than in infusions (163.6±3.5 and 64.7±1.3 µg/mL of preparation, respectively), likely due to the higher mushroom-to-water ratio in decoctions. Interestingly, the overall relative bioaccessibility of terpenes was greater in infusions (36% on average, $p<0.05$). Specifically, lucidenic acid A, the second major compound in infusions, showed the highest bioaccessibility (83%) in this preparation, whereas 54% of the content of the most abundant peak composed by coeluted lucidenic acid A and ganoderlactone B was bioaccessible. In contrast, in decoctions, these two respective peaks were only 12% and 3% bioaccessible. These findings suggest that teas made from *G. lucidum* could be an effective way to ingest its bioactive compounds, with the method of preparation significantly influencing compound bioaccessibility. This study underscores the importance of assessing terpene bioaccessibility in *G. lucidum* through various consumption methods, providing insights into their potential health benefits.

Acknowledgments

The authors thank the FCT (Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020), SusTEC (LA/P/0007/2021), and the institutional scientific employment program-contract with L. Barros, besides the European Regional Development Fund (ERDF) and PRR, within the scope of VIIAFOOD Agenda (n.º C644929456-00000040) for the contract of D.B.Rodrigues.

Keywords : Reishi, in vitro digestion, infusion, decoction, LC-MS

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22860) - INFLUENCE OF COOKING TECHNOLOGIES ON THE BIOACCESSIBILITY OF NUTRITIONAL COMPOUNDS IN PLANT- BASED FOOD

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Abstract

Phytochemicals such as phenols, carotenoids, flavonoids and glucosinolates have demonstrated antioxidant, antibacterial, antiviral activities, enzyme modulation and immune system stimulation, preventing some human chronic and neurodegenerative diseases. Bioaccessibility of these nutraceuticals in the human body depends on multiple factors including cooking methods. In this study tomato samples (*Solanum lycopersicum* L.) were cooked by different methods including blanching, concentration to produce tomato sauce and super-heated steam (SHS) as innovative cooking technology. The control was represented by raw tomato materials. Lycopene, the most important nutraceutical compound in tomato, reported a similar content in blanching and SHS methodologies when compared with the controls. Moreover, the sauce induced a decrease of lycopene likely due to the combination of high temperature and cooking time. A strong and significant increase in antioxidant activity performed by ABTS assay, was observed in tomato fruit cooked with SHS methodology. In general, the antioxidant molecules and activities were strongly reduced after *in vitro* digestion. Lycopene content showed a low bioaccessibility, especially after the SHS methodology which induced a reduction of the lycopene content by 99% as compared with control, blanched tomatoes, and tomato sauce. Conversely, ascorbic acid was higher retained after SHS methodology at the end of the digestion when compared with the other cooking treatments, with a bioaccessibility of 60%. The antioxidant activity showed higher values in SHS methodology and tomato sauce after digestion when compared to blanching methodology.

The work evidenced as the cooking technology influenced not only the content of antioxidant molecules but also their bioaccessibility at the human gastro-intestinal tract.

References

Acknowledgments

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Keywords : bioaccessibility, phytochemicals, plant-based food, in vitro human digestion, INFOGEST

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(22941) - HOW DOES IN VITRO DIGESTION CHANGE THE AMOUNT OF PHENOLICS IN MORUS ALBA L. PROCESSED LEAVES? ANALYSIS OF PREPARATIONS AND INFUSIONS

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Abstract

The application of *Morus alba* L. in traditional oriental medicine and cuisine has resulted in numerous studies on its health-promoting effects. However, if the process is not monitored by the manufacturers, the processing of the leaves alters the obtained health-promoting properties and results in different health qualities in the final composition of dietary supplements.

This research aims to analyze changes (using the HPLC/DAD method) in the proposed conditioned mulberry leaves in terms of key compounds (phenolic acids and flavonols) responsible for antioxidant activity after being digested in *in vitro* conditions. The analyzed material was leaves of white mulberry (*Morus alba* L.) cv. Żółwińska wielkolistna, conditioned (1–4 h) and non-conditioned. The conditioning process of mulberry proposed here, e.g., for industry production, resulted in variable transformations of polyphenols during *in vitro* digestion. For many polyphenols, especially those shown in the highest amounts, significant correlations were found between their content and conditioning, as well as the

stage of digestion. In the case of mulberry infusions, the amounts of individual polyphenols were several times lower than in the preparations, which was due to the degree of dilution. Their amounts tended to decrease in the course of digestion.

Taking this into account, it seems justified to continue research on the *in vivo* bioavailability of bioactive components from conditioned *Morus alba* L. leaves.

Acknowledgments

This work was supported by Poznań University of Life Sciences as the research program „First grant", no. 14/2022/Pierwszy grant, by statutory fundings of Department of Gastronomy Science and Functional Foods (PULS), and by project 00015.DDD.6509.00137.2022.15 'Innovative technology for producing frozen vegetable products with the addition of probiotics as an opportunity to improve the profitability of beetroot production and the competitiveness of farms', according to the WSPÓŁPRACA programme, M16, co-financed by the European Union under the Rural Development Program 2014PL06RDNP001 for 2014-2020.

Topic 2: Bioaccessibility/absorption of beneficial and harmful compounds | Poster

(23200) - ANTINUTRIENTS IN PROTEIN FRACTIONS DERIVED FROM PULSES: IMPACT OF DRY-FRACTIONATION AND COOKING

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Abstract

Pulses are a valuable, sustainable and cost-effective protein source worldwide. However, their protein quality can be hindered by the presence of antinutrients such as condensed tannins, trypsin inhibitors and saponins. The presence of antinutrients in the digestive tract reduces the absorption of amino acids and the bioavailability of nutrients. However, various processing technologies such as soaking and cooking can mitigate the content of antinutrients. Dehulling, air classification, and cooking can be applied to pulses, which can have an impact on the chemical, physicochemical, and nutritional properties of the final ingredients. Understanding the distribution of antinutrients during dry fractionation is crucial for optimizing the use of pulses for protein production.

In the present study the protein concentrates derived from yellow peas and faba beans, obtained by dry-fractionation [1], and their corresponding raw materials were characterized for their condensed tannins content, trypsin inhibition activity and total saponin content, following spectrophotometric assays.

Results indicate that trypsin inhibitors are concentrated in the protein fraction. Moreover, the respective cooked raw materials and fractions had a 58-86% reduction in trypsin inhibitors. Regarding the condensed tannin content, the dehulling step is not needed to remove these components when the interest is focused on a low-tannin protein concentrate. Moreover, the saponin content of whole and dehulled peas and faba beans decreased upon cooking. Overall, regardless of the pulse species, the reduction of ANFs upon dry fractionation and cooking followed the same trend. The knowledge generated in this work is a step forward towards the production of protein-rich fractions with improved quality.

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Acknowledgments

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Keywords : yellow peas, faba beans, dry fractionation, cooking, antinutrients

ICFD 2024

TOPIC 3

BIOACCESSIBILITY/ABSORPTION OF BENEFICIAL AND HARMFUL COMPOUNDS

Portugal | April 9-11th, 2024

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21287) - IN SILICO AND IN VITRO PEPTIDE BIOACTIVITY FROM DIGESTED POTATO PROTEIN ISOLATE STRUCTURES

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1 - Aarhus University

Abstract

Predicted *in* silico and *in vitro* peptide bioactivity from simulated gastrointestinal digestion of different food matrix structures from patatin rich-potato protein isolate

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Keywords: potato protein isolate, digestion efficiency, bioactive peptides, timsTOF LC-MS, heat treatment, gastric conditions, peptidomics

Potato protein isolate has gained attention as a promising protein source due to its nutritional profile and functional properties. However, the influence of protein structure and processing history on bioactive peptides derived from this source, remain largely unexplored. We investigated the digestion of four structures: suspension, foam, gel, and heated foam, under varying gastrointestinal conditions using a commercial potato protein isolate. A semi-dynamic *in vitro* digestion model was utilized and digestion products were analyzed using nanoLC-MS/MS to unveil peptide profiles.

In silico bioactivity assessment involved matching the obtained peptide sequences against a database of potato proteins obtained from uniprot.org. We identified specific peptides with potential health benefits, such antioxidant and angiotensin-converting enzyme inhibitory activities. Additionally, ACE-inhibitory and antioxidant free radical *in vitro* activity assays were performed.

Our results revealed that the initial protein structure significantly influenced digestion efficiency and peptide distribution. The heated-foam structure had the highest degree of hydrolysis (96.6±4.4%), which was 13.2% higher than the gel and 20.1% higher than the suspension and foam. Peptide APIYFPPH (93% predicted *in silico* bioactivity) was detected in the suspension, foam and gel at the early stages of intestinal digestion. While a shorter peptide PIYFPPH (91% predicted *in silico* bioactivity) was found at the last intestinal point for all the structures.

Moreover, the gel had a significantly higher ($p<0.05$) DPPH radical scavenging activity (19.6%±2.8) than suspension (11.3±5.0%), foam (6.8±0.6%) and heated foam (2.5±1.8%). Contrarily, the gel had a significantly lower *in vitro* ACE-inhibitory activity (15.3±4.7%) when compared to the foam (58.4±2.2%)

Heat treatment enhanced protein digestibility, leading to distinct peptide patterns and distributions compared to non-heat treated structures. This study confirmed the presence of bioactive peptides from the digesta of potato protein isolates structures by using *in vitro* and *in silico* approached to evaluate ACE- inhibitory and antioxidant activity.

References

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Keywords : potato protein isolate, bioactive peptide, peptidomics, timsTOF LC-MS, ACE-Inhibition

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21396) - EFFECTS OF FOOD PHENOLICS ON DIGESTIVE PROTEASES BY IN VITRO AND IN SILICO APPROACHES

Borgonovi, Sara Margherita (Italy)[1]; Perugino, Florinda (Italy)[2]; Pedroni, Lorenzo (Italy)[2]; Dellafiora, Luca (Italy)[2]; Pinto, Andrea (Italy)[1]; Dallavalle, Sabrina (Italy)[1]; Iametti, Stefania (Italy)[1]; Di Nunzio, Mattia (Italy)[1]

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Abstract

The interactions between various food ingredients and digestive enzymes can alter how food nutrients are absorbed and have an impact on an individual's health. Phenols and polyphenols (PPs) are effective in treating certain chronic diseases and play a function in metabolic regulation. The majority of phenolic compounds (PPs) that are consumed remain in the gastrointestinal tract, where they can have a variety of advantageous effects because phenolic compounds are generally poorly absorbed following food intake. Because they have the purported ability to suppress proteolytic digesting enzymes, PPs are commonly referred to be anti-nutritional factors in this context. The fact that reports on this subject frequently contradict one another, mostly as a result of various experimental setups, emphasizes the necessity of using consistent methodologies to assess the impact of PPs. Using albumin, gluten, and hemoglobin as substrates, the effects of several PPs (at physiological concentrations) were evaluated "in vitro" on the activities of pepsin, trypsin, and chymotrypsin. Results show that PPs may affect proteolytic activity in opposite ways, depending on the protein substrate and the enzyme. Therefore, a computational approach based on molecular docking and molecular dynamics simulations has been applied to investigate the interactions polyphenols may have with the enzymes and substrates. The analysis focused on the chymotripsin-ovalbumin system as a proof of concept to provide a mechanistic explanation for PPs opposite behavior in affecting proteolytic activity described *in vitro*. Results show that all the PPs under investigation can interact both with the enzyme and the substrate. However, it is interesting to note that only the PPs inhibiting the enzyme *in vitro* could induce structure modification on the substrate which could promote its partial denaturation. According to these results, a substrate-dependent inhibition can be hypothesized to explain opposite ways of PPs to affect protein digestion. The evidence gathered here suggests the possibility of considering some PPs as “digestion-promoting agents” in the formulation of functional foods.

Acknowledgments

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Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21399) - PROCESSING OF RAW DONKEY MILK; EFFECT ON PROTEIN QUALITY AND BIOACTIVE PROPERTIES

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Abstract

Non-thermal processing of milk has been considered over the past decade as an alternative or adjunct to thermal processes. UV-C radiation in combination with turbulent flow of opaque liquids seems to be a promising non-thermal method for the reduction of bacterial populations in milk. Apart from confirming the efficacy of UV-C in destroying pathogens and spoilage bacteria, there is a need for assessment of the quality characteristics of the end-product and especially in added-value dairy products where bioactivity of constituents should be preserved during processing. Under this context, freeze-dried donkey milk powder processing by UV-C was studied and the effect on protein quality, digestibility and bioactive properties were assessed after in vitro digestion. Results show that UV-C treatment retains the protein's quality characteristics highly comparable to the not-treated milk (i.e. raw) rather than the pasteurized milk where some deterioration (i.e. lower bioactivities) was detected.

Keywords : Donkey milk, Bioactivity, Non-thermal processing, UV-C,Protein functionality

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21404) - ROBUST AND QUANTITATIVE PEPTIDE ANALYSIS TO GAIN INSIGHT INTO THE DIGESTIVE ENZYMES

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Abstract

Samples from *in vivo* and *in vitro* protein digestion are often characterized for their peptide composition. LC-MS is widely used to determine the amino acid sequence of the peptides. But, there is currently no method to determine their concentrations. We filled this gap and developed a method to quantify peptides. The method uses the UV-absorbance at 214 nm and sequence dependent extinction coefficients to calculate absolute concentrations of individual peptides. The benefit of this technique is that it avoids (isotopic) labelling treatments and does not completely rely on MS-intensity. Furthermore, the method is highly reproducible (≤10 % relative standard deviation on individual peptide concentrations). In recent years, the data-processing routine for peptide identification was carefully automated, enabling fast and robust characterization of hydrolysates. By analysis of different timepoints during *in vitro* hydrolysis, unprecedented insights were obtained on the digestive enzymes trypsin, chymotrypsin and pepsin. For trypsin, the bovine and human variant were hindered by charged amino acids whether the porcine variant was not. For chymotrypsin, the hydrolysis rates of individual bonds in the protein were analyzed to revise its specificity and preference. Moreover, hindrance was observed when proline occupied certain binding site positions. For pepsin, pH affected peptide concentrations during gastric digestion, but not the preference of pepsin towards certain amino acid residues. We think that UV-based peptide quantification is useful for other scientists that characterize peptides in digests.

Keywords : Peptide quantification, UPLC-PDA-MS, digestive enzymes, peptide release kinetics, subsite model

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21408) - EFFECT OF ISOFLAVONES AND PROBIOTICS ON CALCIUM BIOAVAILABILITY IN HUMAN SAOS-2 CELLS

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Abstract

A sufficient calcium intake is essential for bone health prevention and treatment. Developing a nutritional source of easily bioavailable calcium is especially important for people with a deficiency of this element and the risk of developing osteoporosis. The objective of this research endeavor was to assess the influence of tempeh (T), daidzein (D) and *Lactobacillus acidophilus* (LA) within a simulated intestinal environment of Caco-2 epithelium and Saos-2 cells, with a particular focus on their implications for bone mineralization mechanisms. During the initial stage, calcium bioaccessibility from calcium citrate (CaCt), LA, D, daidzein combination D:CaCt:LA (D1:1:1) and tempeh combination T:CaCt:LA (T1:1:1) was evaluated through digestion simulation. The calcium content of both untreated and digested samples was determined through the application of atomic absorption spectrometry (AAS). During the subsequent stage, the digested samples were utilized to induce intestinal absorption in differentiated enterocyte-like Caco-2 cells. The permeable fractions were then assessed in a culture of osteoblast-like Saos-2 cells. Preliminary cellular experiments involved the utilization of the MTT assay to assess cytotoxicity. The results suggested that the analyzed products did not influence the deposition of extracellular calcium in Saos-2 cells that were cultured in the absence of mineralization stimulators. The combined formulation of permeable fractions of digested CaCt, LA, D, and T has the capacity to augment the proliferation of Saos-2 cells. In Saos-2 cells, D, D1:1:1, and LA had no discernible impact on intracellular calcium accumulation, whereas T and T1:1:1 reduced calcium deposits. Furthermore, the mRNA transcripts and alkaline phosphatase (ALP) activity levels of Saos-2 cells cultured in the absence of mineralization induction were not impacted by the analyzed products. An examination of the analyzed products revealed that no discernible effect on ALP activity or mRNA expression during Saos-2 cell differentiation was absent. Our findings suggest that tempeh, daidzein, and *L. acidophilus* do not have a positive impact on cellular calcium deposition in Saos-2 cells. However, tempeh, daidzein and its combination, and *L. acidophilus* that were studied may increase the process of osteogenic differentiation in Saos-2 cells. Nevertheless, this study does not find any synergistic impact on calcium deposition and the process of osteogenic differentiation in Saos-2 cells between isoflavones and probiotics.

Acknowledgments

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Keywords : isoflavones, probiotics, tempeh, calcium, bioavailability

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21409) - CHARACTERISING THE DIGESTION OF WHEY PROTEIN ISOLATE USING OLDER ADULT GASTRIC CONDITIONS

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Abstract

Elderly malnutrition is widespread in all healthcare settings, with around 1 in 4 adult patients in hospital, and more than 1 in 3 patients in care homes being at risk of malnutrition. Older adults have an increased risk of suffering from malnutrition, with an associated reduced quality of life, poor health and increased disease occurrences. Undernutrition has negative health effects associated with it such as sarcopenia, osteoporosis, increased frailty, and a general increase in morbidity and mortality. Prevalence of malnutrition and undernutrition in the elderly is commonly treated using high-quality proteins, food-fortification and/or oral nutritional supplements. These supplements can potentially attenuate issues with muscle protein turnover in older adults and contribute to resolving bone health issues such as osteoporosis, although changes to the digestive system with increasing age can affect the availability of nutrients, especially protein, in this cohort.

Using current *in-vitro* digestion protocols, adaptations to replicate elderly digestive conditions were made to digest whey protein isolate (WPI). Changes to gastric pH and enzymatic quantity associated with increasing age including poor health effects were examined. Digestibility of WPI was characterised by Degree of Hydrolysis using OPA, peptide bioavailability using ultrafiltration methods, molecular weight distribution by SEC-HPLC, and LC-MS analysis. Significant differences in digestibility were observed, particularly due to gastric pH. Results indicate that the characterisation of a wide range of age-related gastric alterations showed valuable information related to the management of older adult nutrition.

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Acknowledgments

Irish Research Council and Tírlan:

Project ID: EPSPG/2022/382

Institute of Food and Health, University College Dublin

Keywords : in vitro digestion, elderly digestion

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21412) - IN VITRO DIGESTION OF METHYLATED LYSINE DERIVATIVES

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Abstract

As one of the canonical amino acids, lysine is a well-known educt for chemical modifications of food proteins e.g. glycation or carbamoylation. The enzyme-mediated methylation also takes place in plants and organisms and plays an important role in metabolism or cell communication [1,2]. The resulting lysine modifications are *Nε*-mono- (MML), *Nε,Nε*-di- (DML) and *Nε,Nε,Nε*-trimethyllysine (TML). In food, TML has been identified and quantified as a free and protein-bound amino acid [3,4]. It is mainly found in animal products such as eggs or meat in elevated concentrations of up to 136 mg/kg [4]. Based on our own data as well as the literature data and the German National Consumption Study [5], a daily intake of 8 mg TML is expected. It is known that human intestinal bacteria convert TML via a Stickland reaction to δ-valerobetaine [6]. Yet it is not known how the human digestion affects the derivatives. For this reason, we performed an *in vitro*-digestion of the methylated lysine derivatives.

Stock solutions of the derivatives as well as a mixture of MML, DML and TML were digested according to the INFOGEST protocol [7]. Qualitative and quantitative analysis of the derivatives as well as identification of possible metabolites was performed by LC-MS/MS.

Initial results indicate a degradation of the methylated lysine derivatives, especially in the gastric phase. Metabolites could not be identified yet.

Based on the initial findings, we assume that a proportion of the methylated lysine derivatives reaches the large intestine after simulated digestion. Therefore, further studies need to address the metabolism of MML and DML in addition to TML by the gut microbiome.

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V T *J Biol Chem*

X Z *Act Biochim Biophys*

L S *PLOS ONE*

X S L *JCI Insight*

M R Inst

K H L *Nat Metab*

M M *Food Funct*

Keywords : methylation, lysine, posttranslational modification, methyl lysine, in vitro digestion

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21432) - ENHANCE NUTRITIONAL COMPOSITION AND PROTEIN DIGESTIBILITY OF SPROUTED BEAN-FORTIFIED BREAD

Borgonovi, Sara Margherita (Italy)[1]; Iametti, Stefania (Italy)[1]; Marti, Alessandra (Italy)[1]; Sergiacomo, Alessio (Italy)[1]; Di Nunzio, Mattia (Italy)[1]

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Abstract

Pulses serve as a vital nutritional source worldwide, providing proteins, carbohydrates, fiber, and phytochemicals. With approximately 21-25% protein content, pulses play a crucial role in meeting protein requirements, particularly for people in developing countries. Despite their health benefits, pulses contain antinutritional factors such as phytate and enzyme inhibitors, affecting digestibility [1]. Currently, sprouting is gaining attention as a low-cost, sustainable, and effective way to increase not just the levels of health-promoting components but also the nutrients' digestibility.

This study explores the nutritional properties and digestibility of bread enriched with flour from 72-hours germinated cowpea (*Vigna unguiculata*). The selected sprouting time corresponds to the almost complete breakdown of the antinutritional factors.

To minimize processability issues and to ensure a significant nutritional impact, 25% of unsprouted or sprouted cowpea flour was added to wheat flour. Samples were characterized in terms of protein content and profile, residual anti-nutritional factors (trypsin inhibitors and phytates), glucose and starch content (total, slowly and rapidly digestible, resistant), and content in gut-fermenting oligosaccharides. The slight increase in total protein in bean-fortified breads was related to the incorporation of a legume-derived component with Mr around 45 kDa. Analysis of the starch fraction revealed a decrease in total, rapidly digestible, and total digestible starch in bread enriched with either sprouted or unsprouted bean flour, whereas the content of resistant starch and glucose increased in the sample containing flour from sprouted beans. The sprouted bean flour breads also exhibited the lowest levels of trypsin inhibitors and phytates.

The bread sample were subjected to in vitro digestion following the INFOGEST protocol to evaluate the degree of protein at the end of the gastric phase, as well as in the middle and at the end of the intestinal phase. SDS-PAGE at various time of “in vitro” digestion showed that large and medium-sized proteins bands were no longer present at the end of gastric phase, which appeared more intense in sprouted cowpea flour-enriched breads. Duodenal digestion produced small-size peptides, most of them are not retain in the SDS-PAGE gel. Further analysis is underway to identify the nature of protein hydrolysis products and their bioaccessibility. All together these data indicated that bread enriched with sprouting bean flour could be a very promise food with improved nutritional properties.

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Acknowledgments

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Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21437) - BIOACTIVITY OF YOGURT ENRICHED WITH MILK PROTEIN FRACTIONS

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Abstract

Milk is a rich source of essential nutrients and biologically active peptides. Bioactive peptides can be revealed by gastrointestinal digestion after dairy products intake. The aim of this research was to evaluate the effect of digestion on angiotensin I-converting enzyme (ACE; EC 3.4.15.1) and dipeptidyl peptidase IV (DPP IV; EC 3.4.14.5) inhibitory as well as antioxidant activities of yogurt enriched with bovine micellar casein concentrate (MCC) and milk serum proteins mixed with buttermilk concentrate (MSPB; with lactose or lactose free). Yogurts with MCC and MSPB were digested and their bioactivities were analyzed. In silico part of the study was carried out using computation tools: UniProt (http://www. https://www.uniprot.org/) and BIOPEP-UWM (http://www.uwm.edu.pl/biochemia/). The MCC and MSPB were prepared by membrane filtration. The digestion according to INFOGEST method was consisted of the following steps: oral, stomach - 1 hour, pH = 3, and duodenal - 1 hour, pH = 7. The degree of hydrolysis (DH) was determined according to the o-phthaldialdehyde method. The digests were analyzed for their enzymes inhibitory and antioxidant activities. The digests were used in a screening for bioactive peptides by reversed-phase high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC/ESI-MS/MS) method. A high degree of defragmentation of milk proteins in yogurts studied was observed after the duodenal phase (from 57.92% for control yogurt to 72.44 % for yogurt enriched with MSPB with lactose). Digests of yogurts studied showed ACE and DPP-IV inhibitory as well as antioxidant activities. The highest ACE inhibitory activity was determined for yogurt with MCC and MSPB with lactose (IC_{50} = 1,556 mg/ml). The highest DPP-IV inhibitory activity and antioxidant activity were determined for yogurt with MCC and MSPB without lactose (respectively: IC_{50} = 0.021 mg/ml and IC_{50} = 0.883 mg/ml). It was possible to find 33 biopeptides in studied digests. The ACE inhibitory (eg. IY, VY, IW, PR), DPP-IV inhibitory (eg.IPM, IPA) and antioxidant fragments (eg. IA, PR) were identified. It can be concluded that, yogurts enriched with bovine milk protein fractions' concentrates can be considered as an interesting source of peptides with biological activity, including ACE and DPP-IV inhibitors, as well as antioxidant peptides released after digestion.

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Acknowledgments

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Keywords : bioactive peptides, digestion, milk protein fractions, mass spectrometry

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21441) - IN VITRO DIGESTION OF HIGH-LIPID EMULSIONS: TOWARDS A CRITICAL INTERPRETATION OF LIPOLYSIS

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Abstract

Investigating the gastrointestinal fate of food emulsions is critical to unveil their nutritional relevance. To this end, the protocol standardized by COST INFOGEST 2.0 is meaningful for guiding in vitro digestion experiments. In contrast with studies addressing emulsions with low dispersed phase volume fraction (φ 0.05–0.1), we presently raise some points for a proper interpretation of the digestibility of emulsions with high lipid content using the pH-stat method. Oil-in-water high internal phase emulsions (HIPEs) were submitted to gastric pre-lipolysis with the addition of rabbit gastric lipase (RGE). Commercial mayonnaise (φ 0.76) was systematically diluted (φ 0.025, 0.05, 0.1, 0.15, 0.25, 0.4, and 0.76) to cover a wide range of enzyme-to-lipid ratios (8.5–0.3 U per µmol for RGE and 565.1–18.6 U per µmol for pancreatin, in the gastric and intestinal phases, respectively). Lipolysis was tracked either by fatty acid titration (NaOH titration) or completed by analysis of lipid classes and fatty acid composition. Gastric lipase resulted in substantial lipid hydrolysis, reaching 20 wt% at low lipid fractions (φ 0.025 and 0.05). Likewise, the kinetics and extent of lipolysis during intestinal digestion were modulated by the enzyme-to-substrate ratio. A logarithmic relationship between lipid hydrolysis and lipid concentration was observed, with a very limited extent at the highest lipid content (φ 0.76). A holistic interpretation relying on FFA titration and further evaluation of all lipolytic products appears of great relevance to capture the complexity of the effects involved. Overall, this work contributes to rationally and critically evaluating the outcomes of static in vitro experiments of lipid digestion.

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Acknowledgments

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Keywords : lipid digestion, high internal phase emulsion, INFOGEST protocol, pH-stat, static in vitro digestion

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21443) - IN SILICO MODELLING OF THE ABSORPTION OF TRIACYLGLYCEROLS AT THE CELLULAR LEVEL

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Abstract

A mechanistic computer code [1] modelling the process of food breakdown, digestion, and absorption is adopted for the understanding of the absorption of triacylglycerols at the cellular level. The process provides important knowledge on the metabolism of dietary triacylglycerols and may have significant impact on human diets and health.

The overall structure of the computer code is introduced, and various functionalities are described. The emphasis of the current paper is on the incorporation of the kinetics of non-esterified fatty acids [2], which make up the bulk of triglyceride molecules, to the absorption process. The absorption process in the intestine relies on the fatty acids being diffused through epithelial cells along the small intestines into the blood stream. This is translated to a source term for fatty acids density in the absorption within such interactive system.

Experimental data of fatty acids concentration in venous and arterial blood vessels are obtained through existing literature. The computer model is used to generate synthetic data that may be extrapolated for the current study. The work here is to examine data driven boundary models along the small intestine surface and into the blood stream. On the other hand, parameters in modelling absorption of triacylglycerols within the intestine are estimated. In addition, uncertainty analysis of the boundary conditions in the absorption process are studied.

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Acknowledgments
This research was supported by the University of Greenwich

Keywords : Absorption, triacylglycerols, fatty acids, intestine, uncertainty analysis

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21454) - BIOACCESSIBILITY OF PROTEIN IN WHITE BREAD CONTAINING INTACT PLANT CELLS

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Abstract

To assess the effect of the structure in the nutritional value of pulse proteins we investigated the in vitro bioaccessibility of protein released from breads prepared with 0% (B0), 30% (B30) and 60% (B60) of wheat flour replaced with cellular chickpea cells containing encapsulated starch and proteins, and compared it to the amounts of individual amino acids measured in the serum of participants after consumption of the breads.

Results from the in vitro digestion showed that the amount of protein bioaccessible increased with the increasing amount of protein incorporated in the breads. At the end of the gastric phase the hydrolysed protein was mostly in the form of small peptides in the three breads, representing 39, 36 and 38% respectively of the protein hydrolysed in B0, B30 and B60. Larger peptides and soluble proteins were 22%, 0.1% and 7% of the protein hydrolysed with 61, 40 and 46% of the protein still undigested in B0, B30 and B60.

At the end of the in vitro duodenal digestion 99%, 90% and 88% of the proteins were hydrolysed in B0, B30 and B60. The amount of free AA released from the food matrix in B60 was higher than the other 2 breads (74, 77 and 97 mg/gdb in B0,B30 and B60), but when the values were normalised by the initial amount of protein in the composition of the breads, the proportion of accessible free AA was very similar in the 3 breads (35, 28 and 30% in B0, B30 and B60 respectively).

Finally, the kinetics of the release of free essential AA was followed during the in vitro digestion at different time points and compared to the amino acid concentrations measured in human serum after consumption of the three breads.

References

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Keywords : protein, bioaccessibility, bread, in vivo, in vitro

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21455) - INVESTIGATING THE PROTEIN DIGESTIBILITY OF UPCYCLED BARLEY PROTEIN IN THE TINY-TIM MODEL

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Abstract

Plant proteins are gaining popularity due to increased consumer emphasis on health, along with the global movement towards developing sustainable food systems and reducing environmental impact. Brewer's spent grain is the most abundant by-product of the brewing industry and represents an ideal raw material for protein isolate production. The current study investigates the techno-functionality and protein digestibility characteristics of EverPro, a novel barley rice protein (BRP) extracted from brewer's spent grain, in comparison to a variety of common protein sources including whey, soy, rice and pea. Protein ingredients were analysed with regards to nutritional composition, foaming, emulsifying, structural and rheological properties, while digestion kinetics were investigated using the dynamic tiny-TIM *in vitro* digestion model. BRP displayed similar behaviour to whey protein in many respects, demonstrating high solubility, high nitrogen bioaccessability (> 90%), and comparable nitrogen digestion kinetics. The *in vitro* digestible indispensable amino acid score (DIAAS) was analysed and was found to be positively correlated with protein solubility and nitrogen bio-accessibility. BRP displayed an *in vitro* DIAAS of 67.3% (lysine as the single limiting amino acid), a higher value than that of soy (38.3 %), rice (37.5 %), and one of the pea protein sources (39.8 %) This study highlights the potential of BRP as a nutritious, multi-functional ingredient with the ability to aid in the shift towards more sustainable food systems.

Keywords : Plant-based proteins, Sustainability, Nutrition, Brewer's spent grain, Protein digestibility

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21464) - PLANT CELL CULTURES: NUTRITIONAL QUALITY AND PROTEIN DIGESTIBILITY IN VITRO

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1 - VTT

Abstract

Plant cell culture (PCC) technology is a new approach among cellular agriculture to supplement or substitute food ingredients produced by conventional agriculture. Bioreactor-grown PCCs could be exploited as an entirely new food biomass for human consumption to tackle the global food challenge. The aim was to examine the potential of PCCs as a food source with a focus on protein digestibility in comparison with plants (oats) and animal (skim milk) protein sources. Two cell lines (rowan and scurvy grass) were cultivated in pilot scale bioreactors, filtered, and freeze-dried. Scurvy grass had the highest concentration of amino acids (37.2 g/100g vs 21.3 g/100g for rowan), while rowan was richer in dietary fibre (27.5 g/100g vs 21.3 g/100g for scurvy grass). Oat protein had 58g/100g of amino acids and 14.4 g/100g of dietary fibre. Both PCCs had an amino acid score higher than 1 for all the essential amino acids, being the lowest score for leucine (1.03 and 1.38 for rowan and scurvy grass, respectively). Limiting amino acid in oat protein was lysine (score = 0.85). Their protein digestibility was evaluated using INFOGEST in vitro model supplemented with an analytical workflow allowing the assessment of protein digestibility (based on primary amines) and DIAAS calculation according to Sousa et al. (2022). Total protein digestibility of scurvy grass (89.9%) reached similar level of oat protein (90.6%) and skim milk powder (93.7%). Rowan had the lowest protein digestibility (61.5%). PCCs and oat protein were then digested in larger scale and dialysed (10 kDa cut off) to produce an un-digestible (non-absorbable) fraction. The metabolism of dietary fibre in the un-digestible fraction will be further evaluated by an in vitro colon model through the assessment of formation of beneficial short-chain fatty acids. Overall, both PCCs had relative high protein and dietary fibre content and good nutritional quality (i.e., beneficial amino acid composition and protein digestibility in vitro), but scurvy grass had better nutritional profile than rowan.

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This work was supported by the Academy of Finland (Cell2Eat, ID: 339182)

Keywords : plant cells, cellular agriculture, protein digestibility, in vitro DIAAS, dietary fibre fermentation

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21468) - TRACKING IN VIVO RELEASE OF BIOACTIVE METABOLITES ALONG THE HUMAN GASTRO-INTESTINAL TRACT.

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Abstract

Over the last decade there has been growing interest in tracking the *in vivo* transition of food within the gut to elucidate its effect on human health. However, calculating rate of digestion of food can be complex task, influenced by rate of gastric emptying, glycaemic response, and ileal starch digestibility. These factors are additionally complicated by several other variables both individual- (e.g. subject gender, gastric motility) and food-related (e.g. meal composition, meal nutrient content, food structure). Current *in vivo* digestion models in humans have proven to be ethically and financially challenging and *in vitro* models do not always accurately replicate the full complexity of human dynamic digestive mechanisms.

Herein, we track the digestion of chickpeas, in various forms (intact cell clusters, intact single cells, and broken cells), through the human gut using nasoenteral feeding tubes to gather synchronous samples of the gastric[1], duodenal[1], and ileal[2] contents across 10 healthy overnight-fasted volunteers. The study was spread over 4 visits –first visit for gastric and duodenal sampling over a 2-hour period, and other three visits for ileal sampling over an 8-hour period. The placement of nasoenteric tubes for gastric and duodenal sampling was checked using the CORPAK (MedSystems, Halyard) feeding tube model that tracks the position of the tube during placement without the need for X-rays[1]. While the placement of the nasoentric tube for ileal sampling was determined using fluoroscopy[2]. This longitudinal dataset was explored using untargeted proton nuclear magnetic resonance (1H-NMR) metabolic profiling analysis[1] to detect and trace hundreds of metabolites released by the chickpeas across the GI tract. The three different chickpea diets allowed for examination of the effect of food structure on digestion parameters, and therefore on macro- and micronutrient release.

This study demonstrates that by collecting and exploring human samples across the gut, we can build a cohesive model of how food structure can influence and evolve metabolic profiles along the gastrointestinal length. The model gives us a more reliable and accurate measure of the complex gut hormone signalling pathways triggered by nutrient release than current investigative tools; and may help in the maintenance of metabolic health by giving us better insight into medical issues such as appetite dysregulation and type 2 diabetes.

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2- DOI: 10.12688/f1000research.17870.2

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A big thanks to our collaborators at the Australian National Phenome Centre (ANPC) and collaborators under Prof. Elaine Holmes and Prof. Jeremy Nicholson laboratories at Murdoch University.

Keywords : Digestion, In vivo, Bio-accessibility, Bioavailability, Gastro-intestinal tract

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21470) - SWELLING BEHAVIOUR OF PECTIN-CHITOSAN BEADS UNDER SIMULATED GASTROINTESTINAL CONDITIONS

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Abstract

Hydrogel beads, a well-established encapsulation method, play diverse roles in food, pharmaceuticals, and biomedical applications. Among natural biopolymers, low methoxylated pectin stands out for creating hydrogels via calcium ion crosslinking, holding promise for microcapsule production. However, there are challenges, such as the early release of small, encapsulated molecules due to diffusion or swelling and diminished cell viability due to low gastric pH. A potential solution involves integrating a secondary biopolymer, like chitosan. The aim of this study was to evaluate the swelling behaviour of pectin-chitosan hydrogel beads under simulated gastrointestinal conditions. Pectin-based hydrogel beads with and without chitosan-coating were prepared to evaluate swelling behaviour. Pectin (35% degree of methoxylation) was used at a content of 2.2%. The solution was dripped into a calcium chloride solution (130mM) or into the same calcium chloride solution containing 2.2% chitosan. For both samples, swelling was investigated in simulated gastric fluid and subsequently in simulated intestinal fluid for up to 3 hours in each medium with sampling every 30 min. The media matched the pH and electrolyte composition as described in the INFOGEST protocol, but no enzymes were included (Brodkorb et al., 2019). Hydrogel bead size and sphericity were determined, as explained by Davarci et al. (2017). The results showed that both samples disintegrated during incubation in simulated gastric fluid; while particle size did not change after 60 minutes, shrinkage and disintegration were observed after 120 min. The hydrogel beads were fully dissolved when were transferred into the simulated intestinal medium. It is worth mentioning that no degradation occurred when incubating the samples just in distilled water at pH 7. Therefore, the observed degradation must mainly be attributed to the presence of the ions in the simulated intestinal fluids. There is sufficient evidence from the literature to postulate that degradation is caused by monovalent ions, which diffuse through the chitosan coating and displace calcium ions in the pectin network. In conclusion, chitosan-coated pectin hydrogel beads showed no change in size and sphericity in simulated gastric juice; however, they showed severe structural loss during incubation in simulated intestinal fluid and represent an innovative delivery system for functional food ingredients.

References

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Keywords : Swelling behaviour, hydrogel beads, Pectin, Chitosan, Simulated gastrointestinal

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21472) - IN VITRO TEST TO EVALUATE DIGESTION PROPERTIES OF MICRO-ENCAPSULATED PROBIOTICS

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Abstract

Probiotic bacteria present different benefits in human health, including, but not limited to, gut health, with continuously expanding scientific evidence. The most common species used for probiotic supplements are *Lactobacillus* and *Bifidobacterium*, as they are predominant in the human digestive track. Probiotic survival in gastrointestinal conditions is essential to ensure their therapeutic effect and impact on the gut microbiome. In this light, micro-encapsulation of probiotic bacteria can provide protection against the challenges of low pH and degradation via proteolytic enzyme activity. In this study, *B. lactis* HN019 and *L. rhamnosus* GG both encapsulated (using plant protein excipient) and free (non-encapsulated, lyophilized powder form) probiotic cultures were subjected to an *in vitro* InFoGest digestion in assay (using pea protein). Aliquots were taken as a function of time post-gastric and post-gastrointestinal incubation at 37°C and were assessed by flow-cytometry (BD FACS CANTO II). Results demonstrate that approximately 50 % of free probiotics lose viability and functionality after exposure to gastric conditions; whereas micro-encapsulated probiotic remain 100 % viable and functional after exposure to gastric and gastro-intestinal environment. In conclusion, these results indicate that micro-encapsulation confers a a protective effect upon probiotic bacteria that may potentially enhance the health benefits of probiotics and the overall gut microbiome.

References

N/A

Acknowledgments

N/A

Keywords : probiotics, microencapsulation, in vitro digestion, flow cytometry

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21476) - FOOD STRUCTURE REGULATES INTESTINAL NUTRIENTS AND GUT HORMONES: A HUMAN INTUBATION STUDY

Cai, Mingzhu (United Kingdom)[1]; Tashkova, Martina (United Kingdom)[1]; Tejpal, Shilpa (United Kingdom)[1]; Garcia Perez, Isabel (United Kingdom)[1]; Serrano Contreras, Ivan (United Kingdom)[1]; Edwards, Cathrina (United Kingdom)[2]; Frost, Gary (United Kingdom)[1]

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Abstract

Background：Plant food structure influences postprandial glycaemia and gut hormones (1, 2). *In vitro* digestion studies suggest intact legume cell walls act as digestion barriers, limiting the access of amylase to intracellular starch (3, 4). This may provide a mechanism for the lower glycaemic responses observed with the consumption of whole legumes, pending confirmations in the human gut. Furthermore, replicating the same physiological feedback in *in vitro* digestion systems as in the human gut poses a major challenge (5), hindering our understanding of how food structure impacts luminal nutrient sensing in gut hormone responses. The state-of-the-art intubation technique (6) allows researchers to access human digestive fluids, facilitating such investigations.

Methods: Ten healthy adults attended one 4-day inpatient visit at NIHR Imperial CRF. On day 1, two nasoenteric tubes were inserted through nostrils into participants' stomach and duodenum. On days 2-4, participants received one of three chickpea-based meals in a randomised order. These meals are nutrient-matched but differed in cellular structures: broken cell walls (Broken), intact single cells (Intact-S), and intact clustering cells (Intact-C). Gastric and duodenal aspirates were collected before breakfast and for 180 minutes postprandially to analyse starch digestion and metabolites. Blood samples were collected for the same period to measure blood glucose, GIP, and GLP1.

Results: The Broken meal resulted in rapid and higher increases in gastric maltose within first 30min, correlating with the incremental peaks of blood glucose, GIP and GLP1 (Spearman's rho=0.64, 0.47 and 0.41, with all $P<0.05$). Metabolic profiling using ^{1}H-NMR spectroscopy showed significantly differences in the luminal metabolites between groups (e.g., at 30 min postprandial, gastric: Intact-C vs Broken, $R^2Y=0.97$, $Q^2Y=0.89$, duodenal: Intact-S vs Broken, $R^2Y=0.99$, $Q^2Y=0.57$). Metabolites including amino acids, carbohydrates, and bile acids were semi-quantified from their NMR spectra to explore their relationships with gut hormones, suggesting that the maltose and glucose were the most relevant signals to GIP.

Conclusion: Processing legumes to disrupt cell walls increased postprandial glycaemia and led to rapid but short GIP and GLP1 responses driven by gastric and duodenal carbohydrate sensing.

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Acknowledgments

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Keywords : Food structure, Gut hormone, Gut metabolites, Starch digestion, Human intubation study

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21478) - CHARACTERIZATION OF THE MUCUS LINING A CO-CULTURE MODEL OF CACO-2/HT29-MTX CELLS

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Abstract

The Caco-2 cell line is commonly used as a model to study various events occuring at the intestinal level such as the physiological impact of toxins or the absorption of nutriments. More recently, co-cultures integrating enterocyte-like and goblet cells types, namely Caco-2 and HT29-MTX cells, have been reported as promising models of a tunable and functional epithelial barrier. One interest of including HT29-MTX cells is their ability to secrete mucins. It is therefore expected that the cell culture will be lined by a mucus layer, with functional consequences on absorption or bacterial adhesion for example. However, the spatial characteristics of this mucus layer (e.g. distribution or volume) is not fully described. The objective of this work was to visualize mucins in a co-culture of Caco-2/HT29-MTX cells, and to set up a method of mucus characterization based on image analysis.

Caco-2 and HT29-MTX cells were routinely grown in DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were seeded at a density of 2.10^5 cells/cm^2 on transwells in 24-well culture plates, at a ratio of 9:1 (Caco-2:HT29-MTX). Incubation was performed in a humidified atmosphere of 5% CO_2 at 37 °C. On day 21, cultures were fixed with 4% PFA and double-stained for F-actin using phalloidin-rhodamine, and for sialic acid using WGA-Alexa488. This aimed at detecting the cytoskeleton (thereby enabling fine contouring of each cell) and mucins, respectively. Images were acquired on a Zeiss-LSM 880 confocal microscope. Two independent culture wells were observed. Five 3-D images (consisting of around 80 stacked images on average) were acquired per well, resulting in a dataset of 10 images.

From a qualitative point of view, it was observed that cells at day 21 formed mostly a monolayer. Rather than being flat, cultures showed a clear topographic pattern, with domes that could reach 135 µm in height. Mucins were detected mainly on the apical side of the cells. Brightly-stained mucin clusters were visible in the extracellular apical space in close vicinity of some cells, most likely HT29-MTX cells. In addition, a more diffuse signal was also observed, sometimes lining large parts of the observation sites. This may correspond to secreted mucins spreading on top of the culture to form a typical intestinal mucus.

With the objective of eventually study the impact of a treatment (e.g. digested food constituents) on this mucus structure, a method of image analysis was developed. Using the open-source software ilastik (Berg et al., 2019), pixels were classified into three categories corresponding to bright staining mucin clusters, diffuse mucin staining and background. This analysis provides quantitative measurements on average mucin intensity and the volume proportion and average heights of mucin clusters.

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Keywords : cell-based model, mucins, quantification, confocal microscopy, image analysis

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21480) - SCREENING THE IMPACT OF PREBIOTICS IN CYSTIC FIBROSIS COLONIC DYSBIOSIS

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Abstract

Aim: Recently, concerns have been raised regarding the traditional recommended "high fat, high energy" diet to patients with cystic fibrosis (CF). In most cases, energy requirements are met with foods high in saturated fat and simple carbohydrates, and low dietary fiber. This pattern could contribute to an altered colonic microbiota. Increasing dietary intake of prebiotic fibres could be a practical approach to modulate intestinal dysbiosis. This study aimed to evaluate the effect of selection of dietary fiber/prebiotic components on the intestinal microbiota of children with CF.

Method: A static in vitro colonic fermentation model was used. The faecal inoculum (a pool of faecal samples from 3 children with CF) was combined with a culture medium and the prebiotic substrate to be tested (β-glucan, pectin, starch, and resistant starch). Samples were incubated for 20 hours at 37 C˚ under agitation (20 rpm) and in anaerobiosis. Metabolites production of ammonium, lactate, and short-chain fatty acids (SCFA) were determined and changes in microbiota were assessed by 16s rRNA gene sequencing. Changes in basal colonic microbiota after colonic fermentation with the substrates were assessed with the Dunnett test for multiple comparisons (two-way ANOVA).

Results: The synthesis of metabolites and the microbiota profile were positively impacted by specific substrates, but to different extents. Pectin and β-glucan caused a reduction in ammonia concentration of 50.68% and 47.32%, respectively, compared to the concentration of the basal microbiota. No statistically significant differences of lactate production were found. Bacterial phyla that are associated with the pathogenic bacteria causing intestinal inflammation, such as Firmicutes and Proteobacteria, were significantly reduced by pectin and β-glucan. There is little evidence regarding prebiotics in CF studies, and this study found viable alternatives like fiber dietary components with the prebiotic potential to improve gut health in CF children.

Conclusion: The results of this study allowed for establishing the effect of different prebiotics on the microbiota, offering new evidence for future research in dynamic models to obtain a response to long-term treatment in CF towards improving colonic microbiota.

References

Acknowledgments
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grant (PAIDPD-22) awarded to Andrea Asensio-Grau.

Keywords : prebiotics, cystic fibrosis, dysbiosis, colonic fermentation

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21481) - IMPACT OF SEMI-DYNAMIC IN VITRO SIMULATIONS ON PULSE DIGESTION: WHY SALIVA MATTERS

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Abstract

Nutrient-rich pulses exhibit a unique microstructural organization with macronutrients enclosed by a cell wall and an intracellular matrix. This organization persists through cooking followed by mechanical disintegration (e.g., chewing), ultimately slowing down macronutrient digestion. While static *in vitro* digestion methods are commonly employed to study macronutrient digestion in pulses, these do not capture transient processes of human digestion. Therefore, pulse digestion was studied under semi-dynamic conditions using a tailor-made multireactor system (MuReDi), applying recommendations of the semi-dynamic INFOGEST protocol.

As a first objective, this study investigated the individual impact of (i) saliva incorporation, (ii) dynamic gastric pH, (iii) gradual pepsin addition, and (iv) gastric emptying and combinations of those on macronutrient hydrolysis kinetics of lentil cotyledon cells. In brief, digestion kinetics were significantly affected by dynamic factors, with the gastric pH profile determining proteolysis kinetics but not the end point. The incorporation of saliva(ry amylase) during the oral phase in combination with a gradual gastric pH decrease caused a more rapid amylolysis reaching higher final levels compared to simulations without saliva.

Secondly, a semi-dynamic digestion protocol representing the digestive tract of older adults was developed. Starch and protein digestion kinetics were studied in lentils considering slower gastric acidification and lower (gastric and pancreatic) enzyme activities measured *in vivo* in older adults. Gastric proteolysis was strongly affected by the slower gastric acidification, leading to fewer and smaller peptides formed upon gastric digestion. These differences were compensated throughout the small intestinal phase, yielding final proteolysis values similar to those obtained under healthy adult conditions. Incorporation of saliva led to an even more pronounced acceleration of amylolysis under older adult conditions compared to healthy adult conditions, explained by the slower gastric acidification (and linked amylase inactivation).

This work highlights the importance of considering relevant gastric pH, importantly affecting *in vitro* amylolysis and proteolysis rates. Additionally, especially in (semi-dynamic) digestion simulations in which the applied gastric pH (gradient) allows salivary amylase activity, saliva should be considered to relevantly study starch digestion patterns.

References

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Acknowledgments

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Keywords : semi-dynamic in vitro digestion, salivary amylase, macronutrient digestion, pulses, process-induced microstructure

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(21483) - ROLE OF IN VITRO DIGESTION ON PHENOLICS AND ANTI-INFLAMMATORY ACTION OF ECHINACEA EXTRACTS

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Abstract

Echinacea purpurea L. (EP) preparations are one of the best-selling herbal supplements worldwide for its medicinal benefits, including anti-inflammatory activities. This study aimed to evaluate for the first time the impact of *in vitro* gastrointestinal digestion (INFOGEST) on phenolic composition, antioxidant, and anti-inflammatory properties on *in vitro* human colonic inflammatory model of different EP plant part extracts (flowers (EF), leaves (EL), and roots (ER)). The most abundant compounds were chicoric acid followed by caftaric acid, in both cases with the highest concentrations observed in EL. All phenolics identified were hydroxycinnamic acids, and the total concentration was notably higher in undigested EL extracts compared to EF and ER extracts. After digestion, the total hydroxycinnamic acid content remained higher in EL, followed by EF, and lastly, in ER. The bioaccessibility of phenolic compounds in EF, EL and ER varied depending on the plant part, these differences are probably attributable to the differences between the food matrices. Inflammatory model was run using subconfluent CCD18-Co cells, which were incubated with a subtoxic dose (0.5% in culture medium) of digested and undigested echinacea extracts obtained after *in vitro* digestion. Cells were co-treated with 1 ng/mL IL-1β for 16 h stimulate the pro-inflammatory responses. Results revealed significant reductions in IL-6 and IL-8 levels for undigested samples, although lower reduction, but significant reduction was also observed for digested EL and EF samples, but not for digested ER. Furthermore, regarding prostaglandins (PGE_2) biosynthesis in IL-1β-stimulated cells, solely digested EF and ER extracts showed no significant effects on decreasing PGE_2 levels. Therefore, the non-significant reduction on IL-8 with digested ER, and with digested ER and EF on PGE_2 levels could be explained, at least in part, by the lower phenolic content in the digested samples of these plant parts. Collectively, our findings indicate the potential of echinacea extracts, especially EL, in alleviating intestinal inflammatory conditions. Furthermore, this study also provides the scientific basis to give an indication of the effective bioactivity of EP preparations for the management of inflammation-related intestinal diseases.

Acknowledgments

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Keywords : INFOGEST digestion, Echinacea, anti-inflammatory activity, chicoric acid, phenolic composition

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(21485) - EVALUATION OF IN VITRO DIGESTIBILITY OF LINSEED OLEOGELS STRUCTURED BY TWO TYPES OF WAXES

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Abstract

Oleogels are matrices formulated through a mixture of vegetable oils and structuring polymers, the purpose of which is the partial or total replacement of saturated and trans fats in many foods. The *in vitro* digestion of oleogels, especially with linseed oil and oleogelators like natural waxes, is a relatively underexplored area in food science. This work aimed to evaluate the *in vitro* digestibility of linseed oil oleogels structured with 5% beeswax or 5% shellac wax. Lipid digestibility was assessed using the INFOGEST protocol for *in vitro* static digestion, adapted for oleogels, simulating the physiological conditions of the gastrointestinal tract, including the oral, gastric, and intestinal phases (Sabet et al., 2022). 0.25 g of sample was mixed with simulated salivary fluid (SSF) in a 1:1 ratio and incubated for 2 minutes at 37°C. Then, the salivary bolus was mixed in a 1:1 ratio with simulated gastric fluid (SGF) and incubated for 120 min at 50 rpm and 37°C. Finally, simulated intestinal fluid (SIF) was added to the mixture obtained in a 1:1 ratio. Fatty acid (FFA) release was monitored by pH-stat using 0.1M NaOH.

The linseed oil exhibited a percentage of free fatty acids (FFA) of 77.68 ± 0.86%. The structured oil with 5% beeswax showed an FFA of 73.89 ± 2.20%, while the structured oil with 5% candelilla wax yielded an FFA of 69.70 ± 2.23%. These results showed statistically significant differences ($p < 0.05$) in digestibility depending on the structuring agents used. According to the literature, the composition and proportions of wax components (wax esters, hydrocarbons, free fatty acids, and fatty alcohols) vary among the different waxes. The wax composition could have a complex influence on the crystalline microstructure of the oleogel and its characteristics. On the other hand, the self-assembled structures within the oleogel could physically obstruct the interaction between the enzyme and lipids, decreasing the release of fatty acids in the oleogel compared to the oil; however, this decrease is less than 10%. INFOGEST made it possible to quantify a high release of FA from the oleogel, which is important for formulating foods with oleogels from linseed oils rich in polyunsaturated fatty acids.

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Acknowledgments

This research was supported by funding from ANID through FONDECYT project 1200942.

Keywords : oleogels, waxes, in vitro digestion

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(21486) - IN VITRO INFANT MODEL WITH GUT MICROBIOTA TO STUDY ALLERGY TO COW'S MILK PROTEINS

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Abstract

The importance of the intestinal microbiota in early life food allergy has recently been highlighted. In the first years of life, one of the foods frequently involved in food allergies is milk. Cow's milk protein allergy (CMPA) can be IgE mediated and non IgE mediated (NIM-CMPA). The latter is often underdiagnosed due to the lack of analytical methods to adequately detect it. In this regard, *in vitro* models that mimic the infant digestive environment and these pathophysiological allergic conditions are very valuable. Currently, only a limited number of studies have been carried out in this sense. Therefore, the objective of the present work was to develop a route map of *in vitro* test models that allow the simulation of infant digestive conditions to evaluate the interactions between cow's milk proteins (CMP) and the patient´s microbiota.

Following the harmonized *in vitro* digestion INFOGEST protocol, adapted to simulate infant conditions, digests were obtained from different types of infant formulas after the simulated oral, gastric and intestinal physiological phases. Extensively hydrolysed formulae (EHF) were supplemented with the main protein allergen in milk (casein or seroproteins) prior to be subjected to tyndallisation and the simulated digestion process. These digests were used together with pre-prepared faecal microbial inocula from patients and controls (healthy infants) to carry out controlled colonic fermentations in an anaerobic mini-bioreactor simulating infant colonic conditions. After the colonic model, microbial populations and short-chain fatty acids were analysed revealing different profiles and signatures depending on the type of EHF and CMPA condition.

Keywords : allergy, microbiota, infant, in-vitro, digestion

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21487) - PLANT PROTEIN DIGESTION AND METABOLISM: IN VITRO AND MEAL STUDY METHODOLOGIES

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Abstract

Plant proteins have gained significant attention in recent years as replacements for animal proteins due to their potential environmental and health benefits. However, there is limited information on the bioaccessibility of nutrients from plant food sources. Therefore, a systematic scoping review was conducted following the PRISMA-ScR guidelines to examine the current evidence based on in vitro and meal study methodologies used in studying plant protein digestion and metabolism. By evaluating the advantages, limitations, and complementary nature of these methods, we identified gaps in knowledge and proposed strategies for enhancing our understanding of plant protein digestion and metabolism. Three databases were searched for articles conducted between the years 2000 and 2023, focusing on high-quality in vitro or meal studies that investigated extracted plant proteins or foods containing them. In total, 125 articles were included in the review. Soy protein was the primary protein investigated in the meal studies, often in the forms of isolate and concentrates, accounting for approximately 80% of the studies. In contrast, in vitro studied used protein sources from more diverse origins. Studies done with meat analogues or texturized proteins and studies considering the effect and impact of gut microbiota were vastly underrepresented. The protein utilization of plant proteins was consistently inferior to animal protein in nearly all studies. The digestibility of proteins, regardless of the plant protein source, was significantly influenced by the food matrix and processing. This can be attributed to differences in protein conformation and chemical interactions with fiber and antinutrients compared to animal proteins. Given the expected increase in plant protein consumption, this review highlights the need for more diverse clinical meal studies to understand the effect of different plant protein sources and formulations on our nutritional status and health. Plant proteins have very different metabolism kinetics and digestibility than animal proteins. Additionally, the substantial variability in digestion kinetics among different plant protein sources necessitates caution when making direct comparisons. To provide more reliable and accurate data on the digestion of plant proteins, attention should be directed to developing methods designed for measuring plant protein digestion behavior.

Acknowledgments

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Keywords : Digestion, Plant proteins, Metabolism, In vitro study, Meal study

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(21488) - BENEFIT OF CASEIN/PLANT PROTEIN ASSOCIATION AS A STRATEGY TO MEET PROTEIN NEEDS IN ELDERLY

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Abstract

Consumers and product manufacturers are increasingly seeking alternative protein sources to address the protein requirements of specific populations, particularly the elderly, aiming to diversify product offerings and enhance dietary profiles. However, novel protein sources, particularly plant-based proteins, often exhibit deficiencies in essential amino acids, such as methionine. One potential mitigation strategy involves the formulation of protein blends, combining deficient proteins with nutritionally complete sources, such as micellar **c**asein.

In this study, the postprandial amino acids (AA) response in healthy older adults (mean age 72.3 ± 3.4 years, BMI 25.3 ± 2.9 kg/m^2) following the ingestion of 20 g of pea protein, micellar casein, and a casein-pea protein blend was investigated. Plasma AA levels were measured before and up to 5 hours after consumption.

Blending casein/pea in a 60/40 mixture resulted in improved plasma AA availability of total (essential) AA and of key AAs methionine and leucine compared to pea only, while preserving the higher availability of arginine. The amino acids' response of the casein-pea blend was demonstrated to have an intermediary profile between its individual constituents, suggesting that blending represents a viable strategy to improve the inherent limitations of lower-quality plant proteins. This finding holds potential relevance for addressing protein needs in the ageing population.

Keywords : Clinical study, Dairy protein, Plant protein, Amino acid

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21490) - IN SILICO APPROACH OF METABOLIC FLUXES OF AMINO ACIDS IN THE SMALL INTESTINE WITH PIG

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1 - INRAE; 2 - Adisseo

Abstract

Numerous *in vitro* and *in silico* studies have been performed to better understand digestion of foods in human, with the purpose to better asses the bioavailability of nutrients. For example, a debate is ongoing on the estimation of the amino acids (AA) requirements and how to assess dietary protein quality with this regard (Moughan, 2021). While the focus is given on digestive processes to assess AA availability, little is known about the importance of intestinal metabolism on AA availability for the rest of the organism, which is at the interface between digestive processes and post-absorptive metabolism. Stoll et al., 1998 estimated that 44 % of dietary AA are metabolized in the small intestine (SI) after their absorption; this figure seems high in comparison to the relative mass of intestinal proteins over whole body proteins. In the present study, our aim was to aggregate current knowledge to build a conceptual model of intestinal metabolism that help us to better understand the dynamics of metabolic fluxes of AA in the SI, taking the example of pig. A mechanistic model was built representing the metabolism of a non-specific AA in the SI. The model was made of a series of functional units of intestine or segments, which all had an identical structure. Each segment was made up of seven state variables representing, depending on their origin (endogenous or dietary), AA as proteins in the intestinal lumen, free AA in the intestinal lumen, free AA in the intestinal tissue and AA as intestinal proteins. These state variables were linked by fluxes representing the main metabolic pathways of AA metabolism, namely hydrolysis of dietary proteins, absorption of resulting AA, synthesis and degradation of protein, endogenous secretions and exchanges with blood. Homeostasis was used as a key principle, driving fluxes of protein synthesis as well as exchanges of free AA with blood. To parametrize the model, data were obtained from the literature and when such data were not available, values assumed reasonable were used. A simulation was performed over 500 segments of intestine during a period of 24 h comprising five meals and an overnight fast. During periods of feed intake, the model simulated an export of AA to the blood, while during fasting it simulated an importation of AA from the blood for protein synthesis, which could be later secreted in the intestinal lumen. Apparent ileal digestibility was 81 % and true digestibility was 95 %, which is in range with results obtained *in vivo* (van der Wielen et al., 2023). Of absorbed dietary free AA, around 50 % were first used for protein synthesis by the intestinal tissue, before being exported to the blood. This result highlights the importance of rapid recycling of endogenous proteins in the lumen, as indicated by Leterme et al. (1996). The present model can serve as a framework to better understand connection between elements in the SI and the resulting emerging properties.

References

Moughan et al., 2021; Stoll et al., 1998; van der Wielen et al., 2023; Leterme et al., 1996

Acknowledgments

Keywords : Metabolism, mechanistic model, first-pass, protein

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(21491) - PROTEIN IN VITRO DIGESTIBILITY OF INSECTS AND SOYBEANS: INFLUENCE OF FOOD PROCESSING

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Abstract

Sustainable protein sources are becoming increasingly important as the world's population grows and the demand for food increases. There is a need to identify viable alternatives to traditional animal foods from Western diets and to characterize their protein quality to ensure human health and well-being. The FAO recommends the DIAAS (digestible indispensable amino acid score) method to assess the protein quality of foods (1).

The focus of this work was to compare the protein quality of alternative protein sources with known high content of essential amino acids, namely, mealworm larvae, adult crickets, and soy products, to chicken breast. All sources were analyzed before and after typical food preparation and processing methods, e.g., soy products were investigated as beans, tofu, and milk; insects were analyzed blanched, oven-dried, and freeze-dried. All products were subjected to the static INFOGEST protocol, followed by the analytical workflow by Sousa et al (2) to determine *in vitro* digestibility and DIAAS. After *in vitro* digestion, peptides were examined by LC-MS; primary amines and amino acids of the separated hydrolyzed fractions (digestible, indigestible) were quantified by the OPA method and UPLC, respectively.

The results revealed a high protein *in vitro* digestibility for the transformed soy products, the insects, and chicken. For soy, a clear improvement of *in vitro* digestibility was achieved, in the transformed products compared to cooked beans. In contrast, heat-treatment and dechitinization of insects negatively impacted *in vitro* digestibility. *In vitro* DIAAS for soybeans was below 60, whereas DIAAS for tofu, soymilk, blanched and freeze-dried insects was above 75, and DIAAS of chicken breast was higher than 100, considering the reference pattern for growing children.

Protein quality does not depend on the protein source alone but also on food processing and preparation steps. Based on *in vitro* results, insects and soy products can be a viable alternative protein sources in human diets if suitable processing methods are applied.

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Keywords : in vitro digestion, protein hydrolysis, insects, soy, in vitro DIAAS

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(21501) - EFFECTS OF IN VITRO DIGESTION ON PASTA FORTIFIED BY TWO DIFFERENT ITALIAN GRAPE POMACE CV

Angilè, Federica (Italy)[1]; Dold, Cathal (Ireland)[4]; Romaniello, Francesco (Italy)[3]; Giblin, Linda (Ireland)[4]; Cavallarin, Laura (Italy)[2]; Gerardi, Carmela (Italy)[1]; Giovinazzo, Giovanna (Italy)[1]; Bavaro, Simona Lucia (Italy)[2]

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Abstract

Grape pomace is a wineries byproduct, mainly composed of the skins, seeds, and stalks, that is a rich source of phenolic compounds (anthocyanins, flavan-3-ols, stilbenes, and phenolic acids) and dietary fibers. In this study, Infogest in vitro digestion protocol was used to compare the carbohydrate and polyphenol contents of cooked pasta fortified with red and white grape pomace flour of two Italian grape varieties. Additionally, the ability of digested samples to modulate the secretion of the satiety hormone GLP-1 in STC-1 cells was evaluated. The analysis of carbohydrate content showed a decreased level of maltotriose and glucose (from 10 to 25%) in both fortified pasta in comparison with control pasta. Moreover, the total polyphenol content of each fortified pasta was higher than control pasta. In particular, a higher increase was found in functionalized pasta by white grape pomace (45%). To gain a deeper understanding of how phenolic compounds are modified, after simulated digestion, a complete characterization of them in fortified pasta was carried out using HPLC-HRMS. The identification of the single molecules was achieved using high-resolution m/z values and MS2 experiments. The data show a general reduction of flavan-3-ol and flavonoid glycoside in fortified pasta, while a good concentration of phenolic acid was maintained, along with the occurrence of hydroxybenzoic acid. Interestingly, the fortified pasta with red wine pomace exhibits a complete loss of anthocyanins and a 10-fold increase in p-coumaric acid concentration compared to white pomace pasta (203,01 ug/L vs 15,87 ug/L). The satiety effects of digested pasta were also investigated on the STC1 cell line. Surprisingly, all digested pasta samples decreased the release of active GLP-1 secretion in STC-1 cells compared to the basal control (Krebs–Ringer buffer) ($p < 0.05$). In conclusion, our results show that durum wheat pasta fortified with whole pomace flour could potentially improve the nutritional properties by increasing antioxidant and polyphenol content. However, from a functional point of view, more experiments are required to deeply understand their possible role in satiety mechanism regulation.

Acknowledgments

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Keywords : Polyphenols, carbohydrate, in vitro digestion, GLP1

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(21517) - CANIM-ARCOL A NEW IN VITRO MODEL OF THE DOG LARGE INTESTINE CAPTURING SIZE-RELATED EFFECTS

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Abstract

Body weight is an important determinant of variations in canine digestive physiology, mainly related to the large intestine. *In vitro* gut models are increasingly used as an alternative to animal experiments for technical, cost, societal and regulatory reasons. However, up to now, none of the available canine *in vitro* gut models has been adapted to reproduce size-related digestive parameters.

To address this limitation, we developed a new *in vitro* model of the canine large intestine, named the CANIneMucosal ARtificial COLon (CANIM-ARCOL), simulating the main physicochemical (pH, transit time, anaerobiosis), nutritional (ileal effluent composition, bile acids profiles) and microbial (lumen and mucus-associated microbiota) parameters of this ecosystem. The model was adapted to three dog sizes, i.e. smallunder 10 kg, medium between 10 and 30 kg and large over 30 kg. To validate this new model regarding microbiota composition and metabolic activities, *in vitro* fermentations were performed during 20 days in bioreactors inoculated with stools from 13 dogs (4 small, 5 medium and 4 large), and results were compared to *in vivo* data in dogs from the literature.

After a 10 days-stabilization period, microbiota profiles clearly clustered depending on dog size. Especially, *Bacteroidota* and *Firmicutes* abundances were positively correlated with body weight both *in vitro* and *in vivo*, while opposite trends were observed for *Actinobacteria* and *Proteobacteria*. As observed *in vivo*, microbial activities as followed through gas, short-chain fatty acids and ammonia concentration measurement, but also bile acid dihydroxylation, increased with dog size *in vitro*. The new model also provided useful data on mucus-associated microbiome, poorly described up to now in dogs.

In line with the 3R regulation, the CANIM-ARCOL represents a powerful platform to study the fate of food and veterinary products in the canine digestive environment, help to elucidate their mechanisms of action in relation with colonic microbiota and promote innovation in these fields. This model will also help to move toward personalized nutrition or medication, by capturing interindividual or breed variabilities in gut microbiome and considering dog body weight.

Keywords : in vitro gut model, dog, large intestine, microbiota, body weight

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(21521) - IN VITRO DIGESTION AND INTESTINAL FUNCTION MODULATION OF MFGM-PROVIDING INGREDIENTS

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Abstract

Intestinal digestive, barrier, endocrine and immune functions are modulated by the nature of the food bolus. We previously demonstrated *in vitro* the benefits of dairy ingredients providing milk fat globule membrane (MFGM) on gut physiology, both in basal and inflammation states, with a dose-effect of phospholipids (PL) on gut barrier integrity in the basal state. Therefore, these ingredients were used in their native form.

Our objective was to further decipher their effects in an experimental protocol approaching better the intestinal physiology i.e. after ingredient incorporation in a food matrix model and following a first step of *in vitro* static digestion.

The effects of three dairy ingredients (Buttermilk, a non-enriched natural source of MFGM, and MFGM-Buttermilk and MFGM-Whey, two enriched MFGM-ingredients) were studied, in an inflammatory environment mimicking the one encountered in elderly, and using an *in vitro* quadricellular (Caco-2, HT29-MTX, NCI-H716 and RajiB) model of the human intestinal epithelium. Ingredients, studied either alone or incorporated in a dairy drink model, were subjected to a first step of static *in vitro* digestion to better approach intestinal physiology, before studying their impact on the quadricellular model. When studied alone, MFGM-enriched ingredients were standardized at 0.15 mg/mL PL whereas buttermilk was used at a lower concentration (0.031 mg/mL) to investigate dose-dependent effects. Due to solubility issues, PL concentration was lower when using the dairy drink model (0.045 mg/mL and 0.009 mg/mL respectively). Digestion was adapted from the INFOGEST 2.0 protocol. Inflammation was induced using TNFα. Cytoxicity was evaluated by lactate-dehydrogenase release and barrier integrity by trans-epithelial electrical resistance (TEER). Expression of genes of interest was quantified by RT-qPCR using a SmartChip device.

An adaptation of the INFOGEST 2.0 protocol, i.e. a reduction of protease concentration, a longer intestinal digestion phase, and a 1/160 dilution of digestion products were necessary to avoid cell toxicity. All MFGM-ingredients improved barrier integrity compared to digestion fluid alone (i.e. 1/160 diluted product of the digestion protocol without ingredient) and had no effect on cytotoxicity. Yet, digestive fluid alone impaired gut barrier integrity, which was restored by MFGM-ingredients. A MFGM-dose dependent effect on cell viability was observed when MFGM-ingredients were incorporated in the dairy drink model, with a significant higher cell viability for enriched ingredients. The analysis of gene expressions is underway to better characterize the underlying mechanisms and investigate intestinal functions such as the digestive, endocrine and immune ones.

These results add to the body of evidence of MFGM benefits in an experimental device mimicking all the steps of nutrition physiology.

References

Acknowledgments

Keywords : phospholipids, multicellular model of intestinal epithelium, barrier and digestive intestinal function, endocrine intestinal function, immune intestinal function

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21522) - LOW-COST DYNAMIC IN VITRO DIGESTION MODEL PROTOTYPE FOR BIOACCESSIBILITY STUDIES

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Abstract

The bioaccessibility of nutrients present in food is an important parameter for studying multicompartmental in the gastrointestinal tract and predicting its importance for the homeostasis of the organism. To determine bioaccessibility, in vitro models have been widely used, especially static models. However, dynamic models tend to provide greater fidelity to the analyses, but the high cost of dynamic model modules is still a hindrance. This work proposes to build a low-cost dynamic in vitro digestion model prototype capable of simulating various parameters of the functioning of the gastrointestinal tract efficiently. The proposed simulation module is low-cost, but incorporates functionalities and automatic control features that enable various nutrient bioaccessibility experiments, differentiating itself from modules available on the market. The pilot scale module consists of a single-compartment system, small scale (up to 2 mL of sample) with minimal enzyme expenditure, allowing parsimonious use of inputs. The module incorporates a microcontroller system for automatic temperature regulation, dynamic pH control, and continuous and controlled agitation of the sample tube. An automatic fluid drip system is performed through syringes actuated by stepper motors allowing to simulate different enzyme injection profiles throughout the simulated digestion process. The experiments enabled by the proposed dynamic digestion module meet the demands of the Chemistry Food Laboratory of the Faculty of Pharmacy of UFMG, being flexible and reconfigurable for a wide range of experiments that demand an objective evaluation of the bioaccessibility of nutrients present in food.

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References

Keywords : Bioaccessibility, In vitro digestion model, Low-cost, Automatic control, Simulation module

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21525) - A NEW IN VITRO HUMAN COLONIC MODEL SIMULATING OBESITY-RELATED GUT MICROBIOTA DYSBIOSIS

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Abstract

Background: Obesity is a complex, multifactorial and highly prevalent disease, strongly associated with nutritional disorders and gut microbiota perturbations. For technical, regulatory, ethical and cost reasons, *in vitro* models simulating the human digestive tract can be a relevant alternative to *in vivo* assays, provided they are fully validated against *in vivo* data in humans. To date, no relevant *in vitro* model reproducing the nutritional, physicochemical and microbial parameters of the obese human colon has been described.

Methods: An intensive literature review was performed to adapt the Mucosal Artificial Colon (M-ARCOL) model[1] to the specific colonic environment of obese patients (pH, retention time and composition of ileal effluents). Stools from 9 donors (4 healthy and 5 obese) were used to inoculate two bioreactors ran in parallel, set-up to reproduce either healthy or obese parameters. Samples were regularly collected during fermentations to determine lumen and mucus-associated microbiota composition by quantitative PCR and 16S Metabarcoding. Gut microbial activities were followed in the atmospheric phase and luminal medium of bioreactors through gas and short chain fatty acid (SCFA) measurement by chromatography.

Results: When applying obese parameters on healthy stool in the M-ARCOL system, significant shifts in microbiota activity and composition were observed, in accordance with *in vivo* data ($P<0.05$). Less methane but more SCFA and associated energy were produced. An increase in obesity-associated marker populations (*Prevotellaceae*, *Veillonellaceae*) and a decrease in healthy-associated marker populations (*Archaea* associated with the significant fall in methane production, *Akkermanciaceae*, *Rikenellaceae* and *Christensenellaceae*) were also observed in lumen and mucus-associated microbiota, together with a lower bacterial diversity. Interestingly, when applying healthy parameters on obese stools opposite trends were obtained demonstrating gut microbiota resilience.

Conclusions: In conclusion, this study shows that the M-ARCOL accurately reproduces gut microbiome changes associated to obesity, even when the model was inoculated with stools from healthy volunteers. M-ARCOL model can represent a powerful platform as an alternative to *in vivo* animal assays in preclinical trials to perform mechanistic studies on gut microbes under obesity-related conditions and evaluate pharmaceutical and/or nutritional strategies aiming to restore gut microbiota eubiosis.

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Acknowledgments

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Keywords : Obesity, Gut microbiota, Dysbiosis, In vitro gut model, M-ARCOL

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21529) - A SEMI-DYNAMIC DIGESTION PROTOCOL ADAPTED TO THE POPULATION UNDER PROTON PUMP INHIBITORS

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Abstract

There are widespread concerns regarding gastric diseases and discomfort, such as heartburn and reflux. The common medicinal solution prescribed is the use of proton pump inhibitors (PPIs). PPIs function by blocking the gastric HK-ATPase, inhibiting gastric acid secretion and leading to a significantly higher pH in the stomach throughout digestion. However, the importance of a gastric acidic environment for food digestion, erosion, and enzyme activity cannot be overlooked, and the impact of PPIs on food digestion and nutrient release remains relatively unexplored.

To address this knowledge gap, our study aims to propose an *in vitro* semi-dynamic digestion protocol under PPI mediation and investigate the effect of PPIs on the digestion of a mixed meal consisting of bread, cheese, and tomato.

The PPI digestion protocol was developed based on the INFOGEST semi-dynamic digestion protocol and the reported influence of PPIs on the acidity and rate of gastric secretions in humans. Gastric fluid and rabbit gastric extract (RGE) were added continuously, and five gastric emptying steps were conducted. Consistently with the *in vivo* literature, a final targeted gastric pH of 4.2 could be obtained by reducing by half the concentration of gastric acid and its secretion rate compared to healthy conditions. The emptied digesta was analysed for the digestion of carbohydrates, proteins, lipids, and minerals using ion chromatography, OPA, thin-layer chromatography, and ICP, respectively.

Results revealed that under PPI conditions, less arabinose, peptides, and minerals (Ca, Mg, P) were released during digestion, while other compounds showed similar patterns with the healthy condition. The observed effects on nutrient digestion are likely due to the higher gastric pH, resulting in less acidic erosion and modified enzymatic activities. These results are consistent with some of the well-known adverse effects of PPIs such as infections, hypomagnesemia, fractures, etc.

In conclusion, this study presents a detailed semi-dynamic digestion protocol under PPI medication, shedding light on the effects of PPIs on macro- and micro-nutrients during gastric digestion. This protocol can serve as a valuable resource for researchers studying food digestion, contributing to a better understanding of the association between PPI intake and clinical side effects, ultimately promoting better health.

References

Acknowledgments

Keywords : INFOGEST semi-dynamic digestion protocol, Proton pump inhibitors (PPI), Mixed meal, Macronutrients, Micronutrients

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(21531) - A NEW IN VITRO MODEL OF THE HEALTHY HUMAN ILEUM AND ITS ASSOCIATED MICROBIOTA

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Abstract

The human small intestine is the main site of food digestion and nutrient absorption. The small intestinal microbiota certainly plays a key role in host health, but until now it was largely understudied due to sampling invasiveness, especially in healthy volunteers. Due to ethical, technical and cost reasons, *in vitro* dynamic models of the human small intestine appear as a great alternative to *in vivo* studies. However, there is no available *in vitro* system simulating the ileal compartment and its associated microbiota, that has been fully developed and validated based on *in vivo* data in humans.

In this context, the Mucosal Artificial Colon (M-ARCOL) was adapted to simulate the nutritional, physicochemical and microbial conditions found in a healthy human ileum, leading to the Mucosal Artificial Ileum (M-ARILE). A wide literature review was performed to set-up this new *in vitro* model with the pH, transit time and nutrient availabilities found under fed and fasted states in the human ileum. In order to validate the newly-developed model, *in vitro* fermentations were performed during 9 days in both conditions (M-ARILE and M-ARCOL), using stool samples from three adult volunteers. Gut microbiota composition (lumen and mucus-associated microbiota), and metabolic activities (gas and short-chain fatty acids) were daily monitored.

Concentrations of acetate, propionate, and butyrate, were significantly lower in the ileal model compared to the colonic one. Total bacteria levels as well as bacterial diversity (observed ASVs) were also decreased under ileal conditions. Some bacterial populations were enriched in the M-ARILE compared to the M-ARCOL, such as *Clostridium*, *Escherichia* and *Streptococcus* genera. All those results were in accordance with *in vivo* data from healthy human ileum.

This new ileal model will provide a powerful platform for mechanistic studies on the human small intestinal gut microbiome. This can help to get significant insights in the role of ileal microbes in human nutrition, drug metabolism and interactions with enteric pathogens targeting the small intestine.

Keywords : in vitro model, gut microbiota, small intestinal gut microbiome, ileum

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21532) - IN VITRO MODELLING OF ORAL MICROBIAL INVASION IN THE HUMAN COLON

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Abstract

Background: The human gut microbiota plays a crucial role in health and disease, with mounting evidence suggesting that oral microbes can potentially influence gut microbial communities. Recent studies have revealed significant detection of oral microorganisms in stools from dysbiotic patients, but potential interactions of these invasive oral bacteria with intestinal microbiota have been poorly investigated.

Methods: We developed an innovative approach combining the Mucosal ARtificial COLon (M-ARCOL), designed to mimic the nutritional, physicochemical and microbial (lumen and mucus-associated) parameters of the human colon with a salivary enrichment protocol to simulate oral-to-gut microbial invasion. Fecal samples from healthy donors were inoculated into the M-ARCOL, and saliva from the same donors was introduced to reproduce the oral-to-gut route. Over an 11-day fermentation period, we collected samples from luminal and mucosal compartments of the M-ARCOL, subjecting them to whole metagenome shotgun sequencing for comprehensive microbial analysis.

Results: We demonstrated a higher species richness within the mucosal compartment of the M-ARCOL, surpassing luminal samples at all time points during *in vitro* fermentations. Most notably, while a few oral microbial species were present in the colonic compartments before saliva addition, during the oral-to-gut invasion simulation, oral microbial species were predominantly detected in the mucosal microenvironment, irrespective of the donor's richness and gut microbial composition.

Conclusions: This study underscores a preference of oral microbial invaders for the colonic mucosal microenvironment, highlighting the critical importance of incorporating the mucosal setup when investigating microbial dynamics in *in vitro* gut models. The enhanced M-ARCOL model offers a valuable platform to gain mechanistic insights into the role of the oral microbiome in various disease processes.

References

Acknowledgments

Keywords : Oral microbial invasion, Gut microbiota, Mucus, M-ARCOL, Metagenomics

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21545) - THE EFFECT OF CONSUMPTION TEMPERATURE OF WHOLE MILK ON IN VITRO GASTRIC DIGESTION USING MRI

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Abstract

This study utilized Magnetic Resonance Imaging (MRI) to investigate the behaviour of whole fat milk at varying temperature during semi-dynamic in vitro gastric digestion. The experimental protocol was designed to closely reproduce the gastric temperature profiles observed in humans after consumption of milk at 4°C, 37°C, and 60°C. Consuming milk at 4°C significantly postponed the onset of protein coagulation during the gastric phase compared to both 37°C and 60°C. MRI lipid quantitative analyses also showed that the fat-rich particles tended to float the top of the digesta in a process similar to creaming, and after a delay that seemed to increase in the order: milk at 4°C < milk at 37°C < milk at 60°C (no floating particles within 2h). However, the released quantities of free amines in collected samples, which indirectly reflect the activity of pepsin, did not significantly vary with the milk temperature. Our findings highlight the significance of consumption temperature in modulating the structural reorganisation of whole milk during gastric digestion and illustrate some of the capabilities offered by MRI to investigate such phenomena. They also open questions on the potential consequences of milk consumption temperature on the nutrient delivery rate into the small intestine and their further breakdown and absorption kinetics.

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Keywords : MRI, Milk, Digestion, Caseins, Lipids

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(21551) - HEAT INACTIVATION OF TRYPSIN INHIBITORS DOES NOT IMPROVE IN VITRO DIGESTIBILITY OF PEA PROTEINS

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Abstract

Plant proteins have low protein nutritional quality due to an incomplete profile of indispensable amino acids and the presence in the plant source of antinutritional factors (ANF) such as trypsin inhibitors (TI) that limit protein digestibility. A common strategy to improve protein digestibility is to inactivate TI by applying heat treatment. However, depending on the temperature applied, heat treatment can also result in protein aggregation decreasing protein digestibility. Therefore, this study aimed to investigate how heat treatment affected the *in vitro* protein digestibility of different pea protein ingredients containing different levels of TI and matrix composition: pea flour (PF), protein-rich fraction (PRF), and pea protein isolate (PI). Samples were mixed with water (40 wt% dry basis) and heated at 90˚C and 140˚C for 20 min. After heat treatment, the samples were digested using the INFOGEST method (slightly modified), and TI activity (TIA) was determined in unheated and heated samples. The molecular weight distribution of undigested and digested samples was determined with SDS-PAGE. Heating all samples at 140˚C resulted in the inactivation of 70% or higher of TIA. Intact bands between 20 and 30 kDa were found in the SDS-PAGE of unheated PF and PRF after the digestion in the intestinal phase. The bands in this molecular weight range might indicate the presence of TI complex with the digestive enzymes. It was expected that the inactivation of TI in the PF and PRF heated would improve the *in vitro* protein digestibility, however, no improvement was observed. On the other hand, the protein digestibility of PI decreased after heat treatment at 140˚C. This might be a result of protein aggregation which was seen in the SDS-PAGE of heated samples. Therefore, no positive impact of heat treatment was observed on *in vitro* protein digestibility, not even with the inactivation of TI. We suggest that protein aggregation plays a major role in limiting protein digestibility in samples heated at high temperatures, even when TI is significantly inactivated.

Acknowledgments

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Keywords : plant proteins, protein digestibility, nutritional quality, trypsin inhibitors

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(21553) - IMPACT OF ELDERLY GASTROINTESTINAL ALTERATIONS ON IN VITRO GASTRIC EMPTYING AND DIGESTION KINETICS

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Abstract

Aging is usually accompanied by physiological changes such as increased gastric pH, decreased secretion of digestive juices and gastric acid, weakened gastrointestinal peristalsis, and decreased activity of digestive enzymes. However, quantitative studies on the effects of these changes on the characteristics of food digestion and gastric emptying in the elderly are lacking. In this study, the dynamic *in vitro* human gastrointestinal system was used to simulate the gastric environments of the elderly and young adult. The microstructure, rheology, gastric emptying, starch/proteolysis of skim milk and Tibetan staple food tsampa (mainly composed of highland barley) under simulated gastrointestinal digestion conditions of the elderly and young, were systematically studied, respectively. The results showed that for both the skim milk and tsampa, a more compact microstructure and higher shear viscosity as well as more significant protein/particle aggregation were shown in the elderly model during gastric digestion. These resulted in a remarkable delay in the gastric emptying (tsampa: 103.4 min *vs*. 83.1 min; skim milk: 26.5 min *vs*. 22.5 min) and a lower proteolysis (tsampa: 15.8% *vs*. 20.1%; skim milk: 37.2% *vs*. 42.3%) for the elderly, mainly due to the reduced secretion of gastric juice (including gastric acid and pepsin), and the weakened gastric contractions. Moreover, milk proteins, particularly β-lactoglobulin, was more resist to hydrolysis throughout elderly digestion. This study is meaningful for future development of milk products and highland barley-based food that are more suitable for the elderly.

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Keywords : gastric emptying, in vitro digestion system, elderly digestion, skim milk

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(21554) - ORAL-GASTRIC BIOACCESSIBILITY OF INGREDIENT FROM AVOCADO SEED WITH ANTI-H. PYLORI ACTIVITY

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Abstract

Gastric adenocarcinomas are one principal global prevalence, developing from risk factors that include genetic, occidental dietary, promiscuous *Helicobacter pylori* infection, and others. *Helicobacter pylori* is a gram-negative bacterial, microaerophilic to colonize the digestive tract, principal the stomach (Azadbakht *et al.,* 2023). The avocado seed has been used in ancient cultures due potential against gastrointestinal diseases, currently, known that it has high concentrations of bioactive compounds. The objective of the work was to develop a food ingredient by phenolic and lipophilic seed avocado compounds and evaluate bioaccessibility changes and antimicrobial potential against *Helicobacter pylori* during oral-gastric digestion *in vitro* and possible mechanism of action with *in silico* tools. Among the main results, was the presence of oleic, linoleic, and avocadenofuran, in the lipophilic fraction. While the phenolic fraction highlighted the presence of catechin, rutin, ellagic, and chlorogenic acid, and others. Observing changes in the profile during oral-gastric digestion, mainly attributed to acid hydrolysis and mechanical changes. The developed ingredient presented a total inhibition of the clinical and reference strains of *Helicobacter pylori* (3.08 µg/mL), with the gastric phase 10 min being where the most inhibition was presented with more than 90% of the strains, followed by the gastric phase 25 min. Likewise, the lipophilic fraction presented a lower inhibition concentration of 2.59 µg/mL regardless of the bacterial phenotypes, having the same inhibitory behavior during oral-gastric digestion. The main mechanisms found *in silico*, inhibition of target proteins such as CAGA, BABA, and MUC5 are observed, involving the mechanisms of adherence and bacterial pathogenicity. The results suggest that the developed ingredient has antimicrobial potential against *Helicobacter pylori*, through phenolic and mainly lipophilic compounds, mainly in gastric phases 10 and 25 min, because phenolic and lipophilic compounds are bioaccessible in these phases.

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Keywords : Avocado seed, Oral-gastric digestion, New ingredients, Anti-Helicobacter pylori, Byproducts

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(21557) - DETERMINATION OF DIGESTIVE ENZYMES ACTIVITIES IN HUMAN DUODENAL FLUIDS UNDER FASTING AND FED STATE

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Abstract

The improvement of *in vitro* digestion models is essential and depends, among others, on a better knowledge of the enzymatic activities in the digestive tract of humans. Within the framework of a collaboration between INFOGEST and UNGAP, a cost action on oral drug absorption, access to intestinal fluids was possible. Human intestinal fluids were collected under fasting and fed state conditions using a catheter placed in the duodenum. Intestinal fluids from 13 healthy adult volunteers were used for the determination of digestive enzyme activities. Intestinal fluids were collected kinetically over 110 min after ingestion of 240 ml of water (fasted) or 400 ml of Ensure Plus vanilla followed 20 min after by 240 mL of water (fed), leading to a total of 88 samples. Immediately after collection, intestinal fluids were mixed with glycerol (1:1) and inhibitors to protect digestive enzymes from enzymatic breakdown. Then, trypsin, chymotrypsin, and lipase activities were determined using the enzymatic assays protocols described in Brodkorb *et al*. (2019), except that the temperature for the trypsin assay was 37°C. The pH of each sample was also measured immediately after collection. Mean values of 64.9 ± 28.5, 24.2 ± 10.2 and 389 ± 253 Units /ml of duodenal content (fasted) and 62.8 ± 28.9, 22.9 ± 9.6 and 1071 ± 60.7 Units /ml of duodenal content (fed) were obtained for trypsin, chymotrypsin and lipase, respectively. Mean pH was 6.3 ± 1.3 and 6.1 ± 0.8 under fasted and fed state conditions, respectively. No effect of nutritional state (fasted vs fed) on the enzymatic nor on the pH was observed. Due to a high inter-individual variability between volunteers, no effect of the time of sampling was observed on the activity level of trypsin, chymotrypsin and lipase in the duodenum. Few data are currently available in the literature and this new set of data is of major importance. More *in vivo* data on enzymes activities in the upper part of the gastro-intestinal tract in different prandial states would be needed to help the scientific community improving *in vitro* digestion models.

References

Acknowledgments

Keywords : digestive enzymes activities

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(21563) - ABSORPTION MODELS BASED ON MONOLAYERS FROM CELLULAR LINES OR PORCINE DUODENAL ORGANOIDS

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Abstract

Foods are essential to give us the macro- and micro-nutrients we require, but they are also carriers of bioactive compounds that can have an impact on our health beyond the nutrition. Both, nutrients and bioactive compounds, need to be (bio)accessible in the foods so that, after the intestinal digestion, they become (bio)available for their absorption, which is the first step to deliver these molecules to the target tissues where they will exert their (bio)activity. Fermented foods carry out a wide variety of nutrients and bioactive molecules; then, to understand the complex mechanisms behind their health benefits, in vitro and ex vivo models, as close as possible to the in vivo physiological state, are required. In this work we have implemented two absorption models mimicking anatomical locations of the gut, a human-colonocyte model, based on the use of commercially available cellular lines Caco2 and HT29-MTX, and a porcine-duodenal model, obtained from organoids generated from the stem cells located in the intestinal crypts of duodenal tissue excised from the carcass of piglets (*Sus scrofa domestica*) from the "Gochu Asturcelta" breed. Parameters determining the selective permeability of the monolayers, such as the transepithelial electrical resistance (TEER) determining the strength of the tight junctions, and the paracellular transit of a fluorescent molecule (lucifer yellow), were different, as also occurs in the two anatomical locations in an *in vivo* situation. In general, the TEER of mixed Caco2:HT29-MTX monolayers (ratio 70:30, 21 days post-seeding) was higher that the monolayers (5 days post-seeding) obtained from pig duodenal organoids. Concomitantly, the apparent permeability coefficient was about 10-times higher for the duodenal monolayer than for the colonic one. These results are in good agreement with the physiological situation since at the colonic location the intestinal barrier is reinforced and is less permeable as a mechanism of protection against the intestinal microbiota; however, in the small intestine which is less populated, the permeability is higher allowing the uptake of nutrients. Finally, the transport of two types of nutrients (riboflavin) and bioactive molecules (GABA) that can be synthesized by bacteria during foods fermentations, also varied between monolayers. Therefore, both models can be proposed to study the absorption of molecules that can be present in different gut niches. Porcine-duodenal organoid-derived monolayers will be useful for testing transport of food digested components, while colonocyte-derived monolayers might be applied to study the absorption of microbiota-derived molecules.

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Acknowledgments

This work was supported by the projects RTI2018-096339-B-I00 (funded by MCIU/AEI/FEDER, UE) and PID2021-127362OB-I00 (funded by MCIN/AEI/ 10.13039/501100011033 and by "ERDF A way of making Europe). The autonomic Government of Principado de Asturias also has financed this work through the AYUD-2021-50910 project (FICYT, supported by FEDER) and the predoctoral "Severo Ochoa" fellowship (BP17-55) granted by H. Tamés.

Keywords : intestinal cellular lines, duodenal pig organoids, absorption, TEER, lucifer yellow

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(21568) - ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOUND CONTENT OF CHIA SEEDS AND SPROUTS AFTER SIMULATED DIGESTION PROCESS

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Abstract

Chia seed (*Salvia hispanica* L.) is an herbaceous plant whose health benefits are mainly attributed to its nutritional properties, since it is composed of a high amount of proteins, polyunsaturated fatty acids (omega 3 and 6), fiber, minerals, and different bioactive compounds with antioxidant action, such as phenolic compounds. Phenolic compounds are substances that have recognized antioxidant capacity and can have their biological activity altered by the digestion process. Furthermore, its antioxidant capacity can also be improved through food processing, such as germination. The objective of this study was to investigate the antioxidant activity and phenolic compound content of chia seeds and sprouts before and after the simulated digestion process. Chia seeds were germinated for 6 days on filter paper suitable for germination. Chia seeds (6g) and chia sprouts (6g) were subjected to a simulated digestion process according to the INFOGEST 2.0 protocol. The content of phenolic compounds was determined using the Folin-Ciocalteau method, and the antioxidant activity was determined using the FRAP, ABTS and DPPH methods. Analyses were carried out before simulated digestion and after the enteric phase in duplicate. The data were treated statistically using analysis of variance (ANOVA), using Tukey's test to verify significant differences, and the significance level adopted will be 5% ($p < 0.05$). The mean and standard deviation of total phenolic compounds before and after the enteric phase of digestion present in the seeds were 2.37 ± 0.05 and 6.78 ± 0.16, respectively. For sprouts, the mean and standard deviation of total phenolic compounds before and after the enteric phase of digestion were 4.34 ± 0.04 and 6.43 ± 0.07, respectively. It was observed that after simulated digestion, the total phenolic contents of chia seed and sprout extracts increased during the intestinal phase compared to the extracts before digestion. In regard to antioxidant activity, there was a reduction after the intestinal phase in all methods analyzed, with the exception of the ABTS method, which showed increased antioxidant activity in the enteric phase when compared to the extract before digestion. Gastric and intestinal fluids interfere with the antioxidant activity of phenolics due to deprotonation of the hydroxyl portions of aromatic rings. The increase in antioxidant activity by the ABTS method after the intestinal phase of digestion may be related to the increase in the content of phenolic compounds after digestion of both chia seeds and sprouts.

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Acknowledgments

This study was partially financed by the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES) – Financing Code 001, National Council for Scientific and Technological Development (CNPq); and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) (project numbers E-26/211.830/2021)

Keywords : germination, sprouted grains, in vitro digestion, seed, functional food

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22577) - COMPARISON BETWEEN THE ABSORPTION OF ANTHOCYANINS FROM EDIBLE FLOWERS USING TWO INTESTINAL MODELS: CACO-2 AND CACO-2/HT29-MTX

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Abstract

Edible flowers are re-emerging as an alternative and healthier food source. Among them, anthocyanin-rich edible flowers are one of the most interesting group. However it is crucial to understand to aspects related to their bioaccesibility and absorption[1]. The absorption of anthocyanins at the intestinal level is a critical aspect of understanding the bioavailability of these dietary compounds [2]. In this study, we employed in vitro cell models to investigate anthocyanin absorption, specifically comparing the widely used Caco-2 model with a more comprehensive co-culture model, Caco-2/HT29-MTX.

Our primary objective was to assess the adequacy of the Caco-2 model in predicting in vivo anthocyanin absorption and determine whether a more complex co-culture model offers a more accurate representation.

Using an in vitro approach with Transwell® systems, Caco-2 and Caco-2/HT29-MTX (9:1) cells were seeded at $3X10^5$ cells/mL and let grow for 21 days until reach stable TEER (350-400 $\Omega.cm^2$ for caco-2 model and 200-250 $\Omega.cm^2$ for caco-2/HT29-MTX model) values with the formation of a cell barrier. The different anthocyanin extracts, derived from the different edible flowers (*centaurea cyanus*, *clitoria ternatea*, *cosmos bipinnatus* and *viola tricolor*) with distinct structural compositions, were applied at a non-toxic concentration of 0.8 mg/mL (verified by MTT cytotoxicity assays). We then monitored transport kinetics over 240 minutes, collecting and analyzing samples via UHPLC-DAD at 520 nm.

Structural variations in anthocyanins influenced absorption in both models. Surprisingly, the co-culture model exhibited significantly lower transport efficiency (2.5-3% after 240 min) compared to the monoculture model (4.5-5% after 240 min) for all anthocyanin extracts, despite a lower TEER. These results indicate that the Caco-2 model may overestimate real absorption rates.

The co-culture model, with a TEER closer to the in vivo intestine (around 120 $\Omega.cm^2$) and mucus-producing cells, emerged as a more relevant representation. Our findings suggest that monoculture models like Caco-2 may not accurately reflect anthocyanin absorption rates, emphasizing the importance of utilizing more sophisticated models for a nuanced understanding of dietary compound absorption.

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Acknowledgments

The authors acknowledge the national funds provided to the development of this work, by Fundação para a Ciência e Tecnologia (FCT) under the scope of the project “AnthoE.Flos” (2022.01014.PTDC).

Keywords : Anthocyanins, caco-2, HT29-MTX, Edible flowers, Transwell

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22586) - COMBINING COMPUTATIONAL AND IN VITRO MODELS TO BETTER UNDERSTAND THE GUT MICROBIOME

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Abstract

The gut microbiome (GM) plays an important role in host health and disease. To better study the GM under controlled laboratory conditions, we previously developed MiGut – a miniaturised adaptation of the triple-stage chemostat with features that dramatically improve reliability, automation, and scalability compared to similar systems [1].

In this work, we outline how a computational model can be used in conjunction with our state-of-the-art in vitro model to better understand the microbial dynamics of the GM. To achieve this, we adapt the generalised Lotka-Volterra equations [2], expanding their scope to reflect MiGut's triple-stage setup and include readings of physiochemical parameters such as pH and adding terms through data-driven machine learning. Using these methods, it is possible to infer features such as microbial growth rates and susceptibility to external perturbations such as antibiotics or dietary interventions. This, in turn, can be used to assess microbiome resilience, identify keystone species, and inform microbe-microbe interactions. Together, outputs of these in vitro and computational models can be used to predict in vivo human responses to a treatment or intervention.

These developments in computational modelling go hand-in-hand with the advancements offered by MiGut – it is only through the system's increased throughput that we have suitably large datasets for training and validating our computational models. As links between the GM and health continue to be uncovered and microbiome-modulating treatments become more prevalent, there is a need for tools that support a mechanistic understanding of the GM. This combination of in vitro and computational models offers significant potential to better understand the complex microbial dynamics that are critical to gut function.

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This work is funded through the Michael Beverley Innovation Fellowship (University of Leeds, UK).

Keywords : machine learning, in silico, computational, simulation, microbiome

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22587) - THE CELLULAR SENSING TO MIXED MICELLES, A PLUG-IN OR A NECESSARY LEG FOR IN VITRO DIGESTION PROTOCOLS?

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Abstract

The potential applications of *in vitro* digestion protocols go beyond quantification of the efficiency of bioaccessibility and/or classification of any significant cause and effect on it. Indeed, their hyphenation with cell culture models of the gut, allow the visualization of the inherent ability of intestinal epithelium to functionally adapt to the stimuli that digested food triggers in the tissue. Definitively, the visualization of such effects is more enriching when the changes during both *in vitro* digestion and assimilation of a complex mixture of target bioactive compounds are followed. This is particularly essential when the bioaccessibility of food lipids is measured without any labelling or tagging process to those lipids because, once they are micellarized and incorporated to the cells, they will follow the cellular lipid metabolism without any chance to distinguish what it is recently incorporated by the cell, it is metabolized or it was already there. Therefore, the aim of this study was to visualize the changes in lipid metabolism of a cellular culture sensing micelles from different food origins. The experimental approach of this study was to determine the lipid profiles of different food matrices supplemented with a concentrate of milk fat globule membrane (MFGM), which provides the food matrix with a wide range of non-polar and polar lipids. The static INFOGEST digestion protocol was applied. Then, the lipid profile was measured in the micellar fractions isolated after *in vitro* digestion and, finally, in the extracts from Caco-2 cells incubated previously with those micellar fractions. Data were statistically analyzed by a partial least-square discriminant analysis to measure the ability of each lipid class in predicting the origin of the food matrix for both non-digested and digested samples. The analysis of significant values for the variable importance in projection scores denoted the lipid classes with discriminant ability. Cell culture sensing to the different micelles and the functional adaptation of the cellular lipid profiles was determined in a similar fashion. Results show that every food matrix provides micellar lipids with a dissimilar composition, while the cells could sense the different micellar lipid contents. Considering the 'food matrix' as the main effector, changes in the cellular lipid profiles were produced. Therefore, Caco-2 cells could sense the micelles originated from changing food matrices where the MFGM was incorporated. The inclusion of cell culture models in the experimental design of *in vitro* digestion protocols applied to lipids in food matrices is essential to understand the early events that take place in the cell culture countering the micellar contents and adapting the cell metabolism.

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Keywords : food lipids, cell culture, cellular sensing, lipid metabolism

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22599) - IN VITRO DIGESTION OF PROTEIN-RICH DAIRY PRODUCTS ADAPTED TO THE SPECIFIC NEEDS OF OLDER ADULTS

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Abstract

Insufficient energy and protein intake can lead to a condition called sarcopenia, characterized by the loss of muscle mass, strength and function. To avoid this condition healthy older adults need to consume nutrient-dense foods and to increase the amount of high-quality proteins in their diet to promote muscle health. However, it is still unclear if changes in nutrients digestibility in old age may affect the anabolic effect of foods. The objective of this study was to investigate *in vitro* the digestion of two high-protein dairy products similar to cream cheese (containing 24% w/w of proteins, and 20% w/w of lipids). Products were formulated with two different ratios of caseins to whey proteins: 80/20 (= WP20) similar to the ratio found in bovine milk, and 20/80 (WP80) in order to increase the leucine supply. The new static *in vitro* digestion model adapted to the general older adult population (≥65 y) proposed by INFOGEST [1] was implemented to investigate the digestion of these products, as well as the standard version of the protocol. Kinetics of proteolysis and lipolysis were compared between both models for each product, in the gastric and intestinal phase of digestion. Proteolysis was studied with the OPA method, SDS-PAGE, and amino acids were quantified by HPLC, while lipolysis was investigated through GC-MS, and the structure of the products in gastric conditions was observed by CLSM. In both products, the degree of protein hydrolysis (DHP) was significantly lower in older adults' conditions than in young at the end of the gastric phase (-19% for WP20, and -44% for WP80), and at the end of the intestinal phase (-16% for WP20, and -20% for WP80). This is most probably due to the reduction in pepsin and pancreatin activities recommended in the older adult model compared to the standard protocol. The degree of lipid hydrolysis (DHL) was also significantly lower in older adults' conditions than in young at the end of the digestion for WP20 (-30%), but interestingly it was not the case for WP80 (similar DHL were measured). Free fatty acids were also released faster from WP80 than from WP20 in both digestion conditions: after 5 min of intestinal digestion DHL was already ≈ 32% for WP80 against 14% for WP20. This was attributed to the different caseins/whey protein ratios in the products, leading to the formation of different gel structures that may in turn result in different patterns of deconstruction in the gastrointestinal tract.

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Acknowledgments

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Keywords : elderly, proteolysis, lipolysis, caseins, whey proteins

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(22610) - IN VITRO DIGESTION OF CHLOROPHYLLS

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Abstract

Despite the high daily consumption of chlorophylls, the availability of scientific data regarding chlorophyll bioaccessibility is scarce. The main reasons are the lability of chlorophylls as well as the diversity of structures. The harmonized INFOGEST 2.0 protocol was developed taking proteins as target compounds. However, the chemical properties of chlorophylls hamper the direct application of the protocol and demand a specific adaptation to these lipophilic phytochemicals. Determinant variables such as type of mixing, the need for gastric lipase and ultracentrifugation were tested. With this aim, three extreme food matrices, one rich in fiber (vegetable puree), one rich in fat (virgin olive oil), and one liquid fruit juice), were assayed for chlorophyll *in vitro* digestion stability, controlling crucial variables. These factors affected the results during the application of the *in vitro* standardized protocol and consequently the steps that can be biased during the estimation of chlorophyll bioaccessibility were identified. As a result, a specific protocol based on INFOGEST 2.0 has been defined for chlorophyll structures (and lipophilic compounds). Applying such a protocol, it was analyzed the effect of food composition on chlorophyll bioaccessibility after *in vitro* digestion. Ten commercial foods (guacamole, virgin olive oil, tortellini, basil hummus, creamed spinach, vegetable pasta, green tea chocolate, avocado and kiwi juices, and pesto sauce), were selected based on their different nutritional (fat, fiber, protein, and carbohydrates) and chlorophyll composition and content. Results showed that independently of the foods' nutritional composition or food matrix, chlorophyll degradation during the *in vitro* digestion was exclusively correlated with the salt content of the digested food. In parallel, independently of the chlorophyll profile, after *in vitro* digestion, digested foods were formed by 90% pheophytins and 10% chlorophylls and pheophorbides.

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Acknowledgments

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Keywords : chlorophyll, gastric lipase, mixing, ultracentrifugation, pheophytin

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(22617) - IN VITRO DIGESTIBILITY OF HONEY CHLORELLA VULGARIS: STRUCTURAL INSIGHTS AND DIGESTIVE BEHAVIOUR IN SIMULATED GASTROINTESTINAL CONDITIONS

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Abstract

Concerns about the current food systems have driven the exploration for novel protein sources for a more sustainable diet. Microalgae, single-cell aquatic microorganisms, are gaining attention due to their rapid growth in bioreactors. *Chlorella vulgaris* has a relatively high protein content and is one of the best-studied species of microalgae. Honey *Chlorella vulgaris* is yellow in colour and has a milder taste, which makes it suitable for diverse food applications. However, the protein is mostly located within the cell wall, which may hinder accessibility to digestive enzymes (1). Understanding its structure, digestion pattern and outcome is quintessential when introducing new dietary sources. Therefore, this study aims to reveal honey *C. vulgaris*' structure prior and post processing and its impact on digestive behaviour in simulated gastrointestinal conditions.

A commercial honey *C. vulgaris* powder with 29 % protein, 45 % carbohydrates, 11 % lipids and 3 % moisture was studied in the form of 4 % protein suspension. To better focus on the physiological changes in the gastrointestinal tract, a standardised semi-dynamic *in vitro* digestion model was employed to explore the digestive performance. Five gastric emptying (GE) points were removed during the 67.5 min gastric phase. For each GE point, the subsequent intestinal digestion was conducted using the static INFOGEST method. The structure of the non-digested sample and the dynamics during the simulated digestion were evaluated by Confocal Laser Scanning Microscopy (CLSM) and particle size distribution. The gastric and intestinal digesta were further evaluated on the bioaccessible protein content by o-phthaldialdehyde (OPA) assay and protein size distribution via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and size exclusion chromatography (SEC).

The preliminary results suggested that the aggregated microalgae cells gradually disassociated during gastric digestion. Because of the low solubility of the substrates, the inhomogeneous system led to different protein breakdowns by pepsin at different gastric emptying points and consequently influenced the intestinal digestion.

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Acknowledgments

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Keywords : Microalgae, protein digestion, in vitro digestion

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(22628) - ON THE PATH TO HEALTHIER COOKIES: THE PARTICLE SIZE OF THE WHEAT RAW MATERIAL AFFECTS THE GLYCAEMIC RESPONSE

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1 - KU Leuven

Abstract

Starch is the main glycemic component in the human diet. Its digestion and subsequent postprandial glycemic response depend *inter alia* on its molecular structure, the surrounding matrix and the processing conditions during food manufacturing. Consumption of slowly digestible starch has been linked to improved glucose homeostasis as it provides sustained energy release. When soft wheat is coarsely milled, the endosperm cell walls of the resulting farina retain a high degree of integrity. These physical barriers in principle can protect the enclosed starch granules and thus retard and/or reduce the extent of starch digestion (Korompokis et al., 2019). We here showed by optical microscopy that most endosperm cell walls in coarse farina (average particle size: 1,000 µm), unlike those in refined flour (85 µm), were still intact. We next produced wire-cut cookies and substituted part of the flour (*i.e.* 20, 40, 60, 65% on starch dry matter basis) by coarse farina. Substitution by 65% resulted in 28% slower and 14% less *in vitro* starch digestion, indicating that the cell walls of soft wheat protected starch from being enzymatically hydrolyzed. These encouraging *in vitro* results led to the execution of a human intervention study, in which the hypothesis that the (s)lower starch digestion observed *in vitro* would elicit lower glycemic responses *in vivo* (Edwards et al., 2015) was tested. The glycemic response to the consumption of two test cookies (i.e. control cookie and cookie made with 65% coarse farina, 85 g per cookie) was determined over 180 min in 55 healthy participants (28 females, 27 males). Analysis of the data revealed that the cookie made with 65% coarse farina was digested faster than the control, with significantly lower blood glucose concentration from 90 min onwards. We hypothesize that the faster absorption of sucrose and lower starch availability as a result of the coarser matrix were responsible for the effect. While the difference in glycemic response between the two cookies was overall not significant ($p = 0.223$), interestingly there was a clear trend in male participants ($p = 0.088$), whereby the control cookie elicited a 31% higher glycemic response than the cookie made with 65% coarse farina. The impact of oral processing behavior and gastric emptying rate on the glycemic response is currently being evaluated. In conclusion, the intelligent choice of raw materials may be a promising strategy to produce highly nutritious, low-moisture starchy foods that provide sustained energy release.

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Keywords : Starch, Cookie, Wheat, Glycaemic response, Particle size

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22631) - IMPACT OF AGEING ON THE PROTEIN DIGESTIBILITY OF CHEESE MATRICES

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Abstract

According to the latest available data, elderly people (> 60 years old) will represent one person in six of the world's population by 2030, (i.e., 1.4 billion people) (WHO 2022). Advancing age is associated with a musculoskeletal weakness due to a reduction in muscle mass and with digestive system malfunctions such as slower gastric emptying, reduced gastric secretion and a more alkaline environment (Kassis et al. 2023). Therefore, to meet the specific needs of this population, SODIAAL aims to formulate cheeses whose nutritional benefits will be demonstrated by studying their digestibility with the static *in vitro* model described by the *INFOGEST* consortium (Menard et al. 2023; Minekus et al. 2014).

As a first step, our work focused on the optimization of the oral phase for semi-solid to solid cheese matrices, considering two parameters: the saliva/food ratio and the particle size distribution of the bolus. As a second step, semi-solid cheese ("Petit Suisse", Yoplait) was subjected to both adult and older adult static *in vitro* digestion models with the objective to understand the influence of age on the rate of proteolysis of a cheese product. For this purpose, total NH_2 groups released during the gastric phase were analysed using a colorimetric method. Moreover, the amino acids released during the intestinal phase were studied by chromatography.

Regarding the optimization of the oral phase, results showed that the saliva/food ratio was dependent of the initial water content of the cheese matrix to reach the targeted viscosity. For the semi-solid cheese ("Petit Suisse"), the saliva/food ratio was 1:0.15 while it was 1:1 for solid cheese ("Comté"). For solid cheese matrices, grinding with a coffee grinder resulted in particles of around 2.7 mm, which mimics as closely as possible what happens after chewing (Jalabert-Malbos et al. 2007).

Regarding the comparison of both digestion models, the monitoring of proteolysis during the gastric phase revealed a slower and lower total release of NH_2 groups for the older adult model. This is in agreement with the enzyme quantities that were used. In the same way, the proteolysis was less pronounced during the intestinal phase for the older adult model. Therefore, there is less essential amino acids released, including branched-chain amino acids involved in muscle synthesis.

The implementation of the static *in vitro* digestion model for semi-solid cheese gives interesting results to better understand the digestion of elderly people. More studies will be necessary to improve the monitoring of solid samples during gastric and intestinal phases, in order to compare the nutritional benefits of different cheese matrices.

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WHO 2022

Keywords : Cheeses, Proteolysis, Amino acids bio accessibility, In vitro digestion

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22639) - IN VIVO AND IN VITRO EVALUATION OF AN INNOVATIVE COMMON BEAN-BASED NOODLE FOR PATIENTS WITH TYPE 2 DIABETES

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Abstract

Consumption of low-glycemic, nutrient-rich foods such as pulses rich in slowly digestible starch, fiber, and minerals, is crucial for managing type 2 diabetes (T2D). This study aimed to formulate a common-based noodle with attenuated blood glucose and insulin responses, specifically targeting the population of patients with T2D. Given the prevalent iron deficiency among patients with T2D particularly in low and middle income countries, cowpea leaf powder was incorporated into the noodle to augment iron and zinc contents.

The noodle product was developed from whole wheat flour (70%), flour of a high-iron and high-zinc common bean variety Nyota (20%), and cowpea leaves (10%). It contained approximately 70% carbohydrates (30% starch), 19% crude protein, 3% crude fat, 5% crude fiber, and 3% ash, with iron and zinc contents reaching 28 and 21 mg/100g dry matter, respectively. Starch and protein digestion kinetics of the bean-based noodle investigated through static *in vitro* digestion (INFOGEST) showed significantly slower starch digestion in the bean-based noodle, combined with a more rapid and extensive proteolysis compared to a purely-wheat based noodle.

Blood glucose and insulin responses as well as subjective satiety responses were evaluated in healthy volunteers. The bean-based noodle showed a glycemic index (GI) of 34 and food insulin index (FII) of 25, significantly lower than an equi-carbohydrate and iso-caloric glucose reference food. These values were also notably lower as compared to those typically reported for wheat-based noodles, i.e. 46-52[1] (GI)[1] and 46 (FII)[2]. Significant differences in hunger, satiety, and prospective food consumption responses were observed over 2 hours following post-consumption compared to the reference.

Next, sensory properties, subjective satiety as well as blood glucose responses were evaluated among the targeted population group of patients with T2D. All participants found the bean-based noodle acceptable, on average scoring values above 8/10 for flavor, texture, and overall acceptability.

In summary, the substitution of wheat flour by bean flour and cowpea leaf powder improved the nutritional profile, sensory attributes, and postprandial response to consumption of this easy-to-prepare noodle product. This suggests the potential of the developed noodle as a promising and suitable option to replace high-GI and FII wheat- and rice-based staples for those managing T2D. Future studies should evaluate mineral bioavailability as well.

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Acknowledgments

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Keywords : bean-based noodle, type 2 diabetes, glycemic index, food insulin index, in vitro-in vivo study

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22645) - A UNIFYING APPROACH TO LIPOLYSIS UNDER INFOGEST CONDITIONS: THE IMPORTANCE OF TOTAL SURFACE AREA AND COALESCENCE

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Abstract

Although the INFOGEST *in vitro* digestion model has been widely used to evaluate lipid digestion, the effect of the underlying lipolysis kinetics is not well recognized. We used WPI-stabilized emulsions (d_{32} = 0.16 or 7.2 μm) with different oil dose (0.28 to 56.35 mM) under different physiological conditions (lipase activity, bile salt concentration).

In general, emulsions with smaller droplets, and less oil show higher lipolysis rates; however, when relating the measured lipolysis rates to the surface area available (either through a variation of droplet size, or amount of lipid used), the lipolysis rates show remarkably similar behaviour when focussing on the initial lipolysis rates. It is important to point out that most probably coalescence plays an important role in reducing the observed lipolysis rates. This points to a reduced surface area effect rather than effects related to the kinetics of the reaction.

The results of this project clearly show that the outcomes obtained under various conditions (which also would imply at different labs) can be unified, that is as long as the surface area is known, including coalescence. This study will contribute to the target-release of oil and lipophilic bioactive compounds in the small intestine.

Acknowledgments

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Keywords : INFOGEST, in vitro intestinal digestion, lipolysis kinetics, coalescence

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22654) - COMPARISON OF AMINO ACID DIGESTIBILITY BETWEEN YOUNGER AND OLDER ADULTS USING THE INFOGEST DIGESTION PROTOCOL

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Abstract

The quantity and the quality of dietary protein intake are key elements to consider for maintenance of good health conditions in adults and elderly people. The quality is determined by the amino acid composition and the digestibility of the protein source. Older adults are suspected to have reduced amino acid digestibility, due to physiological changes in the ageing gastrointestinal tract. However, data are still limited, and any differences observed between 2 populations of different age may depend on the digestibility of the protein sources.

In this study, we aim to compare the amino acid digestibility of three different sources of proteins: milk, sorghum and black beans using the INFOGEST static protocol mimicking adult and elderly gastrointestinal conditions1,2 which are characterized by lower concentration of enzymes in the gastric (-40%) and intestinal phase (-20 to -30%) for elderly conditions. For testing the plant proteins, black beans were first soaked for overnight followed by cooking in boiling water for 60 min while sorghum was cooked in boiling water for 80 min before subjected to Infogest protocol. At the end of the intestinal phase, the digested samples were separated into digestible (potentially absorbable) and un-digestible (non-absorbable) fractions by precipitation with 80% (v/v) methanol.

Skim milk powder and cooked black beans exhibited very high digestibility both in adults and elderly in vitro conditions. Skim milk powder: 96.7+/- 3.2% vs 95.2+/- 2.2 % and cooked black beans: 95.5 +/- 2.1 % vs 94.1+/- 0.7% in adult and elderly conditions, respectively. By contrast, digestibility was much lower for the cooked sorghum with 66.0+/- 6.5% yet significantly higher than under elderly digestive conditions: 56.2 +/- 6.6%.

Our data suggest that the digestibility of high-quality proteins such as skim milk or black beans are similar in young and older adults. However, the digestibility for proteins such as sorghum that has a much lower digestibility, seems to be affected in older adults. The validation of these results needs to be confirmed by in vivo studies.

References

1 Sousa et al, 2023 ; 2 Menard et al, 2023

Keywords : plants, proteins, digestibility, senior, adults

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22656) - MILK PEPTIDES FOUND IN HUMAN JEJUNUM INDUCE CCK AND GLP-1 SECRETION AND INHIBIT DPP-IV

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Abstract

During food ingestion, gastrointestinal hormones such as glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK) are released in response to nutrients. GLP-1 has a short life due to its degradation by the aminopeptidase dipeptidyl peptidase-IV (DPP-IV), which is expressed at the gastrointestinal tract with maximum secretion in jejunum and ileum, and also circulates in plasma. The role of the DPP-IV enzyme as well as GLP-1, are crucial in glucose homeostasis, especially in type-2 diabetic patients.

This work aims to characterize the DPP-IV inhibitory activity and GLP-1/CCK secretagogue effect of peptides derived from the major milk proteins previously found in human jejunal digests after casein or whey oral ingestion (1). GLP-1 and CCK secretion was evaluated in STC-1 enteroendocrine cells while DPP-IV inhibition was assayed in non-differentiated Caco-2 cells. The intracellular calcium concentration was measured in order to study STC-1 cell activation. Hormone secretion was found to be sequence-specific. For instance, the consecutive loss of amino acids at the N-terminal end of the peptide ^{6}LNVPGEIVE^{14} reduced stepwise CCK secretion. This same event occurred for peptide ^{81}PVVVPPFLQPE^{91} where deletion of proline at the N-terminal end reduced both CCK and GLP-1 secretion. However, for peptides ^{172}LPVPQ^{175} and ^{59}VYPFPGPIPN^{68} the loss of amino acids at the N-terminal end increased GLP-1 and CCK release. Regarding DPP-IV enzyme inhibition, sequences derived from β-casein ^{8}VPGEIVE^{14}, ^{85}PPFLQPE^{92}, ^{89}QPEV^{92}, ^{60}YPFPG^{64}, and ^{60}YPFPGPI^{66} were shown to have a strong inhibitory potential on the DPP-IV enzyme, supporting the important role of proline in second N-terminal position for this inhibitory effect. In conclusion, peptide sequences found in human jejunum after ingestion of milk proteins are able to significantly stimulate CCK and GLP-1 secretion, while inhibiting DPP-IV, which is important to reduce hormone degradation. Therefore, these sequences have been shown to have the potential to control postprandial glycemia.

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Acknowledgments

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Keywords : glucagon-like peptide-1, dipeptidyl peptidase-IV, cholecystokinin, enteroendocrine cells, milk peptides

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22662) - AGE-OPTIMIZED DIGESTION OF TWO HIGH PROTEIN DAIRY PRODUCTS: A COMPARATIVE STUDY USING THE IN VITRO SEMI-DYNAMIC GASTRIC DIGESTION MODEL FOR OLDER ADULTS

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Abstract

As gastrointestinal changes occur with age, the digestibility of high protein foods tailored for older persons must be established. When examining food digestion targeted for a specific population using static or semi-dynamic models, it is critical to use standardised gastrointestinal parameters established from *in vivo* data. This study was part of a larger multi-approach EU project, Eat4Age. We used both the adult and older adult (>65 years old) standardised gastrointestinal parameters to simulate digestion of two near isocaloric fermented dairy products (modified formulation *vs.* commercial product). As such, *in vitro* semi-dynamic gastric digestion consisting of three gastric emptying points and a simulated small intestinal static phase was done. The fermented modified product had an 80:20 whey protein:casein ratio and was coupled with a dairy based high protein caramel-like base that was enriched with whey proteins to give a final protein concentration of 12% in the combined product. As a control, the commercial Skyr product consisting of mostly caseins was also combined with a commercial dairy derived caramel-like base (*dulce de leche*). We have addressed in detail how the parameters of the older adult, as indicated by the INFOGEST consensus on the parameters of the older adult, were applied to a standardised gastric semi-dynamic model, as this has yet to be precisely optimised in the existing literature. By quantifying the free amino acids present in the digesta following precipitation with methanol or TCA and subsequent acid hydrolysis of the supernatant, we compared the evolution of proteolysis of the two products per emptying time. SDS PAGE and size exclusion chromatography were used to determine the molecular weight distribution of the proteins in the soluble fraction of the digestion. We hypothesise that the modified formulated dairy product will undergo more gastric proteolysis than the commercial control product. Next to that, there may be differences in proteolysis between the adult and older models, particularly in the gastric phase. This study will provide the initial steps towards standardising parameters for older adults in a semi-dynamic gastric digestion setup.

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Keywords : Semi-dynamic digestion; older adult; protein digestion

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22680) - IN SILICO COMPARISON OF VISCOSITY-DEPENDENT AGITATION PERFORMANCE FOR INFOGEST STUDIES

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Abstract

Digestion and fermentation processes are commonly studied *in vitro* due to inaccessibility of lower GI for *in vivo* measurement, but often poor representation or knowledge of appropriate physiological conditions reduces confidence in the outcomes. The standardized INFOGEST protocols [1] for static and semi-dynamic digestion models enable consistent comparison of independent studies and make such models more accessible. However, such models are unable to predict digestion kinetics along the GI tract and often poorly represent the *in vivo* mechanical environment. The latter requires greater understanding how transport, mixing and shear rate influence *in vitro* digestion. A known gap in the protocols is a lack of guidance in selection and operation of agitation methods to sufficiently represent true digestion conditions for different rheologies.

Agitation plays critical roles to: mechanically breakdown food; transport & mix enzymes/acid with gastric digesta; influence gastric slurry phase separation; control size/density "sieving" effects; supply fuel to microbes and stimulate biofilm growth; and degrade/aggregate protein. Inconsistent application of agitation methods and operating conditions can bias outcomes, such as bioaccessibility [2]. Viscosity also influences the digestion process in various ways. High viscosities: modify digesta flow field; reduce protein hydrolysis and lipid digestion (through weaker mixing and reduced interaction between digestive juices and food); reduce mechanical forces on food matrix and subsequent degradation; slow gastric emptying; reduce intestinal bioavailability (through weaker micronutrient diffusion and convective transport limiting wall absorption rates).

Here, we introduce novel *in silico* particle-based fluid dynamics simulations of typical gastric and intestinal stage agitation devices to compare flow and mixing dynamics in each, and then evaluate the effect of digesta viscosity on the hydrodynamics per device. The devices considered include INFOGEST stirrer, Rotary Wheel, Head-Over-Heel (HoH), and Rocker. Simulations were run as batch operations with realistic motion of the real devices applied to the vessel (test tube or beaker). Each fluid preparation was assumed to be Newtonian and these properties were kept the same for all devices. For the standard operating conditions used here all devices produced non-physiologically high shear forces. The INFOGEST stirrer with matched conical vessel showed more physiological rates of shear and mixing. Significant differences in flow field, mixing and shear rates were found across the different devices with insights suggesting more optimal choices of operating parameters for different rheologies. For example, the Rotary and HoH devices were found to produce large spatial heterogeneity in the mixing patterns when agitation period is not matched to viscous transport timescales.

References

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This study was supported by the Microbiome for One System Health (MOSH) Future Science Platform (FSP) of Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia.

Keywords : In Silico, INFOGEST, Digestion, Smoothed Particle Hydrodynamics, Agitation methods

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22681) - ADJUSTING THE INFOGEST DIGESTION PROTOCOL FOR IMPROVED APPLICATION OF LIPID DIGESTION

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Abstract

Background: Lipid digestion in humans results in more than 90 % lipid uptake in the small intestine. Most lipids are digested by the digestive enzymes to form free fatty acids (FFA) and monoacylglycerol (2-MAG). Whereas the majority of FFA and 2-MAG are absorbed by intestinal cells, a part might be used as substrate by the intestinal microbiota. To obtain more insights into dietary lipid intake, lipid digestion and potential effects on microbiota in the small intestine, relevant in vitro digestion models are needed. The INFOGEST digestion protocol is primarily optimized for protein digestion. However, lipid digestion has to deal with other variables than protein digestion, e.g. the lipase in the small intestine needs colipase as a cofactor for lipid digestion. In this study, the INFOGEST digestion protocol is optimized for lipid digestion and is made compatible with subsequent in vitro small intestinal fermentation experiments requiring a high FFA concentration. Since pancreatin contains a lot of material that can influence bacterial fermentations, pancreatin suspensions were processed to lower the residual, potentially interfering material.

Methods: The static INFOGEST digestion protocol for digestion of foods as described in Brodkorb et al was used as starting point. For optimisation of the protocol, different types of intestinal lipases were tested for lipase activity (3) and an ultrasonic treatment was included for dissolving pancreatic lipase. Colipase was extracted from pancreatin (2) for addition in the lipase activity measurements and lipid digestions. Lipid digestions were performed with varying amounts of sunflower oil and the addition of pancreatin or processed pancreatin with extra colipase. The % FFA at the end of the intestinal phase was calculated based on the NaOH consumption during titration together with the back titration of the digestion to pH 11,5 (4).

Results: Testing different types of intestinal lipases, we found that the lipase activity of porcine lipase extract decreased in time, whereas the activity of pancreatic lipase was stable. Ultrasonic treatment of pancreatic lipase lowered the residual material. However, we found that extra colipase was required to reach a similar lipase activity as non-processed pancreatin. Examining varying amounts of sunflower oil showed that digestion of 250 mg of sunflower oil results in 100% FFA compared to 63% FFA when 4 gram of sunflower oil was used. The concentration of FFA in the digest material was highest when 4 gram was used.

Conclusion: Processed pancreatin with lower residual material after ultrasonification, with addition of colipase can be used in lipid digestion and subsequent fermentations. Ratio of lipid versus lipase/colipase quantities matters (1). Despite the incomplete digestibility with 4g of lipid, the high concentration of FFA reached under these conditions is necessary for in vitro fermentations.

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Acknowledgments

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Keywords : Lipid digestion, INFOGEST

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22683) - FROM IN-VIVO TO DYNAMIC IN-VITRO MODELLING: DIGESTIBILITY OF PROTEIN GELS AND DRINKS BY NEAR REAL DIGESTIVE TRACT (NERDT)

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Abstract

Digestion of food is often studied via *in vitro* research methods. These methods usually offer cheaper and faster experiments with less ethical constraints as compared to *in vivo* studies. The standardized static INFOGEST method is very useful and the digestibility of a large number of food products can be measured in a relatively short time. However, this static model does not simulate digesta transit through the gastrointestinal tract and neglect the gradual secretion of gastric and intestinal juices. As these processes are all important for digestion, in the recent years more attention has been paid to the development of dynamic digestion models.

One of the recently developed models is the *Ne*ar *R*eal *D*igestive *T*ract (NERDT) or DHS-IV (Peng et al., 2021). This is a dynamic *in vitro* digestion system which consists of a real average size silicone stomach and simulated gastric peristalsis which is very similar to the real human digestion. Currently, it has been used in various *in-vitro* digestion studies. The goal of this study is to better understand the gastric emptying in the NERDT. To reach this goal we have used recent datasets on the *in vivo* digestion of protein drinks and gels where gastric emptying was measured via Magnetic Resonance Imaging (MRI) as described by Deng et al. (2022) and Roelofs et al. (2023). Sample were whey protein isolate (WPI) gel and pea protein isolate (PPI) drinks and gel. During the *in vitro* experiments with NERDT, the size of the pylorus, rotation angle and size of the sample have been found as major parameters indicating the gastric emptying profile. Regarding PPI digestion for drinks, the gastric emptying profile from NERDT fitted well with *in vivo* data. However, for digestion of PPI and WPI gels, the pylorus width play an important role and needed some re-adjustments which were based on the *in vivo* data. With the proper settings for gastric emptying, protein digestion was measured by degree of hydrolysis (OPA-assay) and peptide characterization (HPSEC). In this way experiments with NERDT can nicely supplement the data of existing *in vivo* studies. Next to that NERDT can be useful to pre-test various conditions before doing an in vivo study or even replace some in vivo studies.

References

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Acknowledgments

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Keywords : Near Real Digestive Tract, Gastric emptying, dynamic in vitro digestion, Pea and whey protein

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22685) - INTESTINAL SACCHARIDE TRANSPORT SYSTEMS ARE MODULATED BY POLYPHENOLS

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Abstract

Background: Excessive saccharide intake is implicated in type 2 diabetes. Transporters in the small intestine facilitate their absorption. Dietary polyphenols interact with small intestine saccharide transporters, affecting glucose and fructose absorption. The effects of dietary factors such as polyphenols and different glucose and fructose levels in the diet on saccharide absorption remain largely unknown.

Objectives: We aim to study the impact of polyphenols on glucose and fructose absorption in the small intestine under different glucose-to-fructose ratios and to investigate the underlying mechanisms by assessing saccharide transporter gene expression.

Methods: Intestinal (Caco-2) cells were differentiated to small intestinal epithelial cells and exposed to the polyphenolic compounds phloretin (1mM), phlorizin (500 µM), or curcumin (200 µM) for 4 hours. Next, glucose-to-fructose ratios containing high glucose (2.5 mM glucose/0.5 mM fructose), low glucose (1 mM glucose/0.5 fructose mM) and low fructose (1 mM glucose/0.2 mM fructose) levels were added for 30 minutes. Basolateral appearance of glucose and NBD-fructose were measured. Gene expression of SGLT1, GLUT5, and housekeepers RPL13a and GAPDH was assessed by qPCR. Experiments were performed with n=2 and N=3. Data were statistically analyzed using one-way ANOVA or Kruskal-Wallis, with significance level set at $P<0.05$.

Results: In all tested ratios, phloretin and curcumin significantly reduced glucose absorption by 50% and 60%, respectively, and decreased SGLT1 gene expression by 50% and 40%. Interestingly, phloretin increased NBD-fructose absorption by 120% and 190% in both low and high glucose ratios, while curcumin enhanced absorption by 80% and 100% under both low and high glucose ratios, respectively. However, in the low fructose ratio, curcumin showed no enhancing effect and phloretin only increased NBD-fructose absorption by 20%. Notably, these effects on fructose absorption were independent of GLUT5 gene expression, which remained either unaffected or decreased in response to phloretin and curcumin. Phlorizin decreased glucose absorption by 25% only in the low glucose ratio without impacting SGLT1 or GLUT5 expression in all ratios. In the low fructose ratio, phlorizin reduced fructose absorption by 80%.

Conclusion: Polyphenols significantly decreased glucose absorption, potentially through SGLT1 gene modulation. Despite no impact on GLUT5 expression, both phloretin and curcumin increased NBD-fructose absorption. In the absence of GLUT5 inhibition and with elevated glucose levels, significant fructose uptake induced by phloretin and curcumin implies an alternative pathway for fructose absorption, potentially mediated by pore forming claudins in the epithelium. Future experiments will delve into mechanisms such as paracellular saccharide uptake involving MLCK1 expression.

References

Acknowledgments

Keywords : Saccharides, Transporters, Polyphenols, Glucose Absorption, Claudin-Mediated Uptake

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22687) - ANOTHER STEP TOWARDS A RELIABLE AND REPRODUCIBLE IN VITRO METHOD TO DETERMINE PROTEIN QUALITY OF COMPLEX FOOD PRODUCTS

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Abstract

Background:
The shift towards a more plant-based diet requires the availability of food products with adequate protein quantity and quality. To obtain more insights into the quality of alternative proteins and the impact of the food matrix and processing steps, robust and reproducible *in vitro* methods are needed. Over the past years great efforts were made by the European COST INFOGEST consortium to develop a standardized *in-vitro* method to determine the digestibility of protein, enabling the comparison over different laboratories [1, 2]. The most updated protocol including methanol precipitation to resemble the small intestinal uptake of protein fragments, resulted in comparable *in vitro* digestible indispensable amino acid ratios (IV-DIAAR) for protein concentrates as reported *in vivo* in growing pigs [3]. In this project we determined the protein quality of more complex food products, for which i*n vivo* data was partly available as well [4].

Methods:
Static *in vitro* digestions of whey protein isolate (WPI), pork, pea-based meat and soy-based meat were performed in triplicate according to the INFOGEST protocol [2, 3], with a few modifications to allow consistent analysis of more complex food products. The amount of test-product equivalent to 40 mg protein was added to protein-free cookie reaching a total weight of 250 mg, simulating a meal composition. Methanol precipitation of the digests was performed followed by amino acid composition analysis of the digestible (supernatant) and indigestible (pellet) fraction according to the modified Waters AccQ-Tag™ protocol [5]. DIAAR were calculated based on the essential amino acid content of the food products (determined by ISO 13903:2005 and EU 152/2009) and the true ileal digestibility (TID%) for each amino acid. The TID% of tryptophan, cysteine, and methionine was determined by the average of all other amino acids.

Results:
Mass balances of protein in total digest, pellet and supernatant ranged between 92-102% (SD<9%), indicating that TID% of the more complex food products could be calculated correctly. IV-DIAAR of WPI, pork and pea-based meat showed small standard deviations and were in the same range as previously reported *in vivo* DIAAR in growing pigs with respectively histidine, valine, and sulphur-containing amino acids as first limiting amino acids. IV-DIAAR of soy-based meat was slightly lower than in *vivo* DIAAR of soy protein isolate [6], potentially attributed to the texturization process.

Conclusion:
The most updated Infogest protocol with minor modifications results in a reliable and reproducible prediction of protein quality in complex food products. The protocol is currently applied in ring trials, working towards a consensus protocol which will facilitate the development of nutritious plant-based food.

References

Minekus 2014; Brodkorb 2019; Sousa 2023; Fanelli 2022; Ariëns 2021; Van den Berg 2022

Acknowledgments
This research was supported by Unilever Foods Innovation Centre.

Keywords : in vitro digestion, amino acid analysis, true ileal digestibility, protein quality

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22692) - LENTIL-BASED MUFFIN IN VITRO DIGESTION: UNRAVELING NUTRITIONAL INSIGHTS

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Abstract

Legume grains provide essential nutrients for human health such as protein, fibre, minerals, vitamins and phytochemical compounds. They suit current dietary trends like flexitarian, vegetarian, vegan and gluten-free preferences. Nevertheless, legume consumption remains below recommendations, demanding the creation of alternative intake strategies. Lentils are distinguished among legumes for their quick cooking time, high arginine and flavonoid content, and low glycaemic index, which helps avoid peaks in blood glucose, improving metabolic control. Furthermore, recent epidemiological findings suggest potential benefits against cardiovascular disease and diabetes mellitus [1].

In recent years the growing focus on healthier food options, especially plant-based alternatives, has gained prominence. However, some current plant-based foods prioritize sustainability and taste over relevant health benefits. Another key point, is the lack of understanding of the behaviour of new alternative protein products in the human gastrointestinal tract, thus emphasising the need for comprehensive insights into digestion and absorption aligned with human nutritional requirements. Methods, including *in vitro* and *in vivo* digestion models, were developed to comprehend food behaviour in the human gut. One of the widely used *in vitro* protocol is the INFOGEST and it has diverse applications such as measurement of macronutrient digestion and bioaccessibility of bioactive agents [2]. This allows for the analysis of the impact of the samples' digestive process and to evaluate digested samples glycaemic index or inflammatory potential, demonstrating how foods influence blood glucose levels and bowel inflammation, crucial for conditions like diabetes or obesity.

Based on this knowledge, a novel muffin recipe was created using green lentil flour as a partial substitute for oatmeal flour. The muffin´s nutritional value was evaluated, and the INFOGEST protocol was employed to determine the glycaemic index and comprehend the inflammatory potential of this food product. The lentil flour-based muffin exhibited improved nutritional properties, including a 25 % reduction in fat, a 47 % increase in protein, and a 16 % rise in fibre, with the presence of beneficial omega-3 fatty acids compared to its oatmeal-based counterpart. It also had a 9% lower glycaemic index. Additional data is currently under analysis to assess the lentil-based muffin's post-digestion anti-inflammatory capacity using *in vitro* cellular models. This work aims to support lentil production, one of the oldest legumes, and create a product suitable for all consumers, including those with diabetes, ultimately boosting the lentil's popularity and market value.

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Acknowledgments

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Keywords : Health benefits, INFOGEST, Legumes, Lentil, Muffin

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22694) - PROTEIN INTAKE IN THE OLDER ADULT: FROM AMINO ACID COMPOSITION TO IN VITRO DIGESTION

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Abstract

Protein intake in the older adult: from amino acid composition to in vitro digestion

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By 2051 there will be 1.6 million people over the age of 65 in Ireland. One important consequence of ageing is the gradual decline in muscle mass and strength, with increased risk of disability due to falls and fractures. An increase in dietary protein of 1.0-2.0 g/kg of body weight/day is recommended to meet the physiological needs of the older adult. However, simply increasing dietary protein may not equate to more bioavailable amino acids. The first aim of this study was to assess individual amino acid intake of the older adult in Ireland (>65 years old). A dataset of 2326 food items, from the National Adult Nutrition Survey (IUNA, 2011), were assessed for amino acid composition and intake. The analysis showed that the older Irish population not only met, but exceeded the recommended daily intake of essential, and conditionally-essential amino acids. With physical disability rates at 47.5% in adults over 75 years, the next question posed is whether or not protein digestion and amino acid bioavailability is reduced in the older gut. A static *in vitro* gastrointestinal model for the older adult (Menard, et al., 2023) was employed to study protein digestion of 3 food matrices (liquid, soft solid and solid). Food type was selected by current protein contribution (i.e. meat, bread and dairy) but using future foods i.e. a lentil beverage, buckwheat bread, and soy-based meat alternative to account for the dietary shift towards sustainable agricultural practices. Digestion samples were collected at 3 time points (gastric G120, gastric G180 and post-intestinal I120). The evaluation of protein digestion included the degree of protein hydrolysis (OPA method), peptide size (size exclusion chromatography) and free amino acid analysis. For buckwheat bread, and soy-based meat alternative, I120 samples from the older adult model had surprisingly significantly increased protein hydrolysis and free amino acids than adult I120 samples ($P<0.05$). For lentil beverage, no significant difference was observed.

In conclusion, there are protein digestion differences in the older adult specific to different food matrices, with structure playing a role in increased free amino acids.

To examine amino acid bioavailability, future work will employ 2 gut barrier models, a Caco2-HT29/MTX for the adult barrier versus an aged barrier (i.e. D-galactose-treated Caco2-HT29/MTX).

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Keywords : older adult digestion

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22696) - IN VITRO SIMULATED DIGESTION FOLLOWED BY ABSORPTION AND METABOLISM USING A CO-CULTURE MODEL: IMPACT ON THE BIOACTIVITY OF PEPTIDES DERIVED FROM BREWING BY-PRODUCTS

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Abstract

Oral intake, either gastrointestinal digestion, intestinal absorption, and/or hepatic metabolism, can affect the bioavailability and, therefore, the expected outcomes of food-derived bioactive peptides. During oral administration, peptides face several proteolytic enzymes and selective barriers that may modify and inactivate them or hamper the active peptides from reaching their targets. Gastrointestinal digestion can be simulated *in vitro* using the standardized protocol of INFOGEST (1). Caco-2 cells are a common model used to mimic the intestinal barrier and absorption. However, there is a lack of available *in vitro* assays to simulate metabolism and liver first pass-effect. Thus, we aimed to develop a protocol to overcome this problem and ensure that the bioactivity of brewing peptides (as angiotensin-converting enzyme – ACE-inhibitors) is retained after sequentially simulate the oral administration processes.

The INFOGEST protocol was applied to peptides obtained from brewing by-products (2), and the digested products were subsequently subjected to simultaneous simulation of intestinal absorption (Caco-2 cells) and hepatic metabolism (HepG2 cells) by using a simple static double-layered system separated by a semi-permeable membrane culture insert. The *in vitro* bioactivity of brewing peptides before and after simulated oral administration (0.86 mg/mL) was assessed utilizing a fluorometric assay kit that measures the activity of ACE. Captopril (1 µM) was used as a positive control. Differences in means were compared using one-way ANOVA followed by Tukey's or Dunnett's *t*-test.

Results showed that oral administration affects the bioactivity attributed to peptides derived from the brewing industry. We obtained an increase in ACE-inhibitory activity after simulation of gastrointestinal digestion, intestinal absorption, and hepatic metabolism, indicating that these processes do not negatively affect the bioavailability of brewing peptides, but they somehow modify their structure promoting an increase in bioactivity. This work alerts researchers to carefully consider the oral administration process before proceeding to animal studies not only for ethical reasons, but also to pre-determine the appropriate therapeutic dose range for *in vivo* testing, thus avoiding unnecessary studies. Furthermore, this study highlights the importance of adequately reproducing the main processes of oral administration to identify promising food-derived bioactive peptides.

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Acknowledgments

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Keywords : brewer's spent grain, brewer's spent yeast, protein hydrolysates, bioactive peptides, ACE-inhibitor

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22698) - COMPARISON BETWEEN DIFFERENT ENZYMATIC INHIBITION METHODS OF STANDARDIZED GASTROINTESTINAL MODEL (INFOGEST): A CASE STUDY OF PLEUROTUS OSTREATUS MUSHROOM BIOMASS

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Abstract

Pleurotus ostreatus (PO) is one of the most cultivated and consumed mushrooms worldwide, playing crucial roles in human health (e.g., prebiotic and immunomodulatory)[1]. Mushroom biomass (MB) combines the mycelium and young fruiting bodies[1,2]. Both parts possess numerous bioactive compounds such as α- and β-glucans[1]. A possible synergetic effect between their distinct macromolecules has been proposed[2]. The authors characterized the PO biomass composition for the first time. The results suggested an interesting nutritional profile, where the main differences between PO biomass and fruiting body are the higher α-glucans (77.49%) content and lower concentration of β-glucans (2.96%), free sugars (0.34%), protein (4.62%) and ashes (1.36%). However, it is imperative to assess the influence of the gastrointestinal tract (GIT) on their bioactive compounds, especially α-glucans.

Accordingly, the standardized GIT model (INFOGEST) was used. Samples were taken from oral, gastric, and intestinal phases to better assess the impact of different GIT phases. The results indicated a loss of approximately 19 and 44% of α-glucans in the oral and intestinal phases. However, we identified some issues, mainly linked to the difficulty in collecting homogenous samples (high variability and time-demanding) and non-enzymatic inactivation. During the INFOGEST protocol, enzymatic inactivation may overestimate the enzymatic activity, playing a key role in the bioactive compound quantification. In this context, we tested different enzymatic inhibition approaches to select the method that allows a more realistic macromolecule quantification. We selected temperature and pH for all enzymes and specific inhibitors (enzymes or other specific reagents) for each GIT enzyme (oral: α-amylase inhibitor from *Triticum aestivum*; gastric: pepstatin A and orlistat; intestinal: 4-bromophenylboronic acid, pefabloc SC and Na_2CO_3). Oral, gastric and intestinal phases were analyzed with independent GIT simulations to minimize heterogeneity. The influence of the freezing or freeze-drying process was also evaluated. The impact on GIT α-amylases, proteases and lipases inhibition was monitored by analyzing α-glucans content, fatty acid, protein and peptide profiles. Overall, the results corroborated the importance of enzymatic inactivation. The protein and peptide profiles did not suggest key differences between inhibition methods. A significantly higher concentration of α-glucans was quantified but no significant differences were generally found between different inactivation methods in this bioactive molecule. In some GIT phases, freeze-drying seems to have a negative impact on α-glucan content and especially on total phenolics (TP) and antioxidant activity. pH showed the most negative effect on TP and antioxidant activity, while temperature usually appears to not influence these parameters.

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Keywords : Mushroom Biomass, INFOGEST, Enzymatic inactivation, Freezing and freeze-drying, Bioactive compounds impact

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22717) - ILEAL DIGESTIBILITY OF STARCH FROM FABA BEAN IN HEALTHY HUMANS

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Abstract

Background: Legumes are recommended for a transition to more sustainable protein sources. They are also starchy food that participate to healthy diet. Faba beans are among the best candidates to develop legume consumption as they are cultivated in different countries and under different climates. The digestibility of the starch component in legumes, and subsequently the part of resistant starch, is poorly studied in humans. In the frame of a study on faba bean protein quality in healthy volunteers (LEG4LIFE, NCT05047757), we aimed to assess the ileal starch digestibility as well as the postprandial glucose metabolism.

Method: Nine healthy volunteers (both sexes, aged 25-56 years) were equipped with a naso-ileal tube and ingested a test meal, which consisted of 250 g of dehulled, cooked mashed faba bean. Ileal contents and plasma samples were collected over an 8 h postprandial period. Respiratory exchanges were measured during 15 min every hour. Residual starch in ileal effluents was measured after hydrolyzing samples with a-amylase. Intestinal and plasma glucose were determined with a colorimetric test. Insulin was measured by an ELISA test. Glucose oxidation was determined from respiratory exchanges.

Results: The amount of starch ingested was 30.7 ± 1.2 g. In the terminal ileum, 7.3 ± 2.4 g of starch was recovered, resulting in starch digestibility of 76.1 ± 8.7%. Postprandial glycemic response was weak in all volunteers, with a maximal value of 1.02 ± 0.08 g/L occurring at 1.5 h (baseline 0.96 ± 0.11 g/L). Insulinemia ranged from 28.9 ±14.5 pmol/L at the baseline to 84.1 ± 53.6 pmol/L at 1.5 h. Respiratory quotient was initially 0.88 ± 0.04, increased to 0.91 ± 0.04 during the first postprandial hour and decreased to 0.86 ± 0.04, indicating a predominant glucose oxidation. The amount of glucose oxidized during 8h after the meal was 80 ± 32 g.

Conclusions: We investigated the ileal starch digestibility of faba bean ingested as cooked seeds in humans. The results indicate that resistant starch account for around 25% of faba bean starch. The low glycemic response supports that legumes induce a low glycemic load and may participate to an optimal glucose homeostasis.

References

Acknowledgments

Keywords : legumes, resistant starch, glycemic load, digestion

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22724) - PRELIMINARY INVESTIGATION ON LOCUSTA MIGRATORIA AS A NEW FOOD SOURCE.

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Abstract

Edible insects are becoming interesting alternative food sources for human nutrition, due to the need of finding a sustainable way to feed a large number of consumers (Ververis et al.,2022). Recently, after the positive safety assessment by the European Food Safety Authority (EFSA) and their inclusion in the Novel Foods group, it is mandatory to investigate the functional, healthy, nutritional, and toxicological aspects that are generated during their intake. In this contest, the Italian National Recovery and Resilience Plan financially supported "OnFoods" which is a foundation including twenty-six public and private organizations taking actions sustainability, working on safety, security, and health. Pavia University is a member of this foundation, and our research group aims to characterize the physico-chemical properties, bioaccessibility, bioactivity, and toxicity of some novel foods components among which the oven-dried *Locusta migratoria* components/nutrients. Firstly, preliminary investigations have been performed on the protein's digestibility and amino acids bioaccessibility by using a simulated in vitro digestion process, such as Infogest 2.0 digestion protocol (Brodkorb et al.,2019), modified by adding the colon phase protocol (Hamzalıoğlu et al., 2014). Nutritional properties (e.i. protein content, chitin content, and digestible essential amino acid scores) have been studied and the changes occurring following the digestion process have been monitored as well as Caco-2 cell biocompatibility (Hammer et al.,2023; Sousa et al., 2023). In addition, physico-chemical studies provided insight into the physical-morphological characteristics of *Locusta migratoria* powder (Xiao et al., 2020). Furthermore, we aimed to study the effects of oxidative damage induced by hydrogen peroxide at different concentrations and changes in antioxidant enzymes in Caco-2 cells. These preliminary investigations on protein and polysaccharide *Locusta migratoria* components underlined its potential nutritional value and it may be considered an auspicious choice to overcome the future demands of new protein sources.

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Acknowledgments

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Keywords : Digestion, Insects, Bioaccessibility, In-vitro, Food-safety

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22730) - COMPARISON BETWEEN IN VITRO AND IN VIVO MODELS ON BIOAVAILABILITY OF LIPID COMPOUNDS

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1 - ITERG

Abstract

The use of alternative methods in animal experimentation is now a necessity with regards to societal concerns about ethical approach to animal experimentation.

In this context, the EFSA Scientific Committee highlights the importance of minimizing tests on animals (in vivo tests) and of promoting alternative approaches, wherever possible. These alternatives include laboratory tests (in vitro) or tests performed by computer simulation (in silico). For example, it is recommended to replace or, when appropriate, to reduce the number of animals used in the experiments and to improve their well-being. The EU legislation on the protection of animals used for scientific purposes establishes guidelines for the ethical use of animals in experimental procedures (Directive 2010/63/EU). These are the "3Rs": "Replacement (substitution and replacing animal models whenever possible), Reduction (reducing the number of animals in experiments) and refinement (optimizing the methodology applied to animals).

Studying the intestinal absorption of a compound and its metabolic fate in the organism is the result of different chemical, enzymatic and mechanical processes that occur simultaneously in the organism under the effect of complex regulatory pathways. In the case of studying the digestion of a compound, different approaches can be considered : in vivo, in vitro or in silico methods. However, in vitro or in silico models do not reproduce the biological complexity of the digestive tract and its metabolism. In a regulatory and societal context, it is important to know the advantages and limitations of the different models/approaches, in order to set up the best model that complete the study objectives, especially in projects dedicated to the evaluation of the absorption and metabolic fate of target molecules.

Through our research program, we defined different alternative methods to animal studies to follow the digestion and absorption of specific compounds. Also, a comparative study between in vitro and in vivo methods has been implemented to evaluate the intestinal absorption of nutrional molecules. The aim is to potentially identify an in vitro method that allows to all or a part of an approach on in vivo model.

Keywords : lipid digestion, cellular uptake, fatty acids, lipid compounds, intestinal cells

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22731) - FROM STATIC TO DYNAMIC IN VITRO DIGESTION: IMPACT OF STRUCTURE ON DIGESTION KINETICS

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Abstract

In the evolving landscape of food digestion studies, one of the major trends is the transition from static models towards (semi-) dynamic *in vitro* models, aiming for a more realistic representation of *in vivo* digestion (Duijsens et al., 2022). Our previous study demonstrated significant alterations in macronutrient digestion kinetics in lentils between static and semi-dynamic models (Verkempinck et al., 2022). The semi-dynamic digestion was performed in a custom-made automated system (multireactor) developed at the Laboratory of Food Technology (KU Leuven, Belgium). The multireactor showcased advantages over static models, allowing gradual enzyme and pH additions.

The next step to simulate digestion under conditions even more closely mimicking the physiological reality, is dynamic *in vitro* digestion. In that regard, a state-of-the-art dynamic digestion system was recently developed at Dr. Bornhorst lab (UC Davis, USA) (Nadia et al., 2023). This system also simulates gastric emptying and the peristaltic contraction of the gastric phase (limitations of the semi-dynamic multireactor system) using a simulated stomach bag. Recently published work revealed large similarities between the characteristics in the remaining *in vitro* digest and those evaluated *in vivo* in growing pig stomachs (Nadia et al., 2023).

To integrate the scattered information regarding the effect of *(i)* basic, but high throughput static, *(ii)* more advanced semi-dynamic, up to *(iii)* multifactorial dynamic digestion conditions on obtained digestion kinetics, all three of these approaches were applied to study macronutrient digestion in lentils. In this way, our explorations could shed light on the comparative aspects of these more simple to more complex digestion models. Moreover, digestions were performed on lentil purees with open and closed cotyledon cells to investigate the impact of microstructure on digestion kinetics during *in vitro* digestion under these very distinct digestion systems.

Our findings contribute significantly to understanding the nuances across various digestion models. As different research facilities are currently implementing simple to complex digestion models, our insights underscore the importance of selecting simulation conditions wisely. Therefore, understanding how digestion kinetics are derived through various simulation approaches of different complexities (ranging from static to semi-dynamic to dynamic) is essential.

References

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Keywords : dynamic in vitro digestion, Human Gastric Simulator, lentil microstructure, macronutrient digestion, static in vitro digestion

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22733) - TOWARD A MICROBIAL ALTERNATIVE TO PORCINE PEPSIN: AN ACID PROTEASE PREPARATION FROM ASPERGILLUS NIGER

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Abstract

Background: Porcine pepsin (PP) is used in simulations of gastrointestinal digestion to model human gastric pepsin activity. Because PP supply is inconsistent and costly, we aimed to develop a microbial alternative. The objective of this study was to determine if a food-grade, acid protease from *Aspergillus niger* (AnAP) parallels peptic digestion in vitro. **Methods:** AnAP and PP were compared in the INFOGEST static simulation of oro-gastric (OG) digestion. The OG simulation included a 2-min oral phase with porcine amylase and a 120-min gastric phase with PP (per INFOGEST protocol [1,2]) or AnAP at 37.5, 75, 150, 300, and 600 spectrophotometric acid protease units (SAP) per serving. Substrates included: 1) a canned test meal (CTM) containing canned chicken, canned peas, and instant potatoes, 2) Ensure® oral nutritional supplement (ONS), 3) whey protein concentrate (WPC), and 4) kappa-casein (KC). Analytical testing of gastric digestas included spectroscopy to measure free amino nitrogen (FAN), HPLC for total free amino acid (TAA) concentrations, polyacrylamide gel electrophoresis (PAGE), size exclusion chromatography (SEC), and LC-MS. Experiments were performed in triplicate. Data were compared by 1-way ANOVA and Tukey's multiple comparisons tests. **Results:** Following OG digestion of CTM, WPC, and KC, average digesta FAN concentrations were similar between PP and 37.5 SAP AnAP (all $p > .480$), yet increased up to 2.2-fold more than PP with increasing doses of AnAP (all $p < .04$). With ONS, even the 37.5 SAP dose of AnAP increased FAN 19.9% compared to PP ($p < .001$). ONS, WPC, and KC digesta TAA concentrations were comparable between 37.5 SAP AnAP and PP (all $p > 0.256$), while CTM showed 28.2% significantly higher TAA than PP ($p = .036$). Higher dose AnAP showed up to 89.8%, 3.3-, 4.1-, and 6.7-fold greater TAA concentrations than PP for CTM, ONS, WPC, and KC, respectively (all $p < .001$). For CTM, SEC analysis showed a similar distribution of peptides < 10 kDa between 37.5 SAP AnAP and PP, but less peptides > 10 kDa with AnAP. For ONS, 37.5 SAP AnAP showed reduced levels of most peptides compared to PP. SEC patterns were less generalizable for WPC and KC. Across all substrates, higher doses of AnAP were associated with incrementally more peptides < 10 kDa and less peptides > 10 kDa. PAGE analysis corroborated some of the SEC data, while specifically suggesting that ≥ 300 SAP AnAP is needed to approximate PP-mediated hydrolysis of β-lactoglobulin from WPC. According to LC-MS, PP hydrolyzed KC into peptides covering approximately 85% of the KC protein sequence. AnAP (37.5 SAP) hydrolyzed KC with 92% protein sequence coverage, yielding a mix of shorter and longer peptides compared to PP, likely due to target cleavage site differences. **Conclusions:** These observations warrant further testing of AnAP at doses slightly < 37.5 SAP and in combination with other microbial proteases to better approximate pepsin's endoprotease activity.

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Acknowledgments

Keywords : digestion, enzymes, INFOGEST, pepsin, protease

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(22738) - INSIGHTS INTO THE NUTRACEUTICAL POTENTIAL OF CHESTNUT SHELLS EXTRACTED BY SUBCRITICAL WATER EXTRACTION UPON IN-VITRO GASTROINTESTINAL DIGESTION AND INTESTINAL PERMEABILITY

Pinto, Diana (Portugal)[1]; Silva, Ana Margarida (Portugal)[1]; Dall'acqua, Stefano (Italy)[2]; Sut, Stefania (Italy)[2]; Vallverdú-Queralt, Anna (Spain)[3]; Delerue-Matos, Cristina (Portugal)[1]; Rodrigues, Francisca (Portugal)[1]

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Abstract

In the last decades, the nutraceutical market has expanded with food by-products being explored as sources of antioxidants intended to be valorized as nutraceuticals [1]. Chestnut (*Castanea sativa*) shells (CS) are a plentiful undervalued by-product with health benefits ascribed to phytochemicals [1,2]. Nevertheless, the European legislation for nutraceuticals validation remains vague, highlighting the importance of a comprehensive assessment of *in-vitro* and *in-vivo* bioactivity, phytochemicals composition, and intestinal absorption to guarantee their efficacy and safety.

This study explores the nutraceutical potential of CS by assessing the effects of gastrointestinal digestion and intestinal permeability on the bioaccessibility, bioavailability, and bioactivity of phenolic compounds extracted by subcritical water extraction. The intestinal permeability was studied using a Caco-2/HT29-MTX co-culture model and LC/DAD-ESI-MS was performed to analyze the phenolic composition. The inhibitory responses of acetylcholinesterase (AChE), α-amylase, and antioxidant enzymes were investigated.

The results demonstrated higher phenolic concentrations retained after gastric and intestinal digestion. The antioxidant/antiradical properties improved in the following order: oral < gastric ≤ intestinal digests < undigested extract, reaching almost 40% of bioaccessibility. The CS extract showed antioxidant/antiradical, hypoglycemic, and neuroprotective properties after *in-vitro* digestion. Ellagic acid was the main phenolic compound identified in the digested and undigested extract, while a pyrogallol–protocatechuic acid derivative was only quantified in the digests. Moreover, the CS extract showed potential mild hypoglycemic (≤25% of α-amylase inhibition) and neuroprotective (≤75% of AChE inhibition) effects before and after *in-vitro* digestion, along with upmodulating the antioxidant enzymes' activities and downregulating the lipid peroxidation. The intestinal permeation of ellagic acid achieved almost 25% after 240 min. Taken together, these findings sustain the valorization of CS extract as a promising nutraceutical ingredient with proven bioactivity even after *in-vitro* digestion and intestinal permeation.

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Acknowledgments

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Keywords : Castanea sativa; phenolic compounds; in-vitro digestion; intestinal model; pro-healthy effects.

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22740) - IN-VITRO AND IN-VIVO EVALUATION OF KIWIBERRY (ACTINIDIA ARGUTA) LEAVES EXTRACT AS NEW NUTRACEUTICAL INGREDIENT

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Abstract

Kiwiberry (KB; *Actinidia arguta*) is a small grape-sized fruit, characterized by hairless skin and a pleasant aroma and flavor, that has a huge beneficial impact on human health, mainly due to the different biological effects associated with its consumption, such as antioxidant, anti-inflammatory, and antidiabetic activities [1, 2]. Worldwide, the KB production is increasing due to the biological properties associated. During KB production different by-products are generated, such as fruits without caliber to be commercialized, pomace, or leaves, that are removed to increase the solar exposure [1, 2]. The potential application of leaves as active ingredient for nutraceutical and food sectors should be supported by *in-vitro* and in-vivo studies that effectively committed the bioaccessibility and bioavailability of the bioactive compounds present. Recently, our research team reported the richness of KB leaves obtained by ultrasound-assisted extraction (UAE) in phenolic compounds, particularly neochlorogenic and chlorogenic acids, caffeoylquinic acid, catechin, kaempferol-3-*O*-glucoside and isorhamnetin-3-*O*-rutinoside [3]. In addition, a good scavenging efficiency was observed against superoxide anion radical ($O_2^{\bullet -}$) and hypochlorous acid (HOCl). Nevertheless, the potential of KB leaves valorization as a source of natural antioxidants with attractive applications in food and nutraceutical fields should be proved by *in-vitro* and *in-vivo* models to reinforce the health-promoting properties. The aim of this study was to screen the *in-vitro* permeation, through an intestinal co-culture model, of the principal phenolic compounds from KB leaves extracted by UAE and, most important, to appraise the *in-vivo* antioxidant activity in rat serum and tissues (liver and kidneys), followed the oral administration of the extract to rats (50 and 100 mg/kg body weight (bw)). The *in-vitro* intestinal permeation was assessed by HPLC-MS, while the *in-vivo* antioxidant activity was evaluated using commercial kits. The principal phenolic compounds permeated the intestinal co-culture model or were retained inside cells. The results attested the upregulation of the antioxidant enzymes activities (superoxide dismutase, catalase, and glutathione peroxidase) and the downregulation of lipid peroxidation, with rats treated with KB leaves extract 100 mg/kg bw achieving the best outcomes. The data obtained support that KB extract possessed a good antioxidant *in-vivo* activity, which is typically denoted by the values of the treatment group when compared with control (vitamin C). Ongoing studies are focus on the quantification of phenolic compounds metabolites in liver and kidney.

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Keywords : Intestinal permeation, Animal Assays, Antioxidants, Valorization, Fruit by-product

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22747) - IMPACT OF BBM ENZYMES ON INFOGEST IN VITRO DIGESTION MODEL: A STEP FORWARD TO MIMIC THE INTESTINAL PHASE.

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Abstract

Brush border membrane (BBM) enzymes greatly affect the bioaccessibility and bioavailability of food nutrients. Despite their physiological importance, a step simulating the final step of intestinal digestion has not been included yet in the harmonized protocols of in vitro digestion, primarily due to the challenges to replicate the dynamic of intestinal degradation. We previously optimized the extraction of pig jejunum BBM enzymes and demonstrated by proteomics that pig hydrolases are suited for the use in sequential models of simulated human digestion (Mamone and Picariello, 2023). Herein, we propose a step forward a more physiologically relevant method, completing the harmonized oral-gastric-duodenal digestion INFOGEST models with the missing phase of digestion.
Skim milk powder (SMP) as a model protein food was subjected to the in vitro static digestion, including BBM hydrolases purified from pig jejunum. INFOGEST- digestion model without pancreatic was preferred to prevent the inclusion of high amounts of proteins and free amino acids interfering with the evaluation of the ability of BBM hydrolases to degrade peptides. Immediately after the duodenal phase, omitting any enzymatic deactivation treatment, digesta (chyle) were diluted with buffer pH 7.2 and supplemented with BBM with 0.180 mU of total peptidase activity per milligram of milk. The BBM peptidases activity-to-substrate ratio was optimized according to Shan et al (2002). To comply with the dynamic nature of the intestinal digestion and to balance for the spontaneous inactivation of hydrolases, digesting batches were supplemented with the same concentration of BBM in the course of the experiments each two hours, up to 6 h incubation time. Peptide degradation was monitored at each stage of digestion, by amino acid analysis, TNBS assay, RP-HPLC, high resolution mass spectrometry and bioinformatic tools.
Hydrolysis by BBM amino- and carboxy-peptidases led to a significant increase of free amino acids, up to levels comparable with the expected release of amino acid from milk proteins (Sousa et al. 2023). LC-MS/MS analysis demonstrated that BBM hydrolases erode progressively the peptides released by gastroduodenal processing up to a "core" of resistant peptides.
This approach is particularly relevant if the endpoint is the identify the peptide sequences that cannot be further hydrolysed by digestive enzymes and for determining the amino acid bioaccessibility.

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Keywords : BBM, amino acids, intestinal phase, proteomics

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22753) - IT'S NOT JUST A MATTER OF TIME. MECHANICAL STIMULATION ALSO REGULATES THE DEGREE OF DIFFERENTIATION OF A CO-CULTURE MODEL OF INTESTINAL EPITHELIAL CELLS

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Abstract

Caco-2 cell monocultures have been widely used to model human intestinal epithelium *in vitro*, particularly for permeability studies. However, they have limitations in accurately mimicking human enterocytes. Co-culturing these cells together with mucus-secreting HT29-MTX goblet cells produces a more representative model of intestinal cell diversity *in vivo*. However, it remains a challenge to optimize and balance key conditions such as seeding ratio, culture medium, and time to design an *in vitro* model that better mimics human intestinal morphology and permeability. To improve this co-culture model, we characterized expression of differentiation-related genes (*CDH1*, *TJP1*, *ALPI*, *DPP4*, *SI*, *MUC5AA*, and *MUC2*) and transepithelial electrical resistance (TEER) in Caco-2/HT29-MTX co-culture (9:1 ratio) grown in Transwell from confluence to differentiation under static and dynamic conditions. Although mechanical stimulation slightly affects TEER, we found that growing the enterocyte co-culture under gentle shaking (55 rpm) for 21 days showed an upregulated expression of *MUC2* and *MUC5AC* resulting in an improved mucin secretion profile compared to the static condition, better mimicking the intestinal environment.

In the second part of the study, we coupled the standardized semi-dynamic *in vitro* digestion method, which better simulates gradual gastric secretion, acidification, and sequential emptying, with the improved dynamic Caco-2/HT29-MTX co-culture to develop a novel *in vitro* approach. The integrated model was tested on a simplified food matrix such as skimmed milk with some modifications (*e.g.*, no bile salts, filtration with 3 kDa MWCO and with 0.22 µm filters) to ensure the biocompatibility of the digesta with cultured cells. Different supplementations were performed by applying a 1:3, 1:10, and 1:20 (v/v) dilution of the final small intestinal digesta in serum-free DMEM to the apical compartment of the transwell co-culture, and incubating for 3 h. Confocal microscopy was used to confirm cell integrity and co-culture morphology by staining cells with MitoTracker vital dies, fluorescent lectins, and nuclear labelling in the differently treated samples. We observed that all tested dilutions were not cytotoxic.

This integrated *in vitro* approach combining a semi-dynamic simulated digestion model with a representative intestinal epithelium will be tested in subsequent experiments by adding titanium dioxide nanoparticles to milk at the maximum concentration allowed in food by the FDA [1].

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Keywords : Caco-2/HT29-MTX co-culture, digesta detoxication, nanoparticles, semi-dynamic in vitro digestion model, titanium dioxide

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22756) - THE INFLUENCE OF PROCESSING ON THE BIOACCESSIBILITY AND BIOAVAILABILITY OF FERULIC ACID FROM CEREAL DIETARY FIBRE

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Abstract

Ferulic acid is abundant in cereals, but the lack of free ferulic acid (FA) limits its bioavailability. Many studies claim that cereal processing can release the FA and finally impact its antioxidant capacity. In this study, FA content in different cereals, the effect of milling, thermal processing, and bioprocessing on FA release were compared. The optimized processing methods were used in combination to formulate a food product (biscuit). The digestion of this product and the absorption of nutrients and bioactives were determined *in vitro* (Infogest model) and *ex vivo* (Ussing chamber), respectively. A significant difference is observed in the bioaccessibility and bioavailability of FA in the formulated product.

Keywords : Ferulic acid, Processing, Bioavailability, Bioaccessibility, In vitro digestion, Ex vivo absorption

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22759) - IN VITRO DIGESTION OF COFFEE BREW AND THEIR FRACTIONS: THEIR RELEVANCE TOWARDS CARDIOPROTECTIVE POTENTIAL

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Abstract

Coffee brew is well prevalent in human diet and several benefits for health have been described (Machado et al. 2023). These bioactive properties have been related with the chemical diversity which composes the coffee beverage, although structure-function relationships are still overlooked. One of the compounds most present in coffee brew are the soluble fibres, composed by arabinogalactans and galactomannans polysaccharides, and melanoidins, which may impact on cholesterol metabolism (Silva et al. 2021).

Using a simplified in vitro intestinal model, the effect of chemically characterized coffee and polysaccharides rich fractions on cholesterol bioaccessibility was accessed. Coffee as well as the arabinogalactans and galactomannans polysaccharides rich fractions were shown to decrease cholesterol solubility, due to their capacity to sequestrate bile salt.(Coreta-Gomes et al. 2020) Moreover, coffee brew also was tested to evaluate their effect on the bioavailability of a cholesterol analogue through Caco-2 cell line model, mimetizing intestinal epithelium. The results show that in the presence of coffee extracts, a decrease of the sterol permeability coefficient to half was observed, which was attributed to an increased sterol precipitation and its deposition on the apical epithelial surface.(Pires et al. 2022)

Coffee soluble fibres, namely arabinogalactans and melanoidins rich fractions were also evaluated regarding the outcome of their fermentability using an in vitro colonic fermentation model. Both fractions decreased the acetate: propionate ratio, which is indicative of a potential HMG-CoA reductase inhibition. Furthermore, melanoidin rich fraction also show to decrease the conversion of primary to secondary bile salts. As secondary bile salts are more prone to emulsify cholesterol, their decrease will have impact on cholesterol bioaccessibility and bioavailability, leading to a lower cholesterol absorption.

This work provides evidence that digestion of coffee and their high molecular weight extracts, namely polysaccharides and melanoidins, have cardioprotective potential, opening avenues to develop functional food ingredients based on coffee which can aid on the combat of cardiovascular diseases, which are one of the deadliest illnesses worldwide.

References

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Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22764) - INVESTIGATING THE ROLE OF GLUCOSINOLATE CONTENT IN COLORECTAL CANCER PREVENTION AFTER IN VITRO DIGESTION OF CRUCIFEROUS VEGETABLES (BRASSICACEAE)

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Abstract

Introduction: Cruciferous vegetables from the Brassicaceae family, such as broccoli, cabbage, rocket, radish, and watercress, are widely represented in the traditional Mediterranean diet. Their consumption has been associated with numerous health benefits, including anticancer properties[1]. These properties have been attributed to the presence of glucosinolates in their composition that, when hydrolyzed by the enzyme myrosinase during digestion, originate biologically active breakdown products, mainly isothiocyanates, active in different cancer types, including colorectal (CRC)[2]. Thus, the amount of glucosinolates and isothiocyanates following digestion is of extreme relevance. In this work, we aimed to obtain extracts from cruciferous vegetables, to simulate its digestion *in vitro* and afterwards to evaluate their activity on the CRC cell lines WiDr, C2BBe1, and LS1034.

Methods: Extracts from broccoli, cabbage, radish, rocket, and watercress were obtained through three different processes, namely decoction, maceration, and ethanolic extraction at 80%. In all extractions, 10g of plant were used. For decoction, the plants were mixed with water and subjected to boiling temperature for 30 minutes. In the case of maceration, the plant was mixed with water and crushed for 2 minutes. In the ethanolic extraction, the plant was mixed with an 80% ethanol/water solution and stirred for 1 hour protected from light. Subsequently, all extracts were filtered and concentrated using a rotary evaporator. Following evaporation, all extractions were filtered with 0.7µm glass fiber filters and kept frozen at -20ºC.

For the *in vitro* digestion process, the extracts were further subjected to simulated salivary, gastric, and intestinal fluids at physiological temperature. In the gastric step, the enzyme pepsin was added to each digestion tube, while pancreatin and bile solution were added to the intestinal fluid following the INFOGEST static protocol.

Results: Overall, 30 extractions were obtained of the selected plants. For the digestion simulation, extracts of raw and cooked vegetables were also performed. In total 50 *in vitro* static simulated digestions were performed.

Conclusion: Our results indicate the in vitro digestion process is effective in producing digested bioaccessible fractions. These will be next used to determine the bioaccessibility of glucosinolates, and in parallel the activity of these bioactive breakdown products will be evaluated in CRC cell lines.

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Keywords : Brassicaceae, glucosinolates, colorectal cancer, in vitro digestion

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(22767) - LIPIDS AND PROTEINS BIOACCESSIBILITY IN DHA-ENRICHED FORTIFIED INFANT FLOURS AND FORMULAS USING A STATIC IN VITRO 6-MONTH-OLD INFANT DIGESTION MODEL

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Abstract

Infancy period is marked by digestive immaturity characterized by limited secretory capacity and enzymatic activity, thus affecting the bioaccessibility of nutrients. Despite WHO recommendations for exclusive breastfeeding for 6 months followed by mixed breastfeeding, only 34% of 6-month-olds are breastfed. To ensure adequate intake during the dietary diversification, follow-on infant formulas (IFF) and infant flours (IF) are formulated specifically to meet the nutritional needs of infants from 6 months onward. Their formulation is linked to major challenges of nutritional optimization including the introduction of omega 3 fatty acids especially long chain ones like DHA which has become mandatory in Europe since 2020 in IFF. Such introduction raises issue in terms of oxidation and nutrients bioaccessibility. This study therefore had two objectives: i) optimize oxidative stability and the nutritional composition of IFF and IF, and ii) assess the bioaccessibility of lipids and proteins in these optimized products (n=3) compared with commercial products (n=2) using an *in vitro* digestion process adapted to the digestive conditions of 6-month-old infants. Optimized IFF (IFF_OP) and IF (IF_OP) were formulated according to overviews of products currently on the market (91 IFF and 96 IF) and by introducing ingredients of interest for infant nutrition, such as milk lipids and sources rich in omega 3 and carotenoids. They were subjected to a static *in vitro* digestion protocol whose conditions were established according to the literature in comparison with commercial products (IFF_REF or IF_REF). The *in vitro* static digestion process included two steps: i) a gastric phase (60 minutes, 37°C, pH=3) with a meal/digestive fluid ratio of 50:50 v/v and an enzyme activity of 295 and 22 U/mL for pepsin and lipase, respectively and ii) an intestinal phase (120 minutes, 37°C, pH=7) with a ratio of 25:75 v/v and an enzyme activity of 90 U/mL for lipase and 3.1 mM for bovine bile. For both types of products, the results showed reduced gastric lipids hydrolysis with the onset of DHA release in this compartment. A high lipolysis degree was observed at the end of the intestinal time with a released fatty acids profile reflecting the lipid composition of the source with the presence of short to medium chain in IFF_OP due to the introduction of dairy lipids. The bioaccessibility of omega 3 fatty acids was good and not matrix-dependent, although IF were less rich in lipids than IFF. Vitamin E bioaccessibility was low (7.1-20.7%) and intestinal proteolysis rates moderate (less than 30% released NH_2 out of total) possibly influenced by large droplet size (0.8 µm) in IFF_OP and the presence of anti-nutritional factors in IF_OPTI. When optimized these two types of products are good nutritional carriers but the digestive fate of hydrophilic vitamins (A and E) and their oxidation products needs to be investigated further in the near future.

References

Acknowledgments

Keywords : Infant digestion, Polyunsaturated fatty acids, Vitamins, Proteins, Bioaccessibility

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22768) - THE APPLICATION OF A STATIC IN VITRO DIGESTION MODEL ADAPTED TO THE GENERAL OLDER ADULT POPULATION (INFOGEST) AS AN ASSISTANCE TOOL FOR THE DEVELOPMENT OF FOOD FORMULATIONS ADAPTED TO THE ELDERLY - DIET65+ PROJECT.

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Abstract

According to the United Nations data, the proportion of older people - individuals aged 65 years and over (65+) - is growing faster than any other age group. Indeed, the number of people aged 65+ worldwide is expected to more than double by 2050. In Portugal the National Statistics Institute, estimates that this percentage will reach 3 million individuals in 2050. These demographic changes require societal and governmental responses to adequately address the challenges that are inherently associated with aging. In the elderly, taste, smell, vision, hearing, and touch undergo significant changes, with dysgeusia and hyposmia beginning around 60 years of age and progressively worsening with age. In addition, poor sensory stimulation can compromise some metabolic processes since salivary, gastric, and pancreatic secretions are induced by this initial sensory system. Some degree of gastrointestinal function impairment is also characteristic amongst the elderly and are generally assumed to be a decrease in the secretion of enzymes, juices, and mucus, as a result of atrophic gastritis and the consequent inability to produce gastric acid (hypochlorhydria) impairing the absorption of nutrients as well as promoting bacteria overgrowth [1].

The Diet65+ project - High nutritional and functional value food products integrated with tradition and sustainability adapted to elderly +65 consumer-, intends to develop food products tailored and fully adapted to preserved food's taste, color, and flavor, enhancing the palate and enabling more adaptability, towards a nutritional pattern fulfilling 65 years plus (65+) individual specific nutritional requirements but, at the same time respecting individuals' taste and dietary habits. These nutritional enhanced and adapted products will encompass organic food items developed in line with 65+ consumers' traditional dietary habits. In this context, a variety of food formulations were developed and in a first step the impact of the gastrointestinal tract is being assessed using a static *in vitro* digestion model adapted to the general older adult population (INFOGEST consensus) [2]. The use of an *in vitro* digestion model adapted for older people is of extreme relevance in understanding the fate of food in this specific population, facilitating the development of foods adapted to their nutritional needs. This first screening, using this type of *in vitro* model will allow the understanding of the feasibility of the food formulations developed to fulfill the mentioned dietary requirements in terms of both high fiber and protein requirements. These results will have a determinant role in selecting the best food formulations to be used in further clinical trial studies.

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Keywords : elderly, food formulation, high protein, high fiber, gastrointestinal digestion in vitro models for elderly

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22771) - DIGESTION OF WHEAT PROTEINS AND ACCUMULATION OF GLUTEN-DERIVED IMMUNOGENIC PEPTIDES ALONG THE GASTROINTESTINAL TRACT OF THE GROWING PIG MODEL

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Abstract

The process of gluten and its immunogenic peptides breakdown along the gastrointestinal tract (GIT) is not well understood. This study aimed to investigate the digestion of wheat proteins (gluten) and the accumulation of immunogenic peptides derived from gluten in along the GIT of the growing pig model. Additionally, the study examined the effect of a natural exogenous protease, actinidin, on gluten digestion. Entire male pigs (n=54, 21.2 ± 2.1 (SE) kg bodyweight) were fed whole wheat soda bread either with yellow kiwifruit (0 U/mL actinidin as fresh pulp) or green kiwifruit (27.0 ± 1.2 U/mL actinidin as fresh pulp). Fasted pigs received the last meal (178 g bread + 26 g green or yellow kiwifruit) before being euthanised at 0 (fasted animals), 20, 60, 120, and 300 min postprandially. Entire gastrointestinal contents were collected to determine the hydrolysis of wheat proteins in the stomach and the presence of immunogenic peptides along the GIT. In the stomach, the average rate of digestion of wheat proteins of the pigs was 0.03%/min/g of bread. The total degree of gastric hydrolysis of proteins after 300 min digestion was 9.5%. The average rate of reduction of immunogenic R5 epitopes (peptides with five amino acid sequences) in the stomach was 3.4 mg/min/g of bread. At 20 min of digestion, 88.5% of the total R5 epitopes remained in the stomach but at the end of 300 min, this amount reduced to 11.5%. The average rate of release of R5 epitopes to the small intestine was 3.2 mg/min/g of bread and the average rate of disappearance of R5 epitopes in the small intestine was 3.0 mg/min/g of bread. After 300 min postprandial, R5 epitopes reached the large intestine (e.g., 16.0 mg at 300 min post-feeding). All these values were influenced ($P<0.05$) when actinidin was present in the meal. For instance, actinidin doubled the rate of digestion of wheat proteins in the stomach and subsequently reduced the rate of release of R5 epitopes into the small intestine (0.7 mg/min/g of bread) and the amount reached the large intestine (5.5 mg at 300 min postprandial). In conclusion, the digestion of gluten immunogenic peptides is limited along the GIT and this can be enhanced by a simultaneous intake of exogenous enzymes.

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Acknowledgments

Keywords : Actinidin, Digestion, Gluten, Growing pigs, Immunogenic peptides

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22772) - OPTIMIZATION OF AN IN VITRO BIOACCESSIBILITY FISH MODEL: PROTEIN DIGESTIBILITY USING FISH ENZYMATIC EXTRACTS AND COMMERCIAL ENZYMES

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Abstract

Recent advances in aquaculture nutrition have raised the interest on studies regarding new feed products capable of effectively replacing fish meal and oil, which must be thoroughly analyzed in terms of nutrients bioaccessibility to certify their efficacy. *In vitro* models could allow the assessment of nutrients bioaccessibility, particularly proteins, from new ingredients used to replace fish meal, in a faster, more ethical, and less expensive manner when taken into consideration the *in vivo* approach (Wang, 2021). To date, there are few studies focused on *in vitro* simulating the fish gastrointestinal tract, to quantify the feed digestibility, bioaccessibility and bioavailability of nutrients, which motivates further efforts to develop and validate fish digestive *in vitro* models.

This study aimed to optimize and validate a static *in vitro* digestion model for marine fish capable of assessing the bioaccessibility of proteins, using a juvenile fish species (gilthead seabream, *Sparus aurata*) as biological model. Two different procedures were tested to conduct the bioaccessibility analyses of the samples, namely the use of enzymatic extracts obtained from the fish digestive tract (stomach and pyloric ceca+intestine) in comparison with commercial digestive enzymes. The protein content was quantified in the bioaccessible and non-bioacessible (*pellets*) samples using a combustion method of analysis with the FP-528 LECO nitrogen analyzer calibrated with EDTA according to the Dumas method (Saint-Denis & Goupy, 2004).

The results obtained with the protocol using commercial enzymes were promising, revealing high bioaccessibility values, thus confirming the efficacy of the digestive process. Higher values were obtained under the following conditions: 250 mg of feed, 37 ºC and 24h digestion process. However, such results are being validated with *in vivo* trials. Ultimately, this study will contribute to validate a robust model to assess the efficacy of fish meal replacement ingredients and new aquafeeds that in turn could contribute to the achievement of a truly sustainable aquaculture development, while ensuring the production of safe and high quality farmed products for consumers.

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Acknowledgments

This work was supported by the Project MycoFish - Occurrence, bioavailability and mitigation strategies for mycotoxins in farmed fish and associated feed ingredients: Gilthead seabream as case study (PTDC/CVT-CVT/2660/2021).

Keywords : In vitro digestion methods, Fish, Fish feed, Bioaccessibility, Protein content

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22774) - DETERMINATION OF TRUE ILEAL AMINO ACID DIGESTIBILITY OF SPIRULINA IN HEALTHY VOLUNTEERS FOR USAGE AS THE PROTEIN REFERENCE IN THE DUAL ISOTOPE METHOD

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Abstract

The standard oro-ileal balance method for measuring ileal amino acid (AA) digestibility relies on the sampling of ileal digesta, making it highly invasive. In contrast, the dual isotope method has been developed to indirectly evaluate AA digestibility with minimal invasiveness. This approach involves ingesting a 2H-labeled test protein along with a tracer dose of 13C-labeled reference protein. The ratio of plasma appearance of AAs from test to reference proteins with respect to the meal and corrected for the AA digestibility of the reference protein, allows for the determination of the true ileal AA digestibility of the test protein [1]. The knowledge of the AA digestibility of the reference protein is thus essential. While spirulina is usually used as the reference protein, its ileal AA digestibility has only been indirectly determined with the dual isotope method, not the standard oro-ileal balance method. Therefore, our objective was to ascertain the true ileal AA digestibility of spirulina using the standard oro-ileal balance method in healthy subjects.

Six healthy volunteers (3 women, 3 men; 25.8 ± 5.9 y; BMI, 22.6 ± 1.7 kg/m^2) completed the study. They were equipped with a naso-ileal tube positioned at the terminal ileum. After an overnight fast, they received a single dose of 12 mg/kg of 13C-spirulina administrated with eggs. Ileal digesta were collected over an 8-h postprandial period. The 13C-AA enrichments were measured in digesta and meal using isotopic ratio mass spectrometry, to determine ileal digestibility of individual AAs.

Data have been analyzed for 4 volunteers. The mean ileal AA digestibility of spirulina was 88.9 ± 5.1%, with values ranging from 83.8 ± 6.1% for glycine to 95.0 ± 1.8% for methionine. The mean ileal digestibility of indispensable AA was 87.8 ± 5.4%.

The ileal AA digestibility values obtained with the standard oro-ileal balance method were within the same range as those obtained with the dual isotope method in humans (mean AA digestibility of 85-88% [2,3]). Although the results should be updated with the data of the last two volunteers, these ileal AA digestibility values can be applied in the dual isotope method when spirulina serves as the reference protein.

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Acknowledgments

Keywords : Amino acid digestibility, isotope, spirulina, healthy volunteers

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22779) - DIFFERENCES IN DAIRY- AND PLANT-BASED YOGURT DIGESTION EMPLOYING THE HUMAN GASTRIC SIMULATOR (HGS)

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Abstract

The food matrix composition, structure, and buffering capacity are related to the gastric juice secretion rate and gastric emptying time, which may influence the gastric digesta pH and the viability of yogurt bacteria during digestion. To understand how dairy- and plant-based yogurts are digested, commercial low-fat milk, low-fat milk Greek-style, whole-fat milk Greek-style, soy, almond, coconut, and oat-based yogurts were selected and subjected to simulated dynamic gastric digestion using the human gastric simulator (HGS) combined with a static small intestinal digestion model. The gastric juice secretion rate was dynamically modified based on the intragastric pH, to consider the buffering capacities of the yogurts. Samples emptied from the HGS after 30, 60, and 90 min were subjected to static small intestinal conditions for a subsequent 60, 120, or 180 min. Low-fat milk Greek-style yogurt had the highest total buffering capacity before digestion, while oat yogurt had the lowest with values ranging from 125 ± 3 to 45 ± 1 [(mmol H+/g of yogurt)/pH change], respectively. The dry matter gastric emptying from the HGS was similar for all yogurts ($p>0.05$). Overall, 92 ± 6% of yogurt dry matter was emptied after 60 min of gastric digestion. Dairy yogurts had an overall lower pH (3.9 ± 0.2) before digestion than plant-based yogurts (4.3 ± 0.2). Still, no significant differences ($p>0.05$) were observed in the emptied gastric digesta pH between dairy- and plant-based yogurts. Before digestion, low-fat, Greek-style yogurt had significantly higher concentrations of free amino groups, while coconut yogurt had the lowest, which can be related to the initial protein levels. The free amino group level did not change during gastric digestion ($p>0.05$). Instead, quantities of free amino groups increased 6- to 10-fold during 180 min of small intestinal digestion. Low-fat Greek-style yogurt contained the highest viable bacterial cell numbers (8.6 ± 0.1 log10 CFU/g), followed by whole-fat milk Greek-style yogurt (8.4 ± 0.4 log10 CFU/g), and low-fat milk yogurt (8.1 ± 0.4 log10 CFU/g). No significant changes in bacterial viability were observed during gastric digestion. However, bacterial viability declined up to 1.8 log10 CFU/mL of gastric digesta during first 60 min of small intestinal digestion, remaining stable until 180 min, indicating a possible bacterial adaptation to the simulated small intestinal environment. It was observed that the first 60 min of small intestinal digestion is critical for protein hydrolysis (free amino group concentration) and for bacterial viability. There is a lack of studies on yogurt from dairy and plant food matrices in the context of gastric and intestinal digestion, and therefore this study provides a more holistic examination of yogurt digestion and the potential differences in health-promoting effects.

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Acknowledgments
This project was supported by the National Dairy Council (NDC) and by the California Dairy Research Foundation (CDRF) Grant P-22-009-UCD-MM-HHM.

Keywords : dairy, plant-based, yogurt, buffering capacity, dynamic gastric digestion

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(22788) - EFFECT OF PEPSIN HYDROLYSIS ON DIFFERENT PEA PROTEIN FORMS AND THEIR IMPACT ON THE IN VITRO A-AMYLASE AND A-GLUCOSIDASE INHIBITORY ACTIVITY

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Abstract

Pea protein has gained prominence as a valuable dietary source, offering a range of health benefits attributed to its rich composition of essential amino acids, vitamins, and minerals[1]. Additionally, the bioactive peptides derived from pea protein have demonstrated potential health-promoting properties, particularly in managing conditions like diabetes[2]. This study delves into the comparative analysis of peptides released during pepsin digestion of commercially available pea protein products, shedding light on their potential inhibitory activity towards digestive enzymes such as α-amylase and α-glucosidase. It was hypothesised that differences in hydrolysis history of a protein would affect their solubility, release of peptide during digestion and hence their activity towards different enzymes/targets. We examined pre-hydrolysed pea protein (PPH) and non-hydrolysed pea protein isolate (PPI). Protein characterization revealed that PPH exhibited increased solubility at different pH levels (max. 80% at pH 12, $p \leq 0.05$) and higher presence of low molecular weight proteins/peptides (10-16 KDa) compared to PPI. Pepsin digestion showed a significant increase in hydrolysis and peptide release for PPH (64%, 48,000) compared to PPI (41%, 31,000). Enzyme inhibition assays demonstrated dose-dependent inhibition of α-amylase and α-glucosidase by PPH peptides, with the highest activity at 12mg/ml and 20mg/ml (37% and 46%, respectively). Further investigation involves ultrafiltration at 10 KDa to validate low molecular weight contributions and enhance inhibitory effects for determining IC50. This research provides insights into the differences in pea protein hydrolysis profiles and their impact on enzyme inhibition, highlighting potential benefits for health applications.

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Acknowledgments

Not applicable

Keywords : Pea Protein, Bioactive peptides, enzyme inhibition, hydrolysis

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22804) - IN VITRO PROFILING OF ELDERLY ON LIPID DIGESTION AND NUTRIENT BIOACCESSIBILITY

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Abstract

The aging process induces significant physiological changes affecting the digestive system and influencing nutrient digestion and absorption. Elderly individuals undergo alterations in salivary flow, gastric activity, emptying, peristalsis, and intestinal enzyme secretion, collectively impacting nutrient bioaccessibility. These changes may contribute to nutritional deficiencies and malnutrition in the elderly.

This study aimed to investigate the in vitro digestion and bioaccessibility of micronutrients in the elderly, particularly focusing on the influence of aging-related physiological changes on lipid digestion and oxidation.

Two nutritionally balanced model meals (salmon and chicken-based) were processed into puree or masticated forms and then subjected to in in vitro digestion assays. The INFOGEST 2.0 protocol, modified to simulate two elder deficiency conditions, corresponding to gastric and intestinal impairment, was applied in quadruplicate for each meal and digestive system (N = 48), with blank assays (N = 4). Traditional INFOGEST 2.0 protocol served as control. Lipid profile and oxidation status were evaluated using gas and liquid chromatography and visible spectrophotometry (TBARS method), respectively.

The study revealed compromised lipolysis in particular in the elder model where gastric and intestinal impairment where combined. This underscores the potential consequences for nutrient absorption and the associated health risks stemming from incomplete lipid digestion, particularly in the elderly population. Further examination through secondary lipid oxidation analysis demonstrated notable variations between meals and models, with a pronounced impact on meals richer in unsaturated fats. In particular, the soft salmon meal exhibited significantly lower oxidation rates in the intestinal phase for both elderly models, emphasizing the potential protective effect of compromised gastric function on reducing secondary lipid oxidation risk, especially in meals with unsaturated fats.

The promising results of this study suggest that gastrointestinal compromise may exert a detrimental influence on digestion, particularly affecting lipid metabolism. These findings underscore the intricate interplay between aging-associated physiological alterations and nutrient bioavailability, emphasizing the significant role of gastric function in lipid digestion

Acknowledgments
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Keywords : Elderly, Nutrient bioaccessibility, Lipid digestion, Lipid oxidation, In vitro studies

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22805) - QUANTITATIVE PROTEOMICS OF HUMAN PANCREATIC JUICE ENZYMES AND THEIR CONTRIBUTION TO OVERALL PROTEIN DIGESTION/ABSORPTION

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Abstract

Objectives: This study aimed at the relative quantification of all pancreatic enzymes in human pancreatic juice (HPJ) by LC-MS/MS after trypsin cleavage, as well as the overall quantification of these enzymes based on the known amounts of human pancreatic lipase (HPL) present in each juice sample and used as internal standard.

Methods: HPJ samples (n=10) were collected by performing endoscopic retrograde catheterization on the main pancreatic duct in human patients. Juices were collected on ice, lyophilized and stored frozen at -20 °C before analysis. HPL amounts were estimated from lipase activity measurements. LC-MS/MS was performed using an ESI-Q-Exactive Plus mass spectrometer and data were analyzed using Proteome Discover 2.4. software program and UniProt Homo sapiens protein database. After first considering full trypsin cleavage only for processing the data, we then tested the combined cleavage by trypsin, chymotrypsin and elastase, these enzymes being naturally present in HPJ. This approach allowed amplifying the number of peptides detected for each pancreatic enzyme, the number of peptide spectral counts (psm, peptide single match) and protein sequence coverage. Normalization of spectral counting for each enzyme against total number of spectral identifications for the acquisition was used for intra-sample relative protein quantification.

Results: Protein amounts in lyophilized HPJ powder were found to be 31,05 ± 16.16 % w/w, with HPL representing 6,7 ± 4,73 % w/w of total proteins. Relative pancreatic enzyme quantification by LC-MS/MS then allowed estimating the % w/w of each pancreatic enzyme in HPJ. Knowing the amounts of HPL secreted during a meal (200 to 400 mg of protein) from previous studies in healthy volunteers, it was possible to estimate the secretory output of each pancreatic enzymes (to be shown during the presentation) and thus the total amounts of enzymes (2.5 to 5 grams of proteins) secreted during a meal.

Conclusion: The amounts of pancreatic enzymes secreted during a meal represent 5 to 10 % of the daily protein intake (around 50 grams) suggested by the Food and Drug Administration. This has to be taken into account when studying protein digestion and absorption in vitro and in vivo.

References

Acknowledgments

Keywords : pancreatic juice, enzyme, secretion, meal, protein digestion

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(22807) - IN VIVO ASSESSMENT OF WATER SALINITY IMPACT ON THE ABILITY OF EUROPEAN SEABASS TO DIGEST A DIET CONTAINING INSECTS

Costa, Rafaela S. (Portugal)[1,2]; Basto, Ana (Portugal)[2]; Monteiro, Marta (Portugal)[1,2]; Sá, Tiago (Portugal)[2]; Fantatto, Rafaela (Portugal)[3,4]; Ribeiro, Teresa (Portugal)[3,4]; Vieira, Inês (Portugal)[5]; Santos, Marisa V. (Portugal)[5]; Murta, Daniel (Portugal)[3,4,5]; Valente, Luísa M.P. (Portugal)[1,2]

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Abstract

In response to aquaculture's expansion and the increasing demand for novel, sustainable ingredients for aquafeeds, various studies have supported the utilization of insect meal as an alternative protein source. The European seabass (*Dicentrarchus labrax*) – one of the most consumed fish species in Europe – is a euryhaline species that can tolerate a wide range of water salinity (5-50 ppt). Since the osmoregulatory and digestive functions of teleost fish are closely connected, and alterations in water salinity can impact intestinal morphology and nutrient absorption, we hypothesize that water salinity may influence the digestibility of diets containing insect meal, potentially increasing their nutrient bioavailability. Thus, in this study, we aimed to evaluate the impact of two different water salinity levels – 35 ppt and 5 ppt – on the digestibility of European seabass diets containing an insect meal blend of black soldier fly (BSF, *Hermetia illucens*) and yellow mealworm (YM, *Tenebrio molitor*).

Four isoproteic (44.9% dry matter, DM), isolipidic (18.5% DM), and isoenergetic (22.1 kJ/g DM) diets were formulated to conduct an *in vivo* digestibility trial with seabass juveniles: a practical control diet with low levels of fishmeal (15%, a protein source with high biological value for fish) and three diets containing the insect meal blend (50% BSF and 50% YM) in replacement of 3%, 25%, and 50% of the fishmeal. Cr_2O_3 was also added to the diets as an inert marker to estimate nutrient and energy digestibility. The four diets were randomly distributed among triplicate homogeneous groups of fish reared in tanks with a settling column connected to the outlet for faeces collection. For each water salinity, an independent recirculating aquaculture system was used, with optimal conditions for seabass. Fish were fed three times daily with a predetermined quantity of feed, and the faeces in the settling column were collected once daily (before the morning meal) for approximately 25 days. One pool of faeces per tank was created and stored at -20 °C. Before analysis, the faeces were freeze-dried.

The apparent digestibility coefficients of nutrients and energy in the experimental diets will be discussed concerning both the level of insect meal and the water salinity.

Acknowledgments

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Keywords : Dicentrarchus labrax, Apparent digestibility coefficient, Insect meal, Water salinity

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(22809) - UNVEILING A HIDDEN THREAT: UNDERSTANDING HOW DIETARY CONTAMINANTS SHAPE INTESTINAL BARRIER HEALTH THROUGH AN IN VITRO TRIPLE CULTURE MODEL

Araújo, Ana Margarida (Portugal)[1]; Ramos, Helena (Portugal)[1]; Ribeiro, Mafalda (Portugal)[1]; Martins, Zita (Portugal)[1]; Faria, Miguel A. (Portugal)[1]; M. P. L. V. O. Ferreira, Isabel (Portugal)[1]

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Abstract

Understanding the impact of food contaminants on intestinal health stands as a paramount research domain, particularly considering the wide range of substances present in our diet, including pesticides, heavy metals, mycotoxins, heterocyclic amines (HAA) and polycyclic aromatic hydrocarbons (PAHs). Current research often fails to address the complex scenario of multiple contaminants that coexist in food and overlooks the intricate interplay among different cell types within the human intestine. This gap highlights the pressing necessity for more sophisticated and representative models that can better mimic the intestinal environment. Moreover, acknowledging intestinal inflammation as a crucial risk factor is imperative, especially considering the rising cases of inflammatory bowel diseases in modern western societies. Thus, integrating these aspects in our evaluations is crucial for a holistic comprehension of the impact of food contaminants on intestinal health.

In this work, we used an advanced *in vitro* triple culture model (Caco-2/HT29-MTX/THP-1 cells) to replicate both healthy and inflamed conditions[1] within the human intestine. This model facilitated the investigation of cellular responses to a repeated exposure (dual exposure for 3 hours with 1 h rest interval) to a complex mixture of 41 food contaminants – including PAHs, mycotoxins, pesticides, HAA, and heavy metals. This mixture was formulated based on extensive literature mining and analysis of food contaminant occurrence data[2], aiming to simulate realistic daily exposure levels at the 25th (P25) and 95th (P95) percentile found in an omnivore diet.

Monitoring critical parameters such as cell viability, barrier integrity (TEER) and mRNA expression levels in both physiological conditions unveiled significant findings. Firstly, exposure to the P95 non-cytotoxic mixture led to a clear reduction in TEER values in the Caco-2/HT29-MTX monolayer. This reduction was evident in the normal state (22% reduction) but was more pronounced in the inflamed intestine (61% reduction) compared to the initial measurement (0h). Regarding mRNA expression in intestinal cells, alterations were seen for tight junctions and transporters expression levels, as well as differential expression in inflammatory markers of THP-1 cells compared to vehicle control.

These results strongly indicate that the mixture of contaminants at higher although realistic concentrations (P95) significantly impair the intestinal epithelium's barrier integrity, especially in an inflamed state. This suggests a potential exacerbation of effects on an already compromised intestinal barrier in the presence of these dietary contaminants.

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Acknowledgments

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Keywords : Food Contaminants; Co-Culture Model; Intestinal Health; Barrier Integrity; Inflammatory Response

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(22810) - SIMULATED DIGESTION TO STUDY HEALTH EFFECTS OF INULIN RICH NOODLES

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Abstract

Currently, the dietary fiber and prebiotic effects of inulin have been well documented. In animal and human trials, inulin has significant interference effects on blood glucose response and obesity, but the underlying mechanism is not clear, and studies have focused on the relationship between inulin and intestinal flora. This paper aims to explore whether inulin can reduce the blood glucose response by affecting the gastric emptiness rate, and whether it would achieve the control effect of obesity by decreasing appetite. Using semi-dynamic in vitro digestion method and an advanced near real dynamic in vitro human stomach system are to explore the mechanism for effect of rheology of inulin noodle on starch digestibility rate and blood glucose response. At the same time, to prove whether three layers noodle is a good carrier of inulin, which could improve the inclusion rate of inulin and the efficiency of inulin while balancing palatability and acceptability.

Keywords : Semi-dynamic in vitro digestion, Inulin, In vitro human stomach system, Gastric emptying, Glycemic response

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(22814) - INVESTIGATING THE DIGESTIBILITY AND ABSORBABILITY OF SUSTAINABLE OMEGA-3-RICH OILS

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Abstract

The aim of our research is to investigate the bioaccessibility and bioavailability of sustainable omega-3 rich oils in a specialized *in vitro* model. While the health advantages of omega-3 from fish oil, particularly DHA, are well-established, the potential of algae oil as a sustainable alternative remains underexplored, given its distinct chemical properties. This project addresses the gap in knowledge concerning the bioavailability and potential health benefits of omega-3-rich algae oils by testing oils from various companies. We chemically characterized the oils to obtain insight in their composition. Subsequently, we adapted the INFOGEST protocol to make it more suitable for lipid digestion, allowing us to study the bioaccessibility of the oils. The digested oils were applied on a Caco-2 Transwell cell model to assess their bioavailability. By establishing the connection between bioavailability and bioactivity properties of omega-3-rich algae, we get more insight in the sustainable alternative for fish oil.

Keywords : Omega-3, INFOGEST, Caco-2, Algae oil, in vitro

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(22817) - POTENTIAL OF RED BERRIES TO INHIBIT STARCH DIGESTION

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Abstract

Red berries are rich sources of anthocyanins, a subgroup of polyphenols, which have been associated with beneficial effects towards chronic disease such as diabetes, obesity and cardiovascular disease. In particular, the hypoglycaemic properties of anthocyanins have been highlighted, with reference to inhibition of carbohydrate-hydrolysing enzymes alpha-amylase and intestinal alpha-glucosidases. However, berries contain different amounts and types of anthocyanins, hence their biological activity towards enzyme inhibition may differ. The current study compared the enzyme inhibitory properties of different berries, blackberries, blackcurrants, red and black raspberry, and blueberries. Berries differed in their capacity to inhibit both enzymes with blackcurrant, blackberry and raspberry being more efficient compared to blueberry. The IC50 values of blackcurrant compared to blueberry were around 4 times higher for both, alpha-amylase as well as alpha-glucosidase enzyme when normalised to anthocyanin content. Red raspberry in comparison to black raspberry showed high potency for enzyme inhibition, indicating that components other than anthocyanins might be responsible or strongly contribute towards enzyme inhibition.

Acknowledgments
FSN Nutrition and Public Health. University of Leeds

Keywords : berries, alpha-amylase, alpha-glucosidases

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(22819) - THE IMPACT OF PEA PROTEIN HYDROLYSIS ON IN VITRO A-AMYLASE AND A-GLUCOSIDASE INHIBITORY ACTIVITY

Ebira, Arig (United Kingdom)[1]; Bosch, Christine (United Kingdom)[1]; Hernandez Alvarez, Alan (United Kingdom)[1]

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Abstract

Hydrolysis of pea protein has been associated with the generation of bioactive peptides that exhibit properties to inhibit carbohydrate digestion hence contribute to lowering of blood glucose. Multiple factors can affect the degree of protein hydrolysis, including protein structure and amino acid composition as well as specificity and of protease enzyme and its accessibility to the target sequence.

The aim of this project was to compare the behaviour of different pea protein products with differing pre-hydrolysis during pepsin hydrolysis, with regards to digestion kinetics and formation of bioactive peptides with properties to inhibit the enzymes alpha-amylase and alpha-glucosidase.

Protein characterisation (SDS-PAGE and protein solubility) demonstrated increased solubility for pre-hydrolysed pea protein (PHP) at different pH levels with a max of 80% at pH 12 ($p \leq 0.05$) and higher presence of low molecular weight proteins/peptides compared to non-hydrolysed protein (NHP) (10-16 kDa). Pepsin-facilitated digestion demonstrated a significantly increased degree of hydrolysis and release of peptides over time in the case of PHP reaching 64%, in contrast to NHP with 41%, respectively. The findings from enzyme inhibition assays demonstrated that peptides derived from PHP exhibited a dose-dependent inhibition of α-amylase and α-glucosidase, with the highest inhibitory activity observed at concentrations of 12 mg/ml and 20 mg/ml, resulting in a 37% and 46% inhibition, respectively. Further experiments will aim to enrich low molecular weight peptide fractions to confirm and potentially enhance inhibitory effects towards target enzymes.

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Acknowledgments
Not applicable

Keywords : pea protein, bioactive peptides, hydrolysed protein, diabetes, carbohydrate digestive enzymes.

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22854) - ALWAYS STRIVING FOR BEST PRACTICES IN IN VITRO DIGESTION METHODS

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1 - Agriculture and Agri-Food Canada

Abstract

Novel foods emerge daily. Processing technologies and formulations are adapted to be environmentally friendly, to meet specific nutritional requirements or help alleviate health issues. Furthermore, with the world population growing faster than ever, developing solutions with less traditional ingredients such as crop residues, insects or cellular agriculture could become essential for the health of our planet. In vitro digestion approaches can help analyse potential benefits and shortcomings of novel ingredients or foods. Results obtained by in vitro digestion can also influence policy, safety regulations and processing protocols of novel foods before they reach consumers. To guide the future development of feeding options worldwide, in vitro digestion profiles need several features: 1) comparable to physiological digestion and absorption, 2) repeatable, 3) adaptable, and 4) affordable. The INFOGEST static method was initially published in 2014 (1). The INFOGEST static method was further adapted leading to an improved method being published five years later (2). In 2020, a standardised semi-dynamic method for food was proposed (3). As scientific methods are in constant evolution and in vitro digestion protocols are used by an always increasing number of research teams, we are proposing a methodological review of various adaptations of the INFOGEST static method published since 2019 as well as the semi-dynamic method since 2020 to help understand the main challenges encountered and adaptation proposed by current investigators. Striving for constant scientific accuracy, the authors of the INFOGEST static method have paved the way for other collaborative efforts to meet the concerns associated with in vitro digestion. We hope to be able to support their efforts by highlighting and understanding adaptations proposed to the current protocols and suggesting potential solutions and alternatives to improve our collective knowledge of foods for future generations.

References

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Keywords : Best Practice, Food, Meal, Dynamic In Vitro Models, Static In Vitro Models

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22856) - THE EFFECT OF SLIGHT PH CHANGES IN GASTRIC CONDITIONS ON THE SURVIVAL OF LISTERIA MONOCYTOGENES

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1 - Universidade Católica Portuguesa - Escola Superior de Biotecnologia

Abstract

Listeria monocytogenes, the etiological agent of human listeriosis, poses a substantial threat as a lethal intracellular pathogen after the consumption of contaminated foods. Invasive listeriosis is particularly severe in vulnerable populations, including pregnant women, newborns, the elderly, and immunocompromised individuals. While all strains are conventionally considered equally virulent, acknowledging the inherent variability, the absence of reliable biomarkers hampers the differentiation of strains based on virulence. However, resistance to the gastrointestinal tract could be viewed as a potential discriminatory factor, as invading strains must endure the challenges posed by the gastrointestinal environment for successful establishment. Upon ingestion, on its passage through the human gastrointestinal tract, *L. monocytogenes* faces multiple hurdles, which can significantly affect its ability to cause an infection. One of the many hurdles is the harsh environment of the stomach. Hydrochloric acid (HCl) secretion in the stomach plays a pivotal role in raising acidity to levels lethal for most bacteria. In this study, 71 out of 80 *L. monocytogenes* strains, were screened for their ability to survive gastric conditions using a simulated gastric fluid (SGF; pH 3.0 HCl) as recommended by the INFOGEST protocol. All isolates proved to be resistant to such pH with no significant differences encountered between isolates (data not shown). This study aimed to investigate if lowering the testing pH would impact the survival of the pathogen and if this impact could be different between strains. In this context, five strains were selected and their survival to pH values of 2.8, 2.6, and 2.1, was evaluated.

At pH 2.8, a reduction of ca. 2.5 log CFU/mL was observed for four of the five strains tested. The other strain proved to be more resistant than the other four ($p<0.05$) being reduced by ca. 2.0 log CFU/mL. A similar trend was observed at pH 2.6. When exposed to a more acidic condition (pH 2.1), all isolates appeared to be more sensitive (reductions between 3.2 and 3.6 log cycles), and differences between isolates were not statistically significant ($p>0.05$). Further tests with the remaining strains will be now conducted at pH 2.8.

Although preliminary, these results suggest that minimal fluctuations in gastric pH can either enhance the survival of pathogenic bacteria (at pH 2.6 and above) or reduce it (at pH as low as 2.1). Furthermore, resistance to simulated gastric conditions is strain-dependent and these differences were better noted at pH 2.8.

References

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Acknowledgments

This work was supported by Fundação para a Ciência e a Tecnologia(FCT) through project GenoPhenoTraits4Persitence - Genomic and phenotypic traits contributing to persistence of *Listeria monocytogenes* in food processing environment (PTDC/BAA-AGR/4194/2021). The authors would like to thank the scientific collaboration under the FCT project UIDP/00329/2020. Financial support for author Mónica Oliveira was provided by the doctoral fellowship 2021.08345.BD.

Keywords : Listeria monocytogenes, Gastric conditions, pH sensitivity, Gastrointestinal survival

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22857) - COMBINED EFFECT OF THERMAL PROCESSING AND GASTROINTESTINAL DIGESTION ON THE IGG-BINDING CAPACITY OF LUPINE GAMMA-CONGLUTIN

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1 - REQUIMTE-LAQV/FFUP

Abstract

Lupine is a legume commonly used in human diet as a functional food due to its high nutritional content and important technological properties. However, its consumption can lead to the occurrence of adverse reactions, posing significant health issues in sensitized/allergic individuals, who need to avoid lupine-containing foods. Gamma-conglutin is considered a major allergen with an IgE-binding frequency in lupine allergic patients higher than 50% and, therefore, considered one of the most relevant lupine proteins [1]. This work intended to explore the effect of simulated gastrointestinal (GI) digestion combined with thermal treatment on the immunoreactivity of gamma-conglutin from the most economically important lupine species (*Lupinus albus, L. luteus* and *L. angustifolius*).

Three model foods of wheat pasta were prepared containing 35% of lupine flour from each lupine species and were submitted to a boiling process for 5 minutes to simulate home-made pasta preparation. The proteins were extracted with Tris-HCl 100 mM pH 8.0 at 60 ºC during 2 h and further characterized by SDS-PAGE and immunoblotting in non-reducing conditions. Simulated GI digestion was performed on thermally treated pasta using the harmonized digestion protocol from INFOGEST [2]. The IgG-binding capacity of g-conglutin was assessed by immunoblotting and ELISA with specific antibodies. Preliminary SDS-PAGE and immunoblotting results suggest that the protein profile and the IgG-binding pattern of lupine seeds is very different among species. ELISA results demonstrate that the boiling treatment seems to have a different effect on immunoreactivity depending on lupine species. Simulated GI digestion led to an extensive damage of the protein structure, reducing the IgG-affinity to g-conglutin and its potential presentation to immunocompetent cells. Immunoblotting and ELISA results showed that this reduction was more significant in the intestinal phase, leading to the complete disappearance of g-conglutin IgG-reactive peptides above 5 kDa.

These findings enhance the comprehension of how thermal processing and gastrointestinal digestion affect the immunoreactivity of γ-conglutin and its potential allergenicity. This information can offer valuable insights to the food industry for developing products with reduced allergenic properties.

References

1 DOI: 10.1111/1541-4337.12646

2 DOI: 10.1038/s41596-018-0119-1

Acknowledgments

This research was supported by national funds (FCT) through project Hypoallergen (PTDC/BAA-AGR/4005/2021) and the strategic funding from FCT/MCTES (UIDB/50006/2020| UIDP/50006/2020). J.C., I.M. thank FCT funding (2021.03583.CEECIND/CP1662/CT0012, 2021.03670.CEECIND/CP1662/CT0011).

Keywords : Food allergens, Lupine, gamma-conglutin, gastrointestinal digestion, thermal treatment

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22859) - IN SILICO DIGESTION AS A VALUABLE APPROACH TO PROSPECT BIOACTIVE PEPTIDES FROM EDIBLE INSECTS

Teixeira, Carla S. S. (Portugal)[1]; Biltes, Rita (Portugal)[1]; Villa, Caterina (Portugal)[1]; Sousa, Sérgio F. (Portugal)[2]; Costa, Joana (Portugal)[1]; Ferreira, Isabel M.P.L.V.O. (Portugal)[1]; Mafra, Isabel (Portugal)[1]

1 - REQUIMTE-LAQV/FFUP; 2 - REQUIMTE-LAQV/FMUP

Abstract

Edible insects are emerging as an environmentally and economically sustainable source of protein, being considered as alternative foods, particularly to meat. Presently, the house cricket (*Acheta domesticus*) and the migratory locust (*Locusta migratoria*), are two of the four insect species that areauthorized for human consumption within the European Union, being considered as novel foods [1].In addition to its nutritional value, edible insects have been looked at with great interest as they are sources of important bioactive compounds including bioactive peptides [2,3]. In this context, this work proposes an *in-silico* approach to simulate the gastrointestinal (GI) digestion of *A. domesticus* and *L. migratoria* proteins and identify new peptides with the capability to selectively inhibit the C- and N-domains of the somatic Angiotensin-I converting enzyme (sACE), thus contributing with
anti-hypertensive and anti-fibrosis properties, respectively.

A molecular docking protocol was applied to evaluate the binding interactions between the obtainedpeptides and the two catalytic domains of sACE. The evaluation of the intermolecular interactions between the peptides with highest docking scores, the reference inhibitor and the residues lining theactive sites of the two sACE domains, restricted a large pool of peptides to a group of ten - AVQPCF, CAIAW, IIIGW, DATW, QIVW, PIVCF, DVW (from *A. domesticus*) and TCDSL, IDCSR, EAEEGQF (from *L. migratoria*) - with a distinct binding pattern between the two domains.These results suggest that those peptides may act as selective inhibitors of the C- or -N domains of sACE, thus exhibiting potential anti-hypertensive or anti-fibrosis properties. The *in silico* results were further confirmed by experimental data, namely by performing the *in vitro* GI digestion of the whole *A. domesticus*. The digested peptides were submitted to a LC-MS/MS targeted analysis, which confirmed the presence of three (AVQPCF, CAIAW and PIVCF) of the seven peptides previously identified using the *in silico* approach. The same procedure is currently being applied to
L. migratoria in order to experimentally determine the presence of the predicted bioactive peptides.

This work demonstrated that *A. domesticus* and *L. migratoria* are good sources of bioactive peptides with potential anti-hypertensive and/or anti-fibrosis properties and also demonstrated the potential of the *in silico* methodologies as a valuable and reliable tool to simulate the GI digestion of proteins.

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1 Regulation EU 2015/2283

2 DOI:10.1016/j.foodres.2023.112847

3 DOI:10.1039/d3fo04246d

Acknowledgments

This research was supported by national funds (FCT) through project Hypoallergen (PTDC/BAA-AGR/4005/2021), FCT/MCTES (UIDB/50006/2020|UIDP/50006/2020).

S.F.S., J.C. and I.M. thank FCT for funding through 2020.01423.CEECIND/CP1596/CT0003, 2021.03583.CEECIND/CP1662/CT0012 and 2021.03670.CEECIND/CP1662/CT0011, respectively.

Keywords : Edible insects, bioactive peptides, in silico/in vitro digestion, LC/MS-MS

Topic 3: In vitro, in vivo and in silico models of digestion and absorption | Poster

(22864) - COMPARISON OF THREE IN VITRO DIGESTION MODELS TO EVALUATE THE IMPACT OF A PROTEASE ADDITIVE FOR PIGLET FEED ON PROTEIN DIGESTIBILITY.

Mainville, Isabelle (Canada)[1]; Arcand, Yves (Canada)[1]; Courchesne, Dany (Canada)[1]; Turcot, Sophie (Canada)[1]; Langlois-Deshaies, Rachel (Canada)[1]; Lahaye, Ludovic (Canada)[2]; Germain, Isabelle (Canada)[1]

1 - Saint-Hyacinthe Research and Development Center, Agriculture and Agri-Food Canada (AAFC); 2 - Jefo Nutrition Inc.

Abstract

The incorporation of an exogenous protease to piglet feed, especially during weaning, to optimize the degradation of proteins and increase bioavailability of amino acids results in an improved assimilation necessary for health and maintenance of adequate growth performance. Evaluation of the impact of a protease supplement on nutrient accessibility can be evaluated *in vivo*, but an *in vitro* method could be a more affordable and ethically acceptable alternative. A standardized protocol, based on an international consensus developed by the COST INFOGEST network was published in 2014, and later amended to include more precisions on the methodology, in 2019 (INFOGEST 2.0). This method was developed to evaluate the outcomes of food digestion by examining the generated digestion products (such as peptides/amino acids, fatty acids, and simple sugars) and by appraising the liberation of micronutrients from the food matrix. AAFC is currently optimizing a dynamic In Vitro Digestion System (IViDiS). Based on the INFOGEST 2.0 method, a modified protocol (IVD-MOD) was developed to include 3 different pH phases of stomach digestion and add brush border membrane extracts containing peptidases, amongst others. The results of the modified protocol were compared to those of the INFOGEST 2.0 and the IViDiS methods. The enzyme/substrate ratios were compared. When preparing the meal (feed) and normalizing to respect the recommended 4% protein in the 5g meal, using the INFOGEST 2.0 method, the enzyme/substrate (E/S) ratio was 3.83, meaning that most of the proteins came from the enzymes used to digest the feed. Similar results were obtained with the IVD-MOD protocol. Using the IViDiS protocol, the E/S ratio was 0.425. Mass balance closures were measured and were all near 100%. Total amount of available amino acids (TAA) and available indispensable amino acids (IAA) were measured, using all 3 methods. Digestibility values of the feed, with and without a protease additive, were compared. Results show that the increase in digestibility, when protease was added, was not always statistically significant, although some individual IAA did show significant increases. The complexity relies on the interpretation of results: What % of TAA and IAA really come from the feed versus the enzymes used in digestion protocols? This needs to be addressed by the scientific community in future amendments of standardized protocols.

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3- Egger, L. (2022).

Keywords : digestibility, proteins, in vitro, piglet, protease

ICFD 2024

TOPIC 4

IN VITRO, IN VIVO AND IN SILICO MODELS OF DIGESTION AND ABSORPTION

Portugal | April 9-11th, 2024

Topic 4: Food structures, digestion and imaging technologies | Poster

(21406) - PEA SEED WITH REDUCED ANTINUTRITIONAL PROTEINS: A TECHNOFUNCTIONAL AND NUTRITIONAL STUDY

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1 - Quadram Institute Bioscience, Norwich, UK; 2 - John Innes Centre, Norwich, UK

Abstract

Sustainable and nutritious protein sources are becoming increasingly essential due to a growing global population and the rising popularity of plant-based diets. Legumes, such as pea (*Pisum sativum* L.), are excellent alternative protein choices due to the overall nutritional richness of their seeds and environmental sustainability of the crop. However, the industrial application of legume-based proteins is so far limited by sub-optimal techno-functional performance. Furthermore, pulses contain antinutritional proteins, which can hinder nutrient bioavailability. This research aimed to evaluate the techno-functional and nutritional properties of proteins isolated from two distinct pea lines: a wild-type control pea and a mutant line carrying null mutations for three proteins with poor nutritional characteristics. The seeds of the wild-type and mutant pea lines were ground, and the isolated protein used to create emulsions and foams. The emulsions were characterized based on their particle size, zeta potential, microstructure, and stability over time. Additionally, foams were characterized based on their foamability and stability. To gain further insight into the surface activity of pea proteins, the pendant drop method was used to measure their surface tension and interfacial properties. The findings revealed that proteins from both pea lines effectively stabilized emulsions over a 24-hour period. Emulsion stability was observed to have a positive correlation with the following factors: small droplet sizes, high zeta potential, and specific oil and protein concentrations. Minor differences were observed in the foaming properties of the two pea protein isolates. However, it was noted that foaming stability depended on high protein concentrations, a threshold that was not achieved in this study. In conclusion, this research demonstrated the similar techno-functional properties of wild-type and mutant pea lines, underscoring their potential for industrial use. Future research will utilize static *in vitro* simulation of gastrointestinal food digestion (INFOGEST 2.0) to evaluate the impact of pea mutations on starch and protein digestibility of cooked food items. Lastly, the study will assess the reduced allergenic potential of the mutant pea, further expanding the understanding of these promising protein sources.

Acknowledgments

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Keywords : Legumes, Antinutritional proteins, Techno-functional properties, Food allergenicity, Nutrient bioaccessibility

Topic 4: Food structures, digestion and imaging technologies | Poster

(21422) - TOWARDS ANIMAL FREE DAIRY: COAGULATION OF REASSEMBLED CASEIN MICELLES

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1 - Food Quality & Design Group, Wageningen University and Research

Abstract

Precision fermentation of milk proteins is a promising approach towards animal free dairy, i.e. vegan products with similar nutritional and sensorial properties as traditional dairy. Recombinant production of the caseins is however just the first step. In milk, these proteins are present in large aggregates known as casein micelles, which underly the particular coagulating properties of milk during production of e.g. cheese and yoghurt. During digestion this structure is also of importance, as the combination of acid and pepsin in the stomach cause coagulation of casein micelles leading to clot formation and a delayed emptying rate. To recreate the coagulation properties, recombinant casein will need to be assembled into a micellar structure. An assembly method exists, but it is unsure whether this method produces micelles with similar coagulation properties as native micelles. The aim of this study was therefore to compare the coagulation properties of reassembled bovine casein micelles with native casein micelles. In addition to gastric clotting, acid gelation and enzymatic coagulation (renneting) were assessed to elucidate the role of the different coagulation mechanisms.

Reassembled casein micelles were created by a method based on Schmidt et al (1977) and optimized by Fan et al (2024), where a sodium caseinate solution is slowly mixed with milk salts. Native casein micelles were obtained by microfiltration and diafiltration of raw milk. Gastric clotting (pepsin, 37°C, final pH 2.0) was characterized by a semi-dynamic *in vitro* digestion method, based on Mulet-Cabero et al (2020). Clots were assessed by determining moisture content, SDS-PAGE and CLSM. Emptied digests were assessed by DUMAS and OPA. Renneting (chymosin, 30°C, native pH) and acid gelation (no enzyme, 30°C, final pH 4.6) were characterized by oscillatory rheology and CLSM.

Results showed that reassembled and native micelles formed very similar gastric clots and were digested at a similar rate. This was surprising, as both renneting and acid gelation behavior were significantly different between the two samples. Reassembled casein micelles formed stronger rennet gels (G' 82±7 Pa vs 34±12 Pa) and weaker acid gels (G' 0.7±0.2 Pa vs 3.0±1.3 Pa) than native casein micelles. These differences might originate from the fact that reassembled casein micelles were smaller (120±3 nm vs 157±4 nm) and held more water (3.89±0.04 g/g vs 2.61±0.07 g/g), than native casein micelles while protein content and composition were similar. This study showed that effects depended on the coagulation mechanisms, which illustrates the complexity of dairy coagulation. The knowledge gained will help to design animal-free dairy products with similar properties as traditional dairy, while decreasing the impact of dairy production on animal-welfare and the environment.

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Acknowledgments

This publication is part of the 'Animal-free milk proteins' project (with project number NWA.1292.19.302) of the NWA research programme 'Research along Routes by Consortia (ORC)', which is funded by the Dutch Research Council (NWO).

Keywords : gastric digestion, casein micelles, coagulation, CLSM

Topic 4: Food structures, digestion and imaging technologies | Poster

(21426) - DAIRY MATRIX AND DIGESTIBILITY OF FERMENTED DAIRY PRODUCTS WITH DIFFERENT B-CASEINS

Daniloski, Davor (Australia)[2]; Vasiljevic, Todor (Australia)[1]; Brodkorb, André (Portugal)[2]; A. Mccarthy, Noel (Ireland)[3]

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Abstract

This study aimed to investigate if differences in β-casein polymorphic motif affects the properties of A1/A1, A1/A2 and A2/A2 fermented dairy products, namely yogurt and cheddar cheese and their subsequent digestion properties. The onset of gelation during yogurt production occurred significantly later in A2/A2 milk compared to A1/A1 and A1/A2 milks. Alternatively, cheddar cheese containing β-casein A1 possessed a greater storage modulus, water-holding capacity, with lower syneresis and a more compact gel network. Differences in the functionality of yoghurts and cheeses appeared to be related to the presence of β-casein A1 in A1/A1 and A1/A2 milks, as they were characterised by larger casein micelles, greater levels of κ-casein, with lower hydrophobicity compared to A2/A2 milk. Compositional differences, such as a greater level of α-helixes in A1/A1 and A1/A2 yoghurts and cheeses may also explain the observed differences in gel structure. During *in-vitro* digestion, using the semi-dynamic INFOGEST method, all samples showed protein aggregation and coagulum formation within the first 5 min of gastric digestion, at which time the pH ranged from 5.5 to 6.0. During digestion of A2/A2 yoghurt and cheese, the casein breakdown was slower and possessed a tight protein network, compared to that of A1/A1 or A1/A2 products. In this regard, although yoghurts and cheeses produced from milk containing β-casein A1 possessed faster gastric digestibility, they showed slower intestinal digestion compared to A2/A2 products. Therefore, milk with β-casein A2 is less suitable for yoghurt and cheese making, however, the weak gel it produces could potentially be responsible for its proposed easier digestibility. These characteristics will have significant consequences for set-style yogurt and cheese production and potentially have an impact on human nutrition and should be considered carefully when using milks distinguished based on β-casein phenotype.

References

N/A

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Keywords : fermented dairy products, rheology, conformational differences, in-vitro digestion, β-caseins A1/A1, A1/A2, A2/A2

Topic 4: Food structures, digestion and imaging technologies | Poster

(21429) - WHY PEA PROTEOLYSIS IS NOT ALWAYS THE SAME: IMPACT OF EXTRACTION AND PROCESSING VARIABLES

Verkempinck, Sarah (Belgium)[1]; Guevara-Zambrano, Jessica (Belgium)[1]; Dorine, Duijsens (Belgium)[1]; Grauwet, Tara (Belgium)[1]

1 - Laboratory of Food Technology, KU Leuven

Abstract

Interest in incorporating pulse proteins into food products is growing due to their nutrient-rich and sustainable nature. However, the impact of various extraction and processing methods on pulse protein structure, (micro)structural organization, and *in vitro* protein digestion kinetics remains poorly understood. Therefore, the influence of the protein extraction method on digestive functionality was studied by considering three distinct extraction approaches: *(i)* cooking followed by cotyledon cell isolation, *(ii)* alkaline extraction followed by isoelectric precipitation, or *(iii)* salt extraction, and compared to the digestion kinetics of the original whole pea flour. Results revealed that encapsulated, denatured protein within pea cotyledon cells exhibited the (s)lowest digestion, while accessible and more native protein (e.g., pea flour, pea protein salt extract) demonstrated a relatively high protein solubility, linked to the faster and higher level of digestion. Alkali-extracted pea protein showed partial protein denaturation, significantly reducing solubility and *in vitro* digestion kinetics. In the second part, a commercial pea protein isolate (PPI) dispersion was treated using a range of high-pressure homogenization intensities (0 – 200 MPa) to study the impact on digestive functionality. The original, denatured PPI displayed large irregular shell-like structures, low solubility, and limited proteolysis. Through high-pressure homogenization, a reduction in particle sizes was observed with increasing pressure, enhancing protein solubility and digestibility. Overall, the variations in protein structure significantly impacted the structural arrangement in dispersion, thereby influencing *in vitro* protein digestion kinetics across all studied protein samples. These insights showed the necessity for a deeper understanding of the relationships between process, structure, and digestion in novel pulse-based food(s) (ingredients).

Acknowledgments

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Keywords : pulse protein, (micro)structure, in vitro digestion, structural arrangement, kinetics

Topic 4: Food structures, digestion and imaging technologies | Poster

(21433) - EFFECTS OF PECTIN ON BIOACCESSIBILITY OF ANTHOCYANIN DURING IN VITRO DIGESTION

Jiang, Xiyu (Netherlands)[1]; Bi, Jinfeng (China)[2]; Liu, Xuan (Netherlands)[2]; Liu, Meng (China)[3]; Verkerk, Ruud (Netherlands)[1]; Dekker, Matthijs (Netherlands)[1]

1 - Wageningen University & Research; 2 - 2 Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences (CAAS), Key Laboratory of Agro-Products Processing, Ministry of Agriculture and Rural Affairs, Beijing, China; 3 - University of Liège, Gembloux Agro-Bio Tech, Gembloux, Belgium

Abstract

There is a growing trend of including fruit and vegetable (F&V) juices rather than soft drinks in the human diet, and the global demand for high-quality fruit juices with natural materials, minimally processed, and additive-free is constantly increasing. Mixed fruit juices contain several functional substances such as dietary fiber, including pectin, and bioactive compounds, including carotenoids and flavonoids like anthocyanins [1]. Considering their large production volumes, complementary flavors, and nutrient profiles, apple and peach are selected as raw materials to combine in mixed juice with anthocyanin-rich F&V. In the process of juice production, the interaction between anthocyanins and pectin is inevitable, potentially altering the physicochemical properties of the juice and, in turn, affecting its apparent quality and nutritional properties. The physicochemical stability of cyanidin-3-O-glucoside (C3G) under *in vitro* digestion was studied after binding with pectin from different sources (apple and peach) and fractions (Water-soluble fraction (WSF), chelator soluble fraction (CSF) and sodium carbonate soluble fraction (NSF)). Pectin could increase the retention rate and bioaccessibility of C3G after small intestinal digestion. The formation of complex could protect C3G from degradation under intestinal environment. AW-C3G presented the highest retention of the anti-oxidant capacity after *in vitro* digestion compared to other pectin fractions. Pectin linearity was highly negatively correlated with intestinal retention rate while the contribution of the rhamnogalacturonan (RG) region to the entire pectin was positively correlated with intestinal retention rate and bioaccessibility. Less linear structure with more RG region pectin could bind more C3G and retain stability during intestinal digestion. The hydrogen bonding and hydrophobic interaction were considered to be the main way for the binding between pectin and C3G under small intestinal digestion. The results indicate that specific pectin structures could be selectively extracted to optimize the interaction with C3G to achieve better stability and higher bioaccessibility during digestion. These findings could open opportunities for the application of natural sources of pectin and C3G for innovative functional food investigation.

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Acknowledgments

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Keywords : Cyanidin-3-O-glucoside; Pectin; In vitro digestion; Bioaccessibility; Anti-oxidant capacity

Topic 4: Food structures, digestion and imaging technologies | Poster

(21434) - CASEIN STRUCTURES DIFFERENTLY AFFECT POSTPRANDIAL AMINO ACID DELIVERY

Dupont, Didier (France)[1]; Bruno, Pereira (France)[3]; Malpuech-Brugere, Corinne (France)[4]; Remond, Didier (France)[4]; Boirie, Yves (France)[5]; Baniel, Alain (France)[2]; Richard, Ruddy (France)[5]; Boulier, Audrey (France)[2]; Boudry, Gaelle (France)[6]

1 - INRAE - STLO; 2 - Ingredia SA; 3 - University Hospital of Clermont-Ferrand; 4 - INRAE - UNH; 5 - Université Clermont Auvergne; 6 - INRAE - NUMECAN

Abstract

Milk is well-known to coagulate in the human stomach. This is due to the organization of the major milk proteins i.e. the caseins into a 200 nm spherical supramolecular structure called the casein micelles (CM) that consists in tens of thousands of casein molecules. CM aggregate at acidic pH and/or in the presence of pepsin and can be extracted from milk by microfiltration, providing an excellent protein ingredient. Casein can also be extracted from milk by acidification followed by neutralization, leading to the formation of sodium (SC) or calcium (CC) caseinate. SC and CC exhibit the same composition than CM but very different supramolecular structure (11 nm particles, only ~15 casein molecules). The comparative behaviour of CM vs SC has only been only scarcely studied so far.

In the present study, we aimed to assess if casein structure affects its digestion and its subsequent amino acid delivery kinetic. Three independent studies were performed to investigate the digestive behaviour of CM and SC: an in vitro study on the TIM simulator, an in vivo study on pigs and a clinical study on 12 healthy men. Overall, in vitro and in vivo trials gave the same trends. Higher nitrogen levels were recovered in dialysates after in vitro digestions of SC compared to CM. Likewise, plasma indispensable amino-acid concentration peak was higher after SC compared to MC ingestion in healthy volunteers in a randomized, double blind, cross-over study. In pigs, gamma-scintigraphy using labelled meals revealed that SC was mainly localized in the proximal part of the stomach whereas MC was distributed in the whole gastric cavity. Caseins were found in both solid and liquid phases and partly hydrolyzed casein in the solid phase shortly after SC drink ingestion. These data support the concept of slow (MC) and rapid (SC) casein depending of casein structure, likely due to their intra-gastric clotting properties.

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Acknowledgments

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Keywords : in vivo digestion, casein micelle, sodium caseinate, in vitro digestion

Topic 4: Food structures, digestion and imaging technologies | Poster

(21435) - IMPACT OF NOVEL PROCESSING METHODS ON THE MOLECULAR STRUCTURE OF DIETARY FIBRE IN SNACKS

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Abstract

In recent years diets worldwide have shifted towards increased consumption of processed, low-fibre foods. Dietary fibre, a type of carbohydrate that avoids digestion by human enzymes, reaches the large intestine intact where it is fermented by the gut microbiome, producing short-chain fatty acids (SCFA). These serve as a source of energy for the host and are highly beneficial for human health, making them an essential component of a healthy diet.

In this research, we aim to develop healthier food products using novel processing techniques with our industrial partner PepsiCo to incorporate large quantities of vegetables into snacks. We also focus on understanding intermolecular interactions within the food matrix thus giving us an insight into explaining the fibre solubility alternations, water binding capacity and digestibility. Solid-state nuclear magnetic resonance techniques have been used to study the molecular structure of our processed food snacks, as well as changes in molecular mobility [1]. In vitro digestion was also performed to quantify starch amylolysis. Samples were also subjected to the INFOGEST protocol [2] to further evaluate changes in composition and physicochemical characteristics, when subjected to various gastrointestinal conditions.

Our findings show that different processing techniques have an impact on the solubility and water-binding properties of food snacks. These alterations, in turn, affect their digestibility and accessibility to digestive enzymes. Notably, the addition of vegetables to processed snacks enhances their resistance to digestion, potentially benefiting gut health and overall well-being.

References

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Acknowledgments
This work is funded by PepsiCo, Inc. Views expressed are those of the authors and do not necessarily reflect the position or policy of PepsiCo, Inc.

Keywords : Dietary Fibre, Digestion, Food Matrix, Solubility, Snacks

Topic 4: Food structures, digestion and imaging technologies | Poster

(21440) - EMULSION'S INTERFACIAL COMPOSITION INFLUENCES CLOT DISINTEGRATION OF CO-Q10-ENRICHED MILK

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Abstract

The impact of the milk structure on digestion kinetics and nutrient release in the GIT has become more recognized by the scientific community. In the gastric environment, milk undergoes dramatic physical changes from liquid to semi-solid, forming the so-called 'clot'. Several factors affect the structural features of the clot and its disintegration patterns in the gastric phase, such as the presence of fat globules, heat processing, homogenisation, among others. The formation of a clot in the stomach acts as a control delivery mechanism of proteins and lipids to the small intestine, which may have some health implications. In enriched milk, emulsions can be added as delivery systems of lipophilic health-enhancing substances, but the impact of emulsion's interfacial composition on clot formation and disintegration remains unknown. This study investigated the clot formation and disintegration kinetics of bovine milk (UHT vs pasteurised) enriched with coenzyme Q10-loaded emulsions with various interfacial compositions and the impact on nutrient release in gastric fluids. Therefore, four enriched milk samples were prepared using pasteurised or UHT skim milk combined with either Tween 80 emulsion or sodium caseinate emulsion loaded with Coenzyme Q10. An *in vitro* dynamic digestion model (HGS) was used. Changes in the clot structure were analysed by particle size distribution (sieving method) and CLSM. The composition of the emptied digesta (total solids, protein, and lipids) and Coenzyme Q10 content was also monitored. It was found that the emulsion's interfacial composition (Tween 80 or sodium caseinate) dramatically modifies the clot structure; the clot from milk with sodium caseinate emulsion was very fragmented, compared to a tightly knit clot from milk with Tween 80 emulsion. As expected, UHT treatment caused a greater degree of clot fragmentation, leading to faster disintegration. As a result, significant changes in the percentage of total solids, protein, and lipid retention in the HGS during digestion were observed. Coenzyme Q10 release was also strongly influenced by the clot disintegration kinetics. This study contributes to understanding how emulsion's formulation impacts milk gastric digestion.

References

Acknowledgments
Authors thank the Riddet Institute CoRE and the Tertiary Education Commission in New Zealand for providing funding for this research.

Keywords : Milk, Digestion, Food structure, Emulsion, Coenzyme Q10

Topic 4: Food structures, digestion and imaging technologies | Poster

(21442) - IMPROVING PROTEIN QUALITY OF TEXTURIZED SOY PROTEIN THROUGH EXTRUSION PROCESSING

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Abstract

Plant-based meat analogues, produced from texturized vegetable protein (TVP), have found their way into human diets in response to sustainability concerns, yet their impact on human health remains relatively unknown. Processing techniques employed during TVP production can influence the structural and nutritional properties of the proteins and, hence, the overall health impact of TVPs. Therefore, this study aimed to determine the impact of TVP processing on its structural properties, amino acid score, and in vitro protein digestibility. To achieve this, extrusion with various processing conditions (100 – 160 °C and 50 – 70% moisture content) was used to produce soy-based TVPs using either soy protein concentrate (SPC) or soy protein isolate (SPI). The protein quality was assessed by determining the amino acid (AA) score (measured by HPLC) and in vitro protein digestibility (measured by the fraction of released alpha-amino groups after the application of the INFOGEST 2.0 protocol). Structural and mechanical properties of the TVPs, including water-holding capacity, particle size, and resistance to compression, were assessed to establish a link with protein quality. The results show that processing affects the TVP structural properties as hardness and particle size increase and the water-holding capacity (WHC) decreases with increasing severity of extrusion processing. Additionally, extrusion enhanced the overall in vitro protein digestibility for both SPI- and SPC-based TVPs compared to the starting material. However, despite the profound impact of processing on the structural properties, the protein quality of PBMAs remains relatively stable across a wide range of processing conditions (100 – 160 °C and 60 – 70% moisture content)- no trend in AA scores with various processing conditions was observed. Additionally, only the in vitro protein digestibility of the soy-based TVPs decreased at low moisture extrusion (50% moisture content) when compared to high moisture extrusion (60-70%). The impact of low-moisture processing was more pronounced for the SPI-based TVPs compared to the SPC-based TVPs. Overall, the results demonstrate reasonable stability in protein quality for soy-based TVPs at high-moisture extrusion processing.

References

Acknowledgments

We thank Jarno Gieteling (Wageningen University and Research, Food Process Engineering) for assistance with the extrusion process. We also thank the partners Agrifirm, Cargill, Evergrain, Nutris, Roquette, Unilever, and V2 Foods for funding this project.

Keywords : plant-based meat analogue, in vitro digestion, protein transition, processing, amino acid score

Topic 4: Food structures, digestion and imaging technologies | Poster

(21447) - EFFECT OF XANTHAN GUM-MICELLAR CASEIN INTERACTIONS ON THE DIGESTIBILITY OF HYBRID SYSTEMS

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Abstract

Polysaccharides are widely used in the food industry as additives in food products. In the particular case of milk products, some polysaccharides have shown the capacity to stabilize the structure of proteins. The interactions between proteins and polysaccharides are not only relevant to control the techno-functional properties of food products, but they also play a crucial role to modulate protein digestibility. By selecting the appropriate polysaccharide and adjusting its concentration, it becomes feasible to regulate protein digestibility and promote the release of biologically active peptides in the distal intestine.

Accordingly, this study aimed to investigate the *in vitro* digestibility of micellar casein in polysaccharide-protein hybrid structures, where xanthan gum (XG) was chosen as the structuring polysaccharide. The study evaluated the impact of the structure type (hydrogels vs. aerogels) and the XG:casein ratio. Gastrointestinal digestions were performed following the INFOGEST protocol, and the resulting digestion products were characterized to determine the degree of proteolysis and microstructural changes. The results revealed that the addition of XG significantly delayed the digestion process, particularly during the gastric phase. Following intestinal digestion, it was observed that a fraction of casein was strongly bound to XG, remaining in undigested granules, as evidenced by CLSM micrographs. This phenomenon was more pronounced in the case of the aerogels as compared to hydrogels. However, the overall degree of hydrolysis was more influenced by the XG concentration than the type of structure, with higher XG concentrations leading to a lower degree of protein digestion.

Thus, by adjusting the of XG and the developed structure types, it is possible to modify the digestibility of micellar casein, thereby promoting the release of biologically active peptides in the intestine.

Acknowledgments
Funded by the Minister of Science under the Regional Initiative of Excellence Program.

Keywords : aerogel, structure, protein

Topic 4: Food structures, digestion and imaging technologies | Poster

(21456) - DIGESTIBILITY, NUTRITIONAL AND FUNCTIONAL PROPERTIES OF PROTEIN EXTRACTS FROM ULVA SEAWEED

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Abstract

Seaweeds have a great potential as a sustainable source of alternative proteins of non-animal origin. Besides their high protein and dietary fiber contents, seaweeds are attracting a great deal of interest due to their advantages over land-based biomass, as well as their rich mineral and vitamin content, affordability, and acceptability. However, they are still largely unexplored as food ingredients and their techno-functional and nutritional properties are yet to be investigated.

The aim of this work was to characterize the protein digestibility, nutritional quality and functional properties of hybrid protein-polysaccharide extracts obtained from the green seaweed *Ulva spp*. through different extraction protocols and with varying degrees of protein purification. The extraction method was based on a pH-shifting protocol and the application of an ultrasound pre-treatment to disrupt the seaweed cell walls was also evaluated. The gross composition of the extracts was determined, as well as a more exhaustive characterization of the protein and polysaccharide fractions. Subsequently, the digestibility of the whole seaweed and the extracts was determined by means of *in vitro* gastrointestinal digestions following the standardized INFOGEST Protocol.

Although all the obtained extracts were mainly composed of proteins and polysaccharides, the different steps of the extraction protocol had a significant impact on their composition and functional properties, such as solubility and surface charge. In general, all the extracts showed improved digestibility with respect to the native seaweed, whose digestibility was quite low due to the tough cell walls. However, the presence of polysaccharides in some of the extracts, mainly ulvans, which were capable of forming complexes with the proteins, was also determinant on the digestibility.

Our results provide a basis for the rational design of strategies to produce nutritious protein-rich ingredients from *Ulva* and evidence the potential of this seaweed as an alternative protein source.

Acknowledgments

This research work was financially supported by the grant TED2021-129711B-I00, funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR.

Keywords : In vitro Digestion, Seaweeds, Alternative protein-rich sources

Topic 4: Food structures, digestion and imaging technologies | Poster

(21461) - EFFECT OF ULTRASOUND ON PROTEIN STRUCTURE AND BREAKDOWN IN A PEA-WHEY PROTEIN MODEL FOOD

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Abstract

Background: Driven by climate change and animal welfare concerns, flexitarian diets emphasize plant protein intake. However, there are challenges in developing solid foods with plant proteins due to solubility, gelling issues, and off-flavors. High-power ultrasound can address some of these challenges by reducing particle size through cavitation and shear. This study aimed to investigate the impact of ultrasound on a combination of plant and animal protein system, exploring breakdown processes during static gastric digestion.

Methods: Protein dispersions (15% protein content; 3:2 ratio whey protein to pea protein isolate) underwent ultrasound treatment (167-171 W net power) for 7.5 or 15 minutes, or as control (no ultrasound). The dispersion was analyzed for particle size, followed by thermal gelation (heating in a water bath at 90°C for 1 h and cooling at 4°C for 12 h) and in vitro static digestion. Kinetics of gastric fluid uptake and softening were measured. Protein breakdown was assessed through free amino group content and SDS-PAGE.

Results: Ultrasound treatment significantly ($p<0.05$) reduced the particle size in the protein dispersion compared to control (D [4,3] of 30.6 vs. 102 µm and d_{50} of 5.36 vs. 32.47 µm observed in ultrasound 15 min vs. control, respectively). The smaller particle size was hypothesized to result in increased packing of the gel network in the model foods, as the Young's modulus (before digestion) after 15 mins ultrasound treatment was significantly ($p<0.05$) higher than after 7.5 min and control (83.5 vs. 62.8 vs. 64.2 kPa, respectively). Moisture and acid uptake were only affected by the digestion time ($p<0.05$) across all treatments, and not by ultrasound treatment. Over 180 min gastric digestion, the Young's modulus of ultrasound-treated model food did not change, suggesting negligible softening. However, the Young's modulus of the control model food decreased from 64.2 to 54.9 kPa from 0 to 180 min gastric digestion, suggesting differences with respect to the softening behavior in ultrasound-treated model foods. Protein breakdown was affected by the digestion time and the interaction of digestion time and treatment ($p<0.05$). The free amino group content due to protein breakdown was ~42% higher in control and 7.5 min ultrasound compared to 15 min ultrasound in the liquid digesta after 180 min gastric digestion.

Conclusion: Ultrasound treatment of whey and pea proteins prior to thermal gelation enhanced structural stability during gastric digestion, offering potential for developing novel protein-based foods with delayed breakdown for increased satiety.

Acknowledgments
This work was supported by USDA National Institute of Food and Agriculture, AFRI, award number 2020-67017-31258.

Keywords : pea and whey protein combination, ultrasound treatment, model food system, food structure, protein digestion

Topic 4: Food structures, digestion and imaging technologies | Poster

(21462) - SEAWEEDS' PROTEIN DIGESTIBILITY: METHOD OPTIMIZATION AND COMPARISON OF DIFFERENT SPECIES

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Abstract

The changing demands of modern society and the current environmental concerns are pushing the global food system to undergo significant changes. It is necessary to explore sustainable non-traditional protein sources, and there is particular interest in non-animal proteins. In this context, seaweeds are attracting a great deal of interest. Despite their interesting nutritional profile and advantages over land biomass, the potential of seaweeds as protein sources has not been fully exploited by the food industry due to the absence of information on their nutritional and techno-functional properties, and the complex structure of their cell walls, which are high in non-digestible polysaccharides.

The purpose of this work was to investigate the protein digestibility of edible seaweeds belonging to the three different groups: *Ulva spp.* (green), *Porphyra spp.* (red) and *Saccharina latissima* (brown), and assess the impact of their unique composition and cell wall architecture on the gastrointestinal digestion process. To this end, the standardized Infogest method[1] was optimized to perform *in vitro* digestions, taking into consideration the particular behaviour of seaweeds. *In vitro* digestibility, as well as the digestible indispensable amino acid ratio (DIAAR) and score (DIAAS) were determined, and the digestion products were characterized. Our findings reveal that the three species present interesting AA profiles, with essential AA contents ≥29%, being comparable to conventional protein sources. Moreover, they differed greatly in their protein digestibility values due to differences in their polysaccharide composition and cell wall architectures. In particular, *Porphyra spp.* showed more labile cell walls and presented the greatest protein digestibility (~65%).

Our results evidence the great potential of seaweeds as alternative protein sources. Nonetheless, certain species with recalcitrant cell wall structures, such as *Ulva spp.* and *Saccharina latissima,* present low protein digestibilities. In those cases, diverse cooking methods will be assessed to disrupt their cell wall structure and increase their protein digestibility.

References

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Acknowledgments

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Keywords : seaweeds, digestibility, alternative proteins, nutrition, microstructure

Topic 4: Food structures, digestion and imaging technologies | Poster

(21469) - MACRONUTRIENT RELEASE FROM FAVA BEAN COTYLEDONS WITH WEAKENED PLANT CELLS WALLS

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1 - Quadram Institute Bioscience

Abstract

The prevalence of Diabetes Mellitus in the United Kingdom has doubled in the last 15 years highlighting the need for the population to consider increasing consumption of low glycaemic index foods to support blood glucose management. Pulses, such as fava beans, are of interest for the development of low glycaemic food products, however there is a need to understand the exact mechanisms through which they exert these effects. Pulses are comprised of cotyledon cells, in which starch is surrounded by the protein matrix and encapsulated by the plant cell wall. The plant cell wall is already well-known to delay starch digestion by acting as a barrier to digestive enzymes, however the role of the intracellular protein on starch digestion has been less widely studied. Therefore, to better delineate between these mechanisms, we conducted an *in vitro* digestibility study comparing the digestion of fava bean (*Vicia faba*) cotyledon cells with intact versus chemically weakened plant cell walls. The protein matrix remained unaltered by the treatment as evaluated through qualitative and quantitative analyses. It was hypothesised that the cotyledon cells with weakened cell walls will be more digestible *in-vitro* compared to cells with intact cell walls. For the digestions, the simple α-amylase assay and the static INFOGEST 2.0 upper-gut digestion protocol were used. The results revealed that the modulation of the plant cell wall intactness altered the release of macronutrients both at the gastric and intestinal phases of *in vitro* digestion. Overall, this study shed light on the importance of understanding the complex structure of pulses and how cell wall modulation can influence the release of macronutrients during digestion. Further research into this area can contribute to developing dietary strategies to manage blood glucose levels especially for those living with Diabetes Mellitus.

Acknowledgments

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Keywords : pulses, fava bean, digestion, plant cell wall, macronutrients

Topic 4: Food structures, digestion and imaging technologies | Poster

(21475) - BRAZIL NUT PRESS CAKE IN INNOVATIVE SPREADS: BEHAVIOR DURING DYNAMIC IN VITRO DIGESTION

Tonetto, Maria L. (Brazil)[1]; Taha, Ameer Y. (United States of America)[2]; Laurindo, João B. (Brazil)[1]; Feltes, Maria M. C. (Brazil)[1]; Bornhorst, Gail M. (United States of America)[2]

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Abstract

Limited research has been done to understand the digestion of Brazil nut press cake, a coproduct of Brazil nut oil processing that is increasingly used as a food ingredient. This study aimed to investigate the impact of varying concentrations of press cake in spread consumed as part of a meal during in vitro dynamic gastrointestinal digestion.

Simulated meals (~120 g dry matter) consisted of 1.5 slices of toasted white bread with spread (containing Brazil nut paste, cocoa, brown sugar, soy lecithin and 0%, 5%, or 10% of spread mass as press cake) and one meal without spread as control. In vitro oral digestion involved grinding the meal and mixing with simulated saliva (30 sec). In vitro gastric digestion was conducted in a Human Gastric Simulator (HGS) (37°C) containing fasting gastric secretions (35 mL preheated gastric fluids), peristaltic movements (3 contractions/min), secreted gastric juices (4.1 mL/min), and gastric emptying (5.3 mL/min). Samples were collected at 30, 60, 90, 120, 150, or 180 min, and mass, pH, fat, and moisture content were measured. For each gastric time point the pH was adjusted to 7, and simulated intestinal fluid was added for static digestion in a shaking water bath (37°C, 100 rpm), with samples taken every 30 mins for 3 hrs. Protein, starch hydrolysis, and total phenolic content were measured in all gastric and intestinal samples, and fatty acid concentrations were determined in all gastric samples and in each gastric sample after 180 min of small intestine digestion. Analysis of variance was conducted to determine the impact of meal composition and digestion time on digesta properties.

The gastric digesta pH was significantly influenced by meal, digestion time, and interaction ($p<0.0001$). Meals with Brazil nut spread exhibited a pH range of 5.30 to 5.36 after 30 min, gradually decreasing to ~1.4 by 180 min. Treatment and digestion time influenced gastric emptying of dry matter and lipids ($p<0.05$). The bread meal had a distinct pH and gastric emptying profile, with more rapid decreases in pH and gastric emptying. There were no significant ($p > 0.05$) differences between dry matter and lipid gastric emptying for any meals containing Brazil nut spread, possibly due to the formation of emulsified structures. The starch and protein hydrolysis showed maximum release after 60 and 90 min, respectively. Notably, cumulative fatty acids released during digestion increased over time during gastric and small intestinal digestion. For example, in a meal with 0% press cake, 99.95 µg/mg of dry matter of linoleic acid was released after 30 min gastric digestion, which increased to162.38 µg/mg of dry matter after 180 min small intestinal digestion (59% increase).

This study provides insights into Brazil nut press cake utilization within meals, providing evidence of high-quality nutrients released during digestion, and demonstrating its potential as an upcycled product in the food industry.

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Keywords : Bertholletia excelsa, Human Gastric Simulator, Bioaccessibility, Lipid Gastric Emptying, Gastrointestinal lipolysis

Topic 4: Food structures, digestion and imaging technologies | Poster

(21479) - DIGESTION-MEDIATED RELEASE AND ABSORPTION OF AMINO ACIDS FROM DAIRY/PEA PROTEIN BLENDS

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1 - NIZO food research; 2 - Ingredia S.A.

Abstract

Introduction. Environmental challenges dictate that our food consumption pattern may convert to a more sustainable plant-based diet. As a convenient intermediate step, the development of hybrid products, incorporating both plant-based as well as animal-based ingredients, are gaining commercial momentum. The nutritional consequences of such a conversion in terms of amino acid bioavailability are currently not well-understood. We compared the bioavailability of amino acids from dairy and plant-based proteins as well as a mixture thereof.

Methodology. Following *in vitro* standardized static digestion (based on the INFOGEST consensus protocol), amino acid absorption of the digests was evaluated in an *in vitro* transport assay using a human intestinal epithelial cell monolayer (Caco-2). Amino acid quantification was performed using reversed-phase ultra-high performance liquid chromatography in combination with mass spectrometric detection.

Results. Of all the samples tested, proteins from whey and pea showed most extensive amino acid release as a result of *in vitro* digestion. Release of amino acids from mixtures of whey and casein, and of pea and milk proteins were somewhat lower while release from casein was lowest. Consistent for all ingredients, some amino acids (Leu, Arg, Lys, Trp) were more efficiently released upon *in vitro* digestion than others (His, Thr). While differences were observed in the efficiency of amino acid release upon *in vitro* digestion, absorption rates were generally very similar for all amino acids, regardless of the protein source. As an exception, post-transport methionine and arginine concentrations of dairy-pea blends were higher than for pea or dairy alone, respectively.

Conclusions. Dairy-pea blends are well digested and display enhanced bioavailability of the essential amino acid methionine, playing a vital role in various cellular processes, compared to pea protein alone, while maintaining availability of the other essential amino acids.

Acknowledgments
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Keywords : Protein transition, Pea and milk protein, Amino acid bioavailability, in vitro digestion, in vitro absorption

Topic 4: Food structures, digestion and imaging technologies | Poster

(21493) - EXPLORING DIGESTION OF PEA PROTEIN MATRICES DESIGNED BY EXTRUSION OR HIGH PRESSURE COOKING

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Abstract

The growing global concern for sustainable food production and the increasing demand for plant-based protein alternatives has driven the exploration of pea protein as a versatile and sustainable ingredient in the food industry. This study brings new insights on the digestibility of pea protein isolate (PPI) matrices prepared under different conditions of moisture (30, 60 and 80%), temperature (95 ºC and 130 ºC) and shear, using either extrusion (high-shear processing) or under similar conditions, but in the absence of shear, using high-pressure cooking. The complexity of the matrix was also tested by evaluating the effect of the presence of starch. Digestion was conducted using the *in vitro* INFOGEST 2.0 protocol. To investigate differences in the processing history and composition on the digestive kinetics of pea protein, a range of analytical techniques were utilized, including size exclusion chromatography (HPLC-DAD), gel electrophoresis (SDS-PAGE) and o-phthaldialdehyde (OPA) assay, for the degree of hydrolysis (DH) and size distribution and release of peptides. The chemical data was evaluated hand in hand with microstructural information using confocal microscopy during the course of digestion. Protein and starch bioaccessibility were also evaluated in the intestinal phase (i.e., free amino acids and reducing sugars, respectively). Based on confocal imaging, starch inclusion in extruded samples led to a higher degree of particle breakdown in the oral and gastric phases, especially at higher moisture conditions, whereas this effect was less noticeable in samples cooked without shear. Pressure cooking at higher temperatures resulted in a less disrupted matrix during oral and gastric phases, probably due to the greater interplay between the PPI-starch polymers (i.e., lower phase separation). In the gastric phase, the extruded samples exhibited a greater DH than cooked samples. The presence of starch appeared to enhance the DH of both extruded and cooked samples, in agreement with SDS-PAGE observations. Moisture seemed to positively correlate with the higher digestion extent of extruded samples. Furthermore, cooked samples subjected to a higher heating temperature (130°C) exhibited a lower DH and lower molecular weight (M_w) bands than those heated at 95°C. However, in the intestinal phase, all samples displayed a comparable DH, predominance of small M_w peptides (<1 kDa) and amino acids' release. Although all protein was digested at the end of the intestinal phase, the processing history and its processing variables clearly decreased protein hydrolysis and nutrients release to different extents. Therefore, these results clearly indicate the necessity to provide *in vitro* data on digestibility when formulating and designing processes for the development of future foods to obtain maximum nutritional impact.

References

The present work is not yet published

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Keywords : microstructure, digestion kinetics, protein isolate, processing history

Topic 4: Food structures, digestion and imaging technologies | Poster

(21497) - EFFECT OF STRUCTURE OF TEXTURED VEGETABLE PROTEINS ON IN VITRO GASTRIC PROTEIN DIGESTION

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Abstract

Textured vegetable proteins (TVPs) are plant-based protein products with porous structure derived from extrusion. They serve as main ingredients in the preparation of plant-based meat analogues. The digestive and nutritional properties of TVPs and plant-based meat analogue patties (PBMA patties) made from them are underexplored. Especially, little is known about the influence of the micro- and macrostructure of TVPs on their digestion. Filling in this knowledge gap can provide new insights into improving the nutritional values of PBMA patties. This study aimed to explore the impact of micro- and macrostructure of TVPs on *in vitro* gastric protein digestion. Eight TVPs differing in structural properties from two protein sources (yellow pea, soybean) were used. TVPs were ground into fine powders of similar size to remove the impact of structure on digestion. Plant-based patties were prepared using TVP, water, sunflower oil, methylcellulose, pea protein isolate and NaCl to explore the impact of TVP structure on the digestion of patties. The *in vitro* gastric digestion of TVP powders, TVPs and patties was determined following the INFOGEST 2.0 protocol with minor modifications. The structural properties of the TVPs were quantified using X-ray microtomography. The free amino group concentration differed significantly between TVPs differing in microstructure during *in vitro* gastric digestion regardless of the protein source. The free amino group concentration over time of the TVP-based patties followed a similar trend as observed for the TVPs, demonstrating that the *in vitro* gastric digestion of TVPs is not considerably impacted by preparing a patty from the TVPs. When the micro- and macroscopic structural features were removed from the TVPs by milling, all *in vitro* digestion curves superimposed illustrating the strong effect of TVP structure on digestion. We conclude that TVP structure influences the *in vitro* gastric digestion of TVPs, and that the effect of structure on *in vitro* digestion persists in TVP-based patties. The relationships between the structural properties of TVPs and their *in vitro* gastric protein digestion behavior are currently analyzed and will be presented.

Keywords : gastric digestion, plant protein, meat analogues, structure

Topic 4: Food structures, digestion and imaging technologies | Poster

(21503) - ENRICHMENT OF APPLE MICROBIOME AND ITS SURVIVABILITY DURING SIMULATED GASTRIC DIGESTION

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Abstract

Introduction:

While food preservation techniques significantly improve food safety by eliminating the pathogenic and spoilage microorganisms, processing may inadvertently inactivate beneficial and commensal bacteria. As a result, food and nutraceutical industries have concentrated on fermented foods or probiotic supplements to fill this gap using manually selected strains. This study investigated the potential of utilizing the natural microbiome on apples and understanding its survivability during simulated gastric digestion.

Purpose:

To compare the survivability of enriched apple microbiomes from two apple cultivars during simulated gastric digestion in various food matrices; and to investigate the potential of improving their survivability during gastric digestion.

Method:

Fresh Golden Delicious and Empire apples were cut into wedges with peel on and transferred into Tryptic Soy Broth (TSB) with pre-adjusted pH at 7 or 5; or, the apples were directly blended with TSB. The samples were incubated at 30 °C for 1 to 4 day(s), and the microbiome was collected by centrifugation at 5750 rcf for 10 minutes. The microbiome was transferred into different matrices including deionized water, apple sauce, sweet potato puree, chicken puree, or a water-in-oil emulsion. The augmented food matrix samples enriched with apple microbiome were 1:1 mixed with simulated gastric fluid (HCl, NaCl, gastric mucin, pepsin, and amano lipase A) and kept on an orbital shaker in a 37 °C incubator for up to 180 min. The pH of the digesta was adjusted to pH 4 at 0 min, pH 3 at 30 min, pH 2 at 90 min, and pH 1.5 at 150 min. Samples were taken every 30 to 60 min for bacteria enumeration.

Results:

The enriched apple microbiomes reduced from 8.44 ± 0.39 log CFU/ml to 7.55 ± 0.38 log CFU/ml in the first 90 minutes in deionized water for both apple cultivars from both enrichment condition. As pH decreased beyond the initial stage, microbiome inactivation was observed to be matrix-dependent. That is, the inclusion of fat and protein in the augmented food matrices significantly prolonged survivability of the apple microbiomes during the pH 3 digestion stage by 3 log CFU/ml compared to sweet potato puree ($p<0.05$), and the water-in-oil emulsion appeared to significantly protect the microbiome, as concentrations remained above 4 log CFU/ml throughout the entire digestion process. There was no significant difference in inactivation rates observed between the two apple cultivars or two enrichment pH levels within the same food matrix ($p<0.05$).

Significance:

The results demonstrated the feasibility and possible benefits of enriching natural microbiome from apples and the potential of reintroducing the natural microbiome back into other food products. This work will be extended to understand the ecological dynamics of the apple microbiome and implications for food quality and consumer health.

Keywords : natural microbiome, gastric digestion, food matrix, microbial survival

Topic 4: Food structures, digestion and imaging technologies | Poster

(21509) - ADULT AND OLDER ADULT IN VITRO DIGESTION OF A-TOCOPHEROL FORTIFIED YOGURT USING DIDGI®

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Abstract

Age-related losses of physiological functions in older adults are of utmost importance since they directly impact their metabolism, decreasing their ability to digest and adsorb food nutrients. Fortified and functional foods have been regarded as novel alternatives to improve nutraceuticals and bioactive compounds' delivery extent, increasing foods nutritional properties. As such, the development of tailored foods for older adults aims at providing compounds that are expected to decrease the risk of diet-related diseases. That said, it is critical to understand how these biologically-active molecules interact with other food components, what is their behavior in the gastrointestinal tract and if the formulation modulated their bioaccessibility and bioavailability. *In vitro* gastrointestinal models have become very relevant to evaluate food behavior under digestion without the ethical constraints of *in vivo* models. Nonetheless, specific *in vitro* gastrointestinal models that mimic older adults' digestive conditions are still scarce and lack characterization. As such, the objectives of this work were to adapt and apply older adult *in vitro* digestive conditions suggested by INFOGEST in an *in vitro* dynamic system (DIDGI®) to compare and evaluate the influence of *in vitro* digestion protocols on the digestibility and release kinetics of α-tocopherol from fortified yogurts.

Natural fat and sugar free stirred yogurts were supplemented with oil-in-water nanoemulsions containing 15 mg of α-tocopherol. The adult protocol was based on previous literature regarding yogurt digestion on DIDGI®. The older adult protocol was adapted from the static *in vitro* older adult protocol, and had longer gastric emptying time, a slower gastric acidification profile and between 30 and 40 % reduction of enzymatic activity.

Despite the lower enzymatic activity and slower acidification profile applied in the gastric phase of the older adult protocol, particle size distribution analyses demonstrated that the initial degradation of the fortified yogurt was similar at earlier stages of digestion, due to longer gastric half-life. Conversely, at the end of the gastric phase, the adult protocol was substantially more effective in degrading and homogenizing the gastric content.

The α-tocopherol kinetics attained during the intestinal phase were superior in the adult protocol. Gastric phase duration and the obtained by-products affected the consequent intestinal degradation and α-tocopherol release. α-tocopherol was entirely recovered using the adult protocol (97.3 ± 5.9 %), whereas in the older adult was not (79.8 ± 5.2 %). Bioaccessibility was identical in both protocols (ranged from 60 to 80 %), being statistically significantly higher at 3 hours in the older adult protocol.

In sum, α-tocopherol release kinetics extent and profile were greatly affected by the application of different digestion protocols.

References

Acknowledgments

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The authors are thankful to Lipoid for kindly donating a sample of LIPOID P 75 lecithin.

Keywords : Dynamic in vitro digestion, α-tocopherol kinetics, Older adult in vitro digestion, Adult in vitro digestion, Fortified yogurt

Topic 4: Food structures, digestion and imaging technologies | Poster

(21511) - POLYSACCHARIDE BASED GEL-LIKE STRUCTURES AND THEIR IMPACT ON DIGESTION PRODUCTS

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Abstract

The development of new food ingredients with improved techno-functional and nutritional properties is of great relevance to the food industry. To conduct a rational design of these ingredients, knowledge on the structure-functionality relationship is key. Moreover, interactions between different components in food systems are also known to have a high impact on their digestibility.

In this work, two different food model systems, consisting of hybrid protein-polysaccharide hydrogels and polysaccharide-based emulsion gels, were developed and characterized in terms of multi-scale structure and digestibility. The effect of the polysaccharide type (agar vs. carrageenan) and the physical structure (hydrated vs. dry systems) on the structural and mechanical properties of the samples were evaluated. Subsequently, the samples were subjected to *in vitro* gastrointestinal digestions following the Infogest protocol, and the digestion products were characterized to understand their different digestion mechanisms.

Our results suggest that the gel-like structures exerted a protective effect against the hydrolysis of dietary proteins and lipids upon digestion, being this effect dependent on the polysaccharide type and the physical state of the structures. In general, the hydrogels showed a greater protective effect than the aerogels, due to a limited diffusion of the 'encapsulated' components towards the liquid medium and also a limited diffusion of the enzymes into the gel structures. With regards to the digestion mechanism, the released digestion products were seen to interact with the bile salts present in the intestinal digestion medium, leading to the formation of different nanostructures, such as lamellae, micelles and vesicles. This is expected to have a strong impact on the intestinal transport and absorption of the digestion products, thus determining the nutritional properties of the designed food ingredients.

Keywords : Hydrogels, Aerogels, Emulsion gels, Nanostructure, Controlled release

Topic 4: Food structures, digestion and imaging technologies | Poster

(21515) - INVESTIGATING VARIATION IN STARCH DIGESTIBILITY IN HERITAGE POPULATIONS OF BREAD WHEAT

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1 - Quadram

Abstract

Wheat (*Triticum aestivum*) is a worldwide staple, rich in starch, fibre, vitamins and minerals. Despite its nutritional value, concerns exist about the impact of starch-rich foods on chronic diseases such as type 2 diabetes. Genetic variation in heritage wheat populations holds the potential to improve the nutritional profile of modern breeding lines but the lack of rapid, reliable starch-digestibility assays that incorporate cooking (thermal processing) has hindered such approaches.

In this study, we conducted the first analysis of starch digestibility on cooked, wholemeal wheat flour from genetically-diverse heritage wheats. Landraces from the Watkins collection were assayed using a high-throughput starch digestibility assay previously published by Zafeiriou et al (2023). The core collection consists of 118 landraces collected in the 1920s from widely distributed geographic locations and it captures the genetic variation observed across bread wheat landraces. The results for wholemeal cooked flour showed that the core Watkins contained a wider variation of starch digestibility than elite wheat varieties. Additionally, a consistently low starch-digestibility, by screening a subset of the core Watkins grown in 4 different years, was seen for one line, Watkins 777 (W777).

Recombinant inbred lines (RILs), consisting of segments of the W777 genome incorporated into an elite wheat (Paragon), were used for Quantitative Trait Loci (QTL) analysis. Using multiple time points during the digestibility assay (which measure % starch digested over time,) a total of 5 genetic regions (loci) were found to be associated with starch digestibility.

Further analysis of the RILs for the two QTL on chromosomes 4A and 6A demonstrated significant differences between the alleles that increased starch digestibility and those that decreased it. Specifically, RILs carrying the 6A decreasing allele had significantly reduced starch digestion at all but one assay timepoint compared to RILs carrying the increasing allele. Similarly, RILs carrying the 4A decreasing allele had significantly lower digestibility at two assay timepoints.

The digestibility of starch in wholemeal raw flour, cooked flour, and wholemeal bread samples of the RILs carrying the low starch-digestibility QTL was compared with that for Paragon and W777. The results revealed consistently lower starch digestibility in W777 and in the RILs compared with Paragon.

The insights gained from this work highlight the potential for using natural variation in future breeding programs to enhance the health benefits of wheat.

References

Zafeiriou et al. Foods 12.2 (2023): 266

Acknowledgments

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Keywords : wheat, bread, landrace, starch digestibility, high-throughput screening

Topic 4: Food structures, digestion and imaging technologies | Poster

(21530) - AN ORIGINAL, REMOTELY CONTROLLED SET-UP FOR STUDYING IN VITRO DIGESTION BY MRI

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Abstract

Magnetic Resonance Imaging (MRI) has demonstrated its efficacy in characterizing food properties, offering great potential for quantitatively monitoring digestion. If MRI is a technique of choice for monitoring *in vivo* digestion, we believe in its great potential for *in vitro* exploration to provide insights into the composition and multi-scale structure of ingested foods; we are also expecting contributions to the *in vivo* approach, in terms of signal interpretation and proposals for innovative acquisition sequences.

The objectives of this study were to develop a set-up compatible with a whole body MRI scanner to investigate oral-gastric-intestinal food digestion without human intervention in the MRI room, and to use this set-up to gain spatial insights via high-resolution MRI scans into the digestion mechanism of bread structure.

The set-up comprises a compartment (referred to as 'cell') that can suit a wrist radiofrequency receive coil for MRI measurements and contain a ~ 1.5 cm wide food piece to be digested. Connected to the cell, another compartment, 'vessel', is positioned outside the MRI room, linked through a circulating loop controlled by a peristaltic pump (flow rate: 8.4 mL/min). All the manipulations occurred in the vessel. Both the cell and vessel were equipped with water jackets to maintain temperature at 37 °C. The set-up underwent examination for temperature regulation and mixing, in particular for composition homogeneity between compartments. Subsequently, oral-gastric-intestinal digestion of a piece of bread crumb was conducted, with the bread installed in the cell. Manual control and sampling took place in the vessel, while MRI acquisitions, including Multi Spin-Echo and Ultra Short TE (UTE) sequences (for T_2 mapping and morphology), were performed.

Results demonstrated the set-up enabled the successful execution of digestion, with rapid mass transfer and mixing (complete renewal of the fluid in the cell in 4 min; pH and hydrolysed starch concentrations matching in the cell and vessel) and effective temperature regulation. MRI scans provided internal insights, quantitatively measuring bread piece erosion, pore changes, and local composition during digestion. The degradation level obtained from MRI aligned with the degree of digestion determined through analysis of peptides and hydrolysed starch in the digesta.

The present study demonstrated the feasibility of monitoring real-time digestion in a set-up with two separate, potentially distant compartments, facilitated by the circulation of the digestion fluid, which ensures the continuous renewal of the fluid around the food cube. High-resolution MRI images acquired using this set-up offer spatial visualization of bread degradation. The developed setup holds promise for various applications on other foods, providing comprehensive insights into structure changes during digestion.

References

Acknowledgments

We thank Laurent Blondel (UR OPAALE, INRAE) for his valuable input for producing the device.

Keywords : Remotely controlled digestion model, Magnetic resonance imaging (MRI), In vitro digestion, Bread, Structure breakdown

Topic 4: Food structures, digestion and imaging technologies | Poster

(21549) - IN VITRO PROTEIN DIGESTIBILITY OF GLUTEN-FREE PASTA MADE FROM CLIMATE-SMART RAW MATTERS

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Abstract

Traditionally made from durum wheat semolina (DWS), pasta composition has evolved in recent years, incorporating diverse raw materials such as legumes, wholegrain and/or other nutritious flours. The objective of our study is to go deeper in the development of nutritionally optimized pasta by using climate-smart (CS) crops and assess protein bioacessibility in these new food matrices. It is part of the H2020 Innofood Africa project, bringing together European and African partners to explore CS crops and develop new products for at-risk populations (children and women) suffering from malnutrition or obesity. We have developed optimized pasta made from wholegrain, gluten-free, CS African cereals and legumes. Special attention has been paid on their *in vitro* protein digestibility and the nature of the released peptides in relation with protein network structure.

Four cowpea-based pasta formulations were obtained by linear programming and met the FAO's nutritional recommendations for one meal for adult women, ensuring adequate levels of protein, fiber, zinc, iron and B9 vitamin. As all optimized formulations combine cowpea with teff and/or amaranth leaf flours, the impact of adding these two flours individually or in combination on the protein network structure, digestibility and peptides released was studied. The comparison with DWS pasta controls, varying in fiber contents from 3.5 to 16 g/100g d.b allowed to highlight the impact of the high fiber content on the protein structure and digestibility.

In vitro protein digestibility was assessed using the enzymatic Megazyme kit and the COST Infogest digestion model with control of the degree of proteolysis by monitoring free NH2. At the end of the intestinal phase, the protein digestibility of DWS pasta controls whatever their fiber contents was higher than that of the four cowpea-based pasta with the Megazyme kit, while the results were reversed with the Infogest model. These difference can be explained by distinct gastric and intestinal proteolysis conditions that will be highlighted during the presentation. Despite the lower protein digestibility assessed for cowpea-based pasta, the Megazyme kit revealed PDCAAS two times higher for these matrices compared to DWS controls due to their higher content of essential amino acids. The peptidomic study shows that all cowpea-based pasta have same size of peptides released at the end of intestinal phase (1150 *vs* 1120 kDa in DWS controls) and highlights that most peptides were derived from cowpea storage proteins such as vicilin (50%) and legumin (15%) differing thus strongly with gliadin and glutenin derived peptides in DWS controls.

With the comparison of two reference methods for digestion, this work has pointed out that pasta associating cowpea with amaranth leaf flour provide superior nutritional benefits combined along with high protein digestibility.

References

Acknowledgments
We would like to thank Jeremy Claudel for his contribution.

Keywords : Cowpea, Kinetic, Peptidomic, Structure, New sources of proteins

Topic 4: Food structures, digestion and imaging technologies | Poster

(21559) - CELLULOSE NANOFIBERS-STABILIZED PICKERING EMULSIONS: CHARACTERIZATION AND EMULSION DIGESTION

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Abstract

Cellulose nanofibers (CNF) have been widely applied as a Pickering-type stabilizer. However, they are usually obtained from chemical processes (i.e., acid hydrolysis) that generate toxic effluents, which limits their application in the food industry. Therefore, it is of utmost importance to develop eco-friendly alternatives for obtaining CNF, such as enzymatic hydrolysis and/or application of mechanical forces. Knowing the stabilization mechanism and digestive behavior of Pickering emulsion stabilized with CNFs produced from new processes is essential to allow its future application in food products. In this work, we produced cellulose nanofibers using enzymatic hydrolysis combined with a mechanical process (ultrasound). Also, we successfully obtained ethylcellulose (EC) nanofibers by applying only ultrasound. These fibers were named as CNF-ENZ and CNF-EC, respectively. Next, the nanofibers were characterized and applied as Pickering-type stabilizers. The obtained Pickering emulsions were then evaluated by droplet size distribution, microscopy, and *in vitro* digestion. All cellulose nanofibers (0.01-0.05% w/w) could produce Pickering emulsions. CNF-ENZ-based emulsions were stabilized only by the Pickering-type mechanism, in which the nanofibers partially cover the oil-water interface and form a network between the emulsion droplets that prevents the coalescence, while CNF-EC also showed surface activity that contributed to the stability of the emulsion. Furthermore, these latter emulsions showed a greater release of free fatty acids upon *in vitro* digestion, since CNF-EC could be easily displaced from the oil-water interface by bile salts. Thus, a higher lipid hydrolysis and emulsion destabilization in the intestinal environment was observed. Despite that, all the CNF-stabilized emulsions presented a low fatty acid release (below 20%). Therefore, this study may contribute to obtaining CNF through eco-friendly processes and applying CNF-stabilized emulsions in foods that aim to control the rate of lipid digestion.

Acknowledgments

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Keywords : enzymatic hydrolysis, ultrasound, ethylcellulose, in vitro digestion

Topic 4: Food structures, digestion and imaging technologies | Poster

(22588) - IMPACT OF TECHNOLOGICAL MODIFICATIONS IN BREAD-MAKING ON GLUTEN STRUCTURE AND DIGESTIBILITY

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Abstract

Although wheat gluten has unique viscoelastic properties, its stability to gastrointestinal digestion is an important factor in maintaining epitopes, sequences that induce immune responses in susceptible individuals. Wheat is rarely consumed without technological modifications, which can generate structural changes in the protein and affect the way gluten is digested and recognized by the immune system. Several additives have been commonly used in the baking industry with the aim to improve technological properties or products conservation. However, investigations into the use of additives and their effects on gastrointestinal digestion are still scarce. Ascorbic acid is widely used in bread-making as an oxidizing agent to strengthen and stabilize the gluten network. Otherwise, acetic acid improves bread conservation, although it contributes to the depolymerization of the gluten network. The objective of this work was to evaluate the effects of adding ascorbic acid and acetic acid (using vinegar) on the gluten structure and the consequent impact on the digestibility and immunogenicity of the proteins. Thus, breads with added organic acids were subjected to *in vitro* digestion and analyzed by confocal microscopy, SDS-PAGE and ELISA. Confocal images of breads with ascorbic acid showed a cohesive network, formed by starch granules intertwined in a well-distributed and homogeneous protein structure. In contrast, the addition of vinegar resulted in a more open network, with the presence of gaps and protein aggregates. On oral phase, there was a loss of the cohesive structure of the proteins, with greater availability of starch granules, although gliadins and glutenins were observed in SDS-PAGE. At the end of the gastric phase, only low molecular weights peptides (between 10 and 15 kDa) were detected. Although the changes throughout the digestion process were clear, there were no differences in the protein profile between the samples. Finally, ELISA assay demonstrated a drastic drop in immunogenic gliadin content from the oral phase to the gastric phase in all treatments. Comparing the samples at the end of the intestinal phase, samples with the addition of ascorbic acid did not differ in terms of gliadin content in relation to the control. However, the sample with added vinegar showed a significant reduction of 44% compared to the control. Although the latter result suggests a reduction in gluten immunogenicity, more in-depth evaluation of the digesta using other techniques is necessary to confirm the hypothesis. Therefore, understanding the impacts of technological changes for risk assessment, as well as for investigating new strategies to reduce the immunogenic potential of gluten-containing products.

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Keywords : gluten network structure, technological modifications, digestibility, immunogenicity

Topic 4: Food structures, digestion and imaging technologies | Poster

(22589) - IN VITRO DIGESTION OF PLANT-BASED PECTIN-PROTEIN CONJUGATES

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Abstract

Plant-based proteins and polysaccharides such as pectin form conjugates via Maillard reaction under thermal treatment. These new compounds have improved functionality, combining the properties of both polymers, such as the pectin's solubility and the protein's interfacial activity. The conjugates could, therefore, be used to stabilise different food systems or encapsulate functional components. Structural changes resulting from conjugation may result in changes to the digestion properties of the conjugate compared to that of protein or pectin alone. The protein component can be degraded by pepsin during the gastric phase and pancreatin proteases, including trypsin and chymotrypsin in the intestinal phase. Since the enzymatic degradation of proteins depends on their conformation, the protein component can be hydrolysed to varying degrees. In the case of the pectin component, enzymatic degradation by the digestive enzymes is not expected. However, the prevailing environmental conditions, such as acidic pH values of the gastric phase, can lead to chemical and physical changes in the pectin.

Therefore, this study aims to determine whether conjugation can delay the digestibility of plant protein and investigate the effect of pectin type. For this purpose, potato protein was conjugated with different types of pectin (high-, low-methoxylated and low-methoxylated amidated pectin)[1]. The structure of the conjugate was determined by FT-IR. Potato protein-pectin conjugate samples were subjected to static in vitro digestion (Infogest 2.0)[2]. Digestion was monitored by determining the digests' total nitrogen content, free amino groups, and molecular weight distribution. Additionally, confocal microscopy was performed after the gastric phase.

Conjugation altered the protein degradation during both gastric and intestinal digestion. The conjugates and pectin samples gelled under acidic conditions during the gastric phase, which affected the mixing process during incubation. The digestibility of the potato protein was below 60%, and the pectin samples were not affected as expected. When both polymers were conjugated, the protein digestibility was further reduced. Therefore, the conjugation of plant protein with pectin can delay the hydrolysis compared to pure protein. This property may allow these materials to be used in encapsulation to delay the release of functional components during gastrointestinal transit.

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Acknowledgments

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Keywords : Maillard conjugation, potato protein, amidated pectin, Infogest 2.0, degree of methoxylation

Topic 4: Food structures, digestion and imaging technologies | Poster

(22593) - AN IN VITRO SETUP TO MONITOR THE GASTRIC DIGESTION OF SOLID FOODS WITH ULTRASOUND

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Abstract

Background: *In vitro* and *in vivo* digestion models each have their strengths and limitations. Imaging techniques can be used to bridge the gap between *in vitro* and *in vivo* digestion models since they can be applied in both settings (Smeets et al. 2021 [1]). Specifically, imaging of *in vitro* gastric digestion can be used to validate imaging parameters and what exactly they reflect in terms of digestive processes such as breakdown of macroscopic structure or changes in water content. Subsequently, they can be used *in vivo* in humans. The most versatile and most widely used technique in digestion research is magnetic resonance imaging (MRI). In addition to measuring gastric emptying it can be used to examine intragastric processes and digestion in more detail. However, it's relatively expensive and requires participants to be positioned in the tube of a scanner. Ultrasound (US) is less expensive and has a high availability. It has mainly been used to assess the presence of gastric contents in patients before surgery or to assess gastric emptying rate by determining the antral cross-sectional area. Recently, it was shown that US can be used to examine curd formation *in vivo*, i.e. changes in the intragastric content (Sakata et al. 2022 [2]). We postulate that US has potential for tracking changes in the density of gastric contents. However, this requires *in vitro* validation, before attempting *in vivo* validation with US or the current most sensitive method MRI.

Objective and approach: The aim of this work was to construct and test an experimental setup to monitor *in vitro* gastric digestion with US. Design principles were as follows: Fixation of an US probe with seamless connection to a water bath; Presence of an easily accessible gastric compartment that can be repositioned; Use of MRI-compatible materials to allow MRI-scanning of the setup. Accordingly, a plexiglass water bath (30 x 22 x 18 cm) was constructed with a rectangular 'acoustic window' onto which plastic foil can be fixated to allow a seamless interface between the ultrasound probe, which can be fixated with plastic screws, and the water in the water bath. The gastric compartment consists of a polyethylene bag placed in an adjustable holder to allow for image capture at varying distances. A cover minimizes heat loss, and a circulating heater maintains the temperature at 37°C. To test the set-up, US images of the gastric compartment will be obtained at baseline and after addition of human-produced food boli of two types of bread (softer and harder) and pasta (shorter and longer cooking time) over the course of static gastric digestion (INFOGEST) for 2 hours using a Terason uSmart 3300 NexGen portable ultrasound machine with a curved array transducer. Data collection is ongoing. Gastric content changes over time will be examined using image intensity pattern analysis and compared between the product variants. Results will be presented at the meeting.

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[1] DOI:10.1017/S0029665120007867
[2] DOI:10.1093/JN/NXAC158

Acknowledgments

We thank Johan Belgraver of the AFSG Technical Development Studio for building the *in vitro* setup and Iman Ibrahim for the first pilot testing.

Keywords : Gastric digestion, In vitro model, Ultrasound imaging

Topic 4: Food structures, digestion and imaging technologies | Poster

(22594) - INTERACTIONS BETWEEN LIPIDS AND PROTEINS FROM OILSEEDS DURING THEIR IN VITRO DIGESTIBILITY

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Abstract

Background: The limited resources and the ecological impact of animal protein husbandry have led to a rise of plant-based diets. Face to an ever-increasing global demographic growth, the demand for protein products is expected to grow in the next decade, making the search for new protein sources essential. The oilseed proteins, generally present into the animal feed market, could have a potential application in Human nutrition. They offer a double nutritional interest in providing proteins and lipids. However, the nutritional quality of oilseed proteins and the impact of the lipids on their digestibility are not well-known.

Aim: The aim of this study was to determine the *in vitro* oilseed proteins digestibility and to assess the effect of lipids on protein digestibility according to the sourcing of plant proteins, processing method and nutrient composition of oilseed matrices.

Methods: The INFOGEST protocol was applied to determine the *in vitro* digestibility of the different formulas: flour and concentrate of sunflower and rapeseed with or without rapeseed oil. Pea was used as reference. *In vitro* digestates were characterized by calculating the degree of protein and lipid hydrolysis.

Results: The addition of lipids in the reaction medium significantly influenced the *in vitro* digestibility of proteins from oilseeds, according to the nature of plant-based protein matrices (-15% on average for the flour and +10% on average for the concentrates). Similarly, *in vitro* lipid digestibility was affected by the presence of proteins, depending on the intrinsic lipid content in the protein matrix.

Conclusion: This study has shown that protein digestibility can be modulated by the presence of lipids, and reciprocally, proteins can have a notable effect on the degree of lipolysis. In addition, the extent of hydrolysis depends on the type protein, the composition of the matrix and the processing method. The combination of lipids with plant proteins could be a nutritional strategy to improve the essential amino acid bioavailability score of oilseed proteins.

Keywords : Lipids, Proteins, Oilseeds, Digestibility, Interaction

Topic 4: Food structures, digestion and imaging technologies | Poster

(22597) - IMPACT OF BIOACTIVE COMPOUNDS FROM TOMATO ON STARCH DIGESTIBILITY

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Abstract

Nutrient interactions in foods can play a major role in nutrient digestibility and absorption, which ultimately determine their effects on health. Combining nutrients that can act synergistically could lead to lower postprandial glycaemia and lower the risk of non-communicable diseases like type 2 diabetes.

The focus of this study is to build on our current understanding of the interactions between starch, present in staple foods like rice and pasta, and bioactive compounds from tomatoes, carotenoids and polyphenols, which are commonly eaten together with starch-based foods. The hypothesis of this study is that interactions between starch and bioactive compounds influence starch digestibility, and that these effects may vary depending on dose and processing of the ingredients. Most studies to date have focused on bioactive compounds from grapes, berries and tea primarily, while tomatoes are a widely consumed, often in combination with a high-glycaemic starch-based food such as pasta, bread and rice.

Thus, starch and tomato bioactive compounds interaction is now being studied at microstructural level using a single enzyme system, to evaluate the susceptibility to amylolysis of starch in combination with different doses of bioactive compounds extracts. This method is known to correlate well with *in vivo* glycaemic index [1] and to be suitable for use with raw ingredients [2] as well as with complex foods such as bread [3].

Based on the results of this preliminary study, starch and tomato phytochemicals release from a food matrix and digestion will be investigated using the INFOGEST static model. Here, co-digestion and fortification will be used to study the interaction of starch and tomato bioactive compounds at a macrostructural level.

The information generated in this study will provide new mechanistic insight into how tomato bioactive compounds modulate starch digestion when consumed with staple starchy foods and importantly, the availability of bioactive compounds when consumed as part of a meal.

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Acknowledgments
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Keywords : Polyphenols, Carotenoids, Tomato, Starch, Interactions

Topic 4: Food structures, digestion and imaging technologies | Poster

(22601) - IMPACT OF HEATING ON IN-VITRO PROTEIN DIGESTIBILITY AND FUNCTIONALITY OF FAVA BEAN PROTEIN ISOLATES

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Abstract

Fava bean (Vicia faba L.) is a promising alternative protein source because it is rich in protein, containing essential amino acids vital for human health. However, the utilization of Fava bean maybe limited due to inherent anti-nutritional compounds that could lower the protein digestibility. The objective of this research was to investigate the effect of thermal treatment on the *in vitro* protein digestibility and functionality of fava bean protein isolates obtained after different pre-extraction processes. In this study, three fava bean protein isolates were produced after three different pre-treatment of fava beans, i.e., isolates from (i) whole fava beans; (ii) dehulled beans and (iii) overnight soaked beans. Three samples were milled and protein isolates were then obtained using the alkaline solubilization and isoelectric precipitation technique. Part of the fava bean protein isolates underwent heat treatment (100°C for 30 min). Heated and unheated samples were subjected to simulated *in vitro* digestion following the harmonised INFOGEST static method. Total amino acid composition of the digesta showed the presence of all the essential amino acids, whereas sulphur containing amino acids like cysteine and methionine appeared to be the limiting amino acids. Thermal treatment affected the secondary structure of the fava bean protein isolates as determined by Fourier Transformed Infrared spectroscopy (FTIR). The solubility (%) of the heated and unheated protein isolates was determined at pH 3 and pH 7 showed an overall improvement in solubility (35% - 58%). The digestibility of the proteins was evaluated using the proxy *in vitro* digestible indispensable amino acid ratio, the total protein digestibility determined by Total Nitrogen (TN) and by determination of free amine groups ($R\text{-}NH_2$) using OPA analysis. The overall total protein digestibility values from both (TN and $R\text{-}NH_2$) ranged from approximately 66% to 89% for all the samples, treated or untreated, respectively. Heat treatment generally showed a significantly higher overall total protein digestibility, with or without any pre-extraction process applied. This study confirmed that thermal treatment of the fava bean protein isolate can be helpful in increasing their digestibility and other protein qualities of fava bean protein isolates.

References

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Acknowledgments

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Keywords : in vitro digestion, fava bean, limiting amino acids, total digestibility, protein isolates

Topic 4: Food structures, digestion and imaging technologies | Poster

(22605) - MICROSTRUCTURAL CHARACTERIZATION AND DIGESTIBILITY OF POLYSACCHARIDE-GRASS PROTEIN MICROCAPSULES

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Abstract

Encapsulation is a well-known technique to enrich food products with sensitive bioactive compounds. Here we describe an encapsulation method using complexes of polysaccharides and proteins as a wall material for encapsulation systems. Recently, there has been a focus on plant products as an alternative source of protein. Green leaves have being proposed as a source of protein, the main protein being RuBisCO, which has all essential amino acids, hence it may become an interesting nutrient for human health. RuBisCO can be extracted from different green leaves such as perennial ryegrass (GPC). The aim of this study was to develop innovative approaches to produce pectin-protein microcapsules and assess their digestibility and the microstructural changes of microcapsules during static *in vitro* digestion.

For this purpose, pectin from citrus with a low methoxyl degree (DM 35%) (CP Kelco, Lille Skensved, Denmark), and whey protein isolate (WPI) or GPC (1:1 mixture) were used to produce microcapsules by external gelation in $CaCl_2$ using a Vibration Nozzle System. Pectin-protein microcapsules were subject to static *in vitro* digestion following the INFOGEST method (Brodkorb, Egger et al. 2019). The digests after gastric and intestinal phase were characterised in terms of microstructure of the microcapsules (by confocal laser scanning microscopy), particle size distribution (by laser diffraction, Mastersizer 3000, Malvern), percentage of protein hydrolysis and release (by Dumas and OPA) and protein molecular weight (SDS-PAGE and high performance liquid chromatography-size exclusion).

All microcapsules shrunk slightly in simulated gastric fluid (pH = 3) and swelled in simulated intestinal fluid (pH = 7), particularly, the WPI-Pectin microcapsules, where the change was more noticeable than for GPC-Pectin.

Pectin-GPC microcapsules remained spherical after the gastric phase, with minimum release of the protein content. During the intestinal phase, structural changes were observed with loss of sphericity and the identification of broken microcapsules, which was correlated with an increase in the measured particle size and release of the protein.

In conclusion, pectin-protein systems could be used as encapsulation matrices to incorporate bioactive compounds within the capsules, which may be protected during gastric conditions and be released in the intestine.

References

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Acknowledgments

This work has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement N° 872019.

Keywords : Microencapsulation, Grass protein concentrate, Static in vitro digestion, Alternative protein, Low methoxyl degree pectin

Topic 4: Food structures, digestion and imaging technologies | Poster

(22613) - COMPOSITIONAL ANALYSIS AND IN VITRO DIGESTIBILITY OF ALTERNATIVE PROTEIN SOURCES

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Abstract

Current animal protein dependence in the food system is recognised as unsustainable. At the same time, the world population continues to increase which brings an associated increase in demand for dietary protein. As such the search for environmentally friendly alternatives has intensified, focussing on plant-based, insect, microbial and algae solutions. Introducing new protein ingredients into the food supply necessitates a comprehensive nutritional assessment.

This study aimed to assess the compositional analysis and digestibility of 20 ingredients sourced from eight different alternatives: Chlorella vulgaris, rapeseed, oats, fava bean, lentils, chickpeas, bacterial, and insect. Nitrogen content (by Dumas), amino acid composition (by Ion exchange chromatography), in vitro digestibility, trypsin inhibition (1) and in vitro DIAAS (2) utilizing the INFOGEST static protocol were performed.

Protein characterisation results revealed nitrogen content ranges between 2.7 and 15.8% (oat flour at the lower end with fava bean and rapeseed isolates at the higher end). All ingredients were shown to be deficient in Methionine and Cystine. Protein digestibility by OPA ranged from 60 to 100%, with Chlorella vulgaris the least digested protein. Generally, protein isolates had the highest degree of hydrolysis. Chlorella vulgaris was found to lack Lysine and all ingredients lack Tyrosine. In addition, lentil ingredients had the lowest level of Cystine, while oat ingredients had the highest. Microalgae, bacteria, crickets and rapeseed ingredients were good suppliers of Methionine. In terms of trypsin inhibition activity, less purified ingredients seemed to exert the highest inhibition, which could interfere with protein digestibility, with values of up to 9%. This research serves as a crucial basis for identifying protein ingredients that balance nutritional needs with climate needs. However, various processing techniques will impact digestibility and need to be included to further explore the nutritional profile.

References

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Acknowledgments

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Keywords : Alternative proteins, Digestibility, In vitro DIAAS, Amino acids, In vitro digestion

Topic 4: Food structures, digestion and imaging technologies | Poster

(22620) - STRUCTURAL PROPERTIES AND IN VITRO DIGESTIBILITY OF QUINOA PROTEINS WITH GUAR GUM ADDITION UNDER AGING GASTROINTESTINAL TRACT CONDITIONS

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Abstract

Aging leads to several changes in the human body, including a decline in certain gastrointestinal functions. Improving the texture of liquid food by adding thickening agents is a typical intervention for elderly patients with dysphagia. The objective of this study was to characterize conformational changes in quinoa proteins (QPI) with the addition of guar gum (QPI-GG) and to investigate the effect of GG on the digestion of quinoa proteins by simulating aging gastrointestinal tract conditions. QPI dispersions (3% w/v) and QPI with 0.25% guar gum (QPI-GG) were subjected to a static *in vitro* digestion model adapted to the general older adult population (≥65 years), as proposed by the INFOGEST international consortium. QPI and QPI-GG were characterized in terms of charge, turbidity, solubility, intrinsic fluorescence, and secondary structure (FT-IR and Raman spectroscopy). Protein hydrolysis was studied using the OPA method and SDS-PAGE. The results showed that the surface charge of QPI was significantly modified ($p<0.05$) in the presence of GG, and the isoelectric point decreased from 4.7 to 3.1. The intrinsic fluorescence intensity decreased in the order of QPI>QPI-GG, indicating that the hydrophobic amino acids (tryptophan) were oriented toward the center of the proteins, along with the formation of protein-polysaccharide complexes. The solubility of the proteins decreased from 39% to 13% in the presence of GG. FT-IR and Raman spectroscopy analysis demonstrated that conformational rearrangements of the QPI occurred due to interactions with GG. The degree of hydrolysis of QPI (~59%) during intestinal digestion increased with GG (~68%), possibly because the molecular-ordered structure of QPI was significantly affected, increasing the pseudo β-sheet and α-helix in the QPI-GG complexes. This research will enrich the application of QPI in beverages based on plants with modified textures in the elderly population.

Acknowledgments

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Keywords : Quinoa proteins, Protein hydrolysis, Guar gum, Elderly, Plant-based beverage

Topic 4: Food structures, digestion and imaging technologies | Poster

(22634) - RICE AND RAPESEED SIDE STREAMS: IMPACT OF PHYTIC ACID REDUCTION ON GELATION PROPERTIES AND IN VITRO PROTEIN DIGESTION

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Abstract

Valorization of food processing side streams as resources for human nutrition can contribute to global food scarcity. For example, rice bran and rapeseed press cake are side streams containing considerable amounts of nutritionally valuable protein and dietary fibre. However, these side streams also contain the well-known secondary plant component phytic acid. This component affects both technological and nutritional quality of the side streams.

This work aimed at understanding the impact of phytic acid reduction on the techno-functional and nutritional properties of air classified protein-enriched fractions from rice bran and rapeseed press cake. Phytic acid reduction was brought about by enzymatic degradation with phytase, and heat-induced gelation was used as a food processing model. To predict the impact of phytic acid degradation in the fractions as such and before gelation on protein digestibility, the *in vitro* INFOGEST model was employed, and digests analyzed for degree of protein hydrolysis based on the reaction of primary amino groups with *o*-phthalaldehyde (OPA).

During a 2-hour treatment with a food grade phytase the phytic acid content decreased from 20.6 to 3.7 mg/ g dry matter for rice bran and from 7.6 to 2.3 mg/ g dry matter for rapeseed press cake. The subsequent heat-induced gelation of phytase-treated fractions compared to control fractions led to, among others, increased water holding capacity of gels from 54.6 to 77.8% and from 60.1 to 91.3% for gels made from rice bran and rapeseed press cake, respectively. Preliminary results indicate that phytase treatment of side stream fractions leads to reduced protein digestibility *in vitro*, being more pronounced for rapeseed press cake.

Future research will elucidate relations between phytic acid reduction, gel structure, and protein digestibility through *in vitro* digestion using the INFOGEST model.

Acknowledgments

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Topic 4: Food structures, digestion and imaging technologies | Poster

(22637) - IMPACT OF STABILIZERS ON THE DIGESTIBILITY OF HIGH INTERNAL PHASE WATER-IN-OIL EMULSIONS

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Abstract

Emulsões de água em óleo de alta fase interna, também conhecidas como W/O HIPEs, são sistemas coloidais que contêm pelo menos 74% (p/p) de gotículas de água dispersas em uma fase contínua de óleo. Estas emulsões exibem notável comportamento reológico semelhante ao sólido, permitindo a sua utilização como substitutos de gordura em produtos alimentares. Além disso, tais sistemas podem ser utilizados como candidatos interessantes para transportar componentes bioativos hidrofílicos por todo o trato gastrointestinal. Portanto, para ampliar a aplicação das HIPEs, seu comportamento no processo de digestão deve ser investigado. Diante disso, este estudo teve como objetivo avaliar a digestibilidade *in vitro* de HIPEs A/O estabilizados com mistura de cera de girassol e polirricinoleato de poliglicerol (PGPR). O PGPR foi utilizado como emulsificante e ceras de girassol convencionais (SW) ou hidrolisadas (HSW) foram utilizadas para aumentar a estabilidade das emulsões. A concentração de cera variou de 0 a 2% (m/m), enquanto foi utilizada uma concentração fixa de PGPR (0,5% m/m). O método convencional gota a gota foi empregado para produzir os HIPEs A/O (~80% p/p da fase dispersa). A digestibilidade *in vitro* destes sistemas foi avaliada utilizando o protocolo COST INFOGEST. Nossos resultados mostraram que um aumento na concentração de cera em HIPEs A/O frescos resultou em uma diminuição do tamanho das gotas ($d_{3,2}$: SW de 3,75 ± 0,27 para 2,01 ± 0,10 µm e HSW de 6,15 ± 0,65 para 3,90 ± 0,21 µm). A incorporação de ceras desempenhou um papel fundamental no aumento da estabilidade dos HIPEs A/O, particularmente durante a fase gástrica. Todos os sistemas coloidais experimentaram um aumento na distribuição do tamanho das gotículas na fase gástrica, potencialmente induzido pela diluição do sistema. Notavelmente, HIPEs A/O contendo HSW foram mais sensíveis à fase gástrica, especialmente a emulsão com menor concentração de cera que foi a única a apresentar inversão de fase nesta etapa. Este resultado foi corroborado por microscopia óptica e de fluorescência. Os ARS podem apresentar maior resistência à digestão devido à alta hidrofobicidade induzida por longas cadeias de ácidos graxos saturados. Na fase intestinal, HIPEs W/O estabilizados apenas com PGPR apresentaram a menor liberação de ácidos graxos livres (12,64 ± 2,05%) em comparação com sistemas contendo cera (variação: 17,77 ± 0,51 a 33,50 ± 2,37%). Isto enfatiza o papel das ceras no auxílio à lipólise e na preservação do óleo na fase externa, apesar de suas propriedades estruturais superiores. Em essência, nosso estudo contribui para uma melhor compreensão das ceras de girassol como estabilizantes e seu impacto na digestibilidade, abrindo caminho para uma compreensão aprofundada de tais materiais visando sua potencial aplicação em HIPEs A/O.

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Keywords : Sunflower waxes, PGPR, W/O HIPEs, Stability, High viscosity

Topic 4: Food structures, digestion and imaging technologies | Poster

(22640) - RATING THE NUTRITIONAL VALUE OF PROTEIN EXTRACTS FROM GRASS AND OTHER GREEN LEAVES FOR HUMAN CONSUMPTION

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Abstract

Green leaves may be a promising source of proteins due to their high content of RuBisCO, which is an enzyme that participates in the photosynthesis and is considered the most abundant protein in nature (Ellis 1979). However, an extraction process must be applied to release the protein from the undigestible fiber fraction, increase its concentration and remove any anti-nutritional factors, so it can be considered a suitable protein source in the diet. This work discloses the potential nutritional value of protein extracted from different green leaves such as perennial ryegrass and quinoa leaves for human consumption. Different approaches were employed for the protein extraction from the green leaves, focused on the extraction of the soluble compounds, followed by the protein concentration by acid precipitation. The different protein extracts obtained were constituted mainly by RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase), which was identified by electrophoresis (SDS-PAGE), matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) and high performance liquid chromatography-size exclusion (HPLC-SEC).

The digestibility of the protein extracts was assessed through the static INFOGEST *in vitro* digestion method (Brodkorb, Egger et al. 2019), both on the raw protein concentrates and after a heat treatment at 95°C for 30 min to simulate a cooking process. The protein digestibility was determined by the measurement of total amino groups (by OPA), total nitrogen (by Dumas) and total amino acids. And the characterization of the digest after each gastro-intestinal phase was completed by SDS-PAGE, confocal laser scanning microscopy (CLSM) and HPLC-SEC. The protein extracts exhibited very high protein digestibility, close to 100% in the different protein extracts. However, in the grass protein concentrate which contained some residual chloroplast material, the digestibility was reduced from 99 ± 1 to 86 ± 3 % when the cooking simulation process was applied. This could be attributed to the aggregation of chloroplast material during the heat treatment. Finally, the amino acid profile present in all the protein extracts provided all the essential amino acids recommended by FAO (2011), suggesting that proteins from grass or other green leaves may have nutritional potential.

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Keywords : Green leaves, RuBisCO, In vitro digestibility, Nutritional value, Essential amino acid

Topic 4: Food structures, digestion and imaging technologies | Poster

(22644) - DETECTION OF INTACT BOVINE MILK PROTEINS AFTER GASTROINTESTINAL DIGESTION USING UHPLC-HRMS

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Abstract

The presence and relative abundance of intact protein after gastrointestinal digestion is an important aspect of the evaluation of digestion efficiency and the assessment of protein allergenicity. The detection of intact proteins after gastrointestinal digestion is commonly done using gel electrophoretic applications. These are highly sensitive, with limits of detection around 10 ng [1]. However, in some cases gel electrophoretic separation of the proteins does not allow for an unambiguous identification of the food proteins due to 1.) overlap of the bands of the digestive enzymes with the proteins of interest and 2.) the overlap of larger protein break-down products with lower molecular weight proteins. Therefore, this project aimed to explore whether UHPLC-HRMS can be used as a fast, sensitive, and unambiguous tool to monitor intact protein after gastrointestinal digestion. This was done on the example of bovine milk proteins. An UHPLC-HRMS method to detect bovine milk proteins was established for both unheated skim-milk (M-NT) and skim-milk heated at 90 °C for 30 min (M-HT). Both milks were applied to a static *in vitro* gastrointestinal model to simulate infant digestion [2]. Samples were taken after 60 min in the gastric phase (GP60) and after 10 min intestinal digestion (IP10) and measured on an UHPLC-HRMS system (timsTOF Pro 2, Bruker Daltonics, Billerica, Massachusetts, USA). Additionally, samples were applied to sodium dodecyl sulphate gel electrophoresis (SDS-PAGE) to compare the two methods. Detection of β-lactoglobulin variant A was possible in the soluble phase of the digests GP60 and IP10 in M-NT using UHPLC-HRMS, while caseins were not detectable. In M-HT detection of β-lactoglobulin variant A was possible in GP60 but not in IP10. This was in line with the analysis by SDS-PAGE. In contrast, the band of α-lactalbumin (ALA) was visible on SDS-PAGE at both digestion times points independent from the heat treatment, while with UHPLC-HRMS only a protein with a similar ion envelope but ~0.5 kDa lower molecular weight than ALA could be detected. This could indicate that the band for ALA that is visible on SDS-PAGE corresponds to a partially hydrolysed ALA, however this requires further confirmation.

In conclusion, it was shown that UHPLC-HRMS can be used to monitor intact bovine milk proteins after gastrointestinal digestion of unheated and heated bovine milk, respectively. UHPLC-HRMS has the potential to provide further information on the fate of different protein isoforms which are not separated via SDS-PAGE and helps to fast and sensitive confirm the identity of protein bands on SDS-PAGE.

References

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Keywords : mass spectrometry, milk, intact protein, digestion

Topic 4: Food structures, digestion and imaging technologies | Poster

(22648) - EVOLUTION OF THE IN VITRO DIGESTIBILITY OF PROTEINS FROM DIFFERENT LEGUMES ACCORDING TO PROCESS AND TARGET APPLICATION (AAPRO CHAIR)

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Abstract

In order to reduce environmental impact in a sustainable way, the WHO and FAO recommend achieving a balance of 50% animal proteins / 50% plant proteins in our plates. The AAPRO[1] Chair (Advantages and Acceptability of Alternative Proteins) fits into this framework. The aim of this Chair is to help manufacturers and consumers integrate alternative proteins (such as plant proteins derived from legumes) into innovative products. It aims to adapt new product offerings to consumer demand for products that deliver health and environmental benefits, as well as hedonic acceptability. As part of this AAPRO Chair, one of the workpackage focuses on assessing the nutritional quality of proteins derived from different legumes, depending on the process applied or the food application selected. More specifically, in vitro protein digestibility (IVPD) according to the INFOGEST 2019 method was evaluated, and the post-digestion in vitro amino acid profile was obtained. Legume flours (soy, chickpea, yellow pea, lentil and white bean flours) were tested according to the different processes applied. IVPD % ranged from 65% to 81%, depending on the flours tested. Soaking and cooking these seeds in water improved their digestibility (from 81.91% to 88.19%). In addition to this work, food applications based on vegetable egg yolk substitutes containing yellow pea protein isolates were tested. The results showed a digestibility of 68% for vegetable egg substitutes, compared with 72% for conventional egg yolk. These plant-based substitutes were tested in pastry cream as application and their digestibility was compared with the conventional recipe. These results will be presented and discussed.

References

1 : https://fondation.univ-angers.fr/fr/les-chaires/chaire-aapro.html

Keywords : in vitro protein digestibility, legumes, INFOGEST, plant substitutes, AAPRO

Topic 4: Food structures, digestion and imaging technologies | Poster

(22649) - CASEIN STRUCTURE DIFFERENTLY IMPACTS SATIETY BY MODULATING PLASMA AMINO-ACID KINETIC.

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Abstract

Dietary protein is strong appetite inhibitor as it reduces food intake in following meals by signaling directly or indirectly to the brain, modulating eating behavior. However, the type of protein in snacks or pre-loads differently influences food intake, likely due to differences in dietary protein hydrolysis and amino acid bioavailability. We recently observed striking differences in plasma amino acid kinetics as well as intra-gastric behavior between micellar casein (MC) and sodium caseinate (SC). Gastric distension and plasma amino acid levels, in particular that of leucine, both impact food intake. The objective of the present study was therefore to clarify whether the structure of casein impacts its preload effect on subsequent food intake in the pig model.

Overnight fasted pigs (21.5 ± 1.5 kg) equipped with jugular catheters were allowed to consume within 5 min casein drinks differing in casein structure (SC *vs.* MC, 350 kcal, 10% casein, 1.2% glucose in water) in a cross-over study. *Ad libitum* intake of their regular feed was assessed during 1hr, either 1 or 4hr after casein drink ingestion. Gastric emptying of the casein drinks radiolabeled with ^{99}Tc-colloïd was followed during 2hr using gamma-scintigraphy. Plasma kinetics of hormones related to eating behavior (ghrelin, GLP-1, insulin) and of free amino acids were evaluated for 2hr following casein drink ingestion.

The amount of feed consumed 1hr, but not 4hr, after SC ingestion was lower than the amount of feed consumed after MC ingestion (feed consumed at 1h: SC 1306 ± 138 *vs.* MC 1513 ± 79 g, P=0.03). Gastric emptying parameters after both types of casein ingestion were not significantly different ($t_{1/2}$: SC 103 ± 12 *vs.* MC 116 ± 18 min, β: SC 0.67 ± 0.14 *vs.* MC 0.52 ± 0.04, P>0.05). Plasma ghrelin, GLP-1 and insulin kinetics were similar after casein drink ingestion (SC *vs.* MC, P>0.05 for all hormones). Free plasma amino acid concentrations, in particular that of leucine, increased after both SC and MC ingestion but was greater after SC than MC ingestion from 60 to 120 min (plasma leucine at 60 min: SC 87.8 ± 4.8 *vs.* MC 66.0 ± 3.5 mg/L, P=0.009).

In conclusion, ingestion of casein differing in their structure impacts subsequent food intake likely due to difference in amino acid bioavailability. Casein exhibits less anorectic effect when consumed as micellar casein than as sodium caseinate. Such differences might be of importance when designing food dedicated to people with low appetite.

Keywords : milk protein, casein, structure, digestion kinetics

Topic 4: Food structures, digestion and imaging technologies | Poster

(22651) - PLANT PROTEIN DOMINANT ENTERAL TUBE FEEDS ARE NON-COAGULATING AFTER GASTRIC DIGESTION IN CONTRAST TO CASEIN DOMINANT ENTERAL TUBE FEEDS

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Abstract

Rationale:

Enteral Nutrition Products (ENP) are used for the dietary management of patients who require tube feed as they are at risk of disease related malnutrition. We have demonstrated previously that tube feeds with a dairy dominant p4 protein blend (DDp4: 35% whey, 25% casein, 20% soy and 20% pea) are non-coagulating, increase gastric emptying and reduce gastric residual volume when compared to Casein Dominant (CD) tube feeds, which is relevant for upper gastrointestinal tolerance. To support the worldwide protein transition to more plant and less animal proteins, a new Plant Dominant protein blend (PDp4: 16% whey, 6% casein, 46% soy and 32% pea) was developed in line with the Planetary Health Diet composition proposed by the EAT-LANCET commission (Lancet 2019). The coagulating properties of ENP with this new PDp4 are compared to ENP with DDp4 and ENP with CD.

Methods:

Twelve ENP varieties (4 DDp4, 4 PDp4 and 4 CD, each with 4% and 6% w/v protein and with and without fibre) and four 6% w/v PDp4, DDp4, 100% whey and 100% casein solutions were digested *in vitro*. Gastric digestion of a 150 mL bolus was performed in a semi-dynamic *in vitro* digestion model based on adult GI conditions. Saliva and gastric juice flow, as well as the pH curve were mimicked over time. Solid particles present after 100 min of digestion were fractionated according to their size (>2 mm, between 1-2 mm, and between 1-0.25 mm) using mechanical sieving and were quantified by weighing. The particle wet weights were corrected with the weight of sieved undigested fluid. When there was enough sieved digesta available, dry matter was determined.

Results:

The four ENP variants of DDp4 and of PDp4 were found to have a comparable average total particle wet weight of less than one gram, while the four CD ENPs had an higher average total particle wet weight of ~58 gr ($p<0.002$). The particle dry weights of the DDp4 and PDp4 ENP variants were non-detectable, whereas the average total particle dry weight was ~15 gr for the CD ENPs. Protein or fibre content did not affect particle wet or dry weight. The DDp4, PDp4 and 100% whey protein solutions had a minimal total particle wet weight, in contrast to that of 100% casein (~35 gr).

Conclusion:

The ENP with the new plant-dominant 4 protein blend can be considered non-coagulating after *in-vitro* gastric digestion, similar to the dairy-dominant 4 protein blend. This effect has been proven to be independent of protein density and the presence of dietary fibers. Non coagulating plant-dominant enteral nutrition products might support upper gastrointestinal tolerance in a more environmentally sustainable manner.

Keywords : medical nutrition, gastric coagulation

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(22652) - IN VITRO DIGESTION OF 3D PRINTED FORTIFIED CARROT PUREE ADAPTED TO THE OLDER ADULT POPULATION

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Abstract

In the present context, it is acknowledged that an individual's physical and mental well-being is significantly influenced by their dietary choices. Functional foods have increasingly captured the attention of the scientific community and the food industry, serving as an ideal complement to the diet of populations with specific deficiencies (e.g., vitamin deficits, gastrointestinal diseases and disorders) or preferences (e.g., sports or aesthetic nutritional requirements). One approach involves the incorporation of bioactive compounds to enhance, fortify or enrich common foods, aiming to provide a higher supply of nutrients with beneficial biological activity. To overcome the limited physicochemical stability of these compounds, nano-encapsulation emerges as a promising alternative, providing enhanced protection and improved bioavailability, ultimately resulting in a more favorable cost-benefit ratio. More recently, 3D food printing has emerged, allowing for the customization of food properties, adapting functional (e.g., nutritional profile) and sensory (e.g., structure and texture) characteristics of diverse food matrices to specific contexts, such as those faced by the elderly, who commonly experience chewing and swallowing difficulties and nutritional deficiencies. This study delves into the digestion of a 3D printed carrot puree, previously structured with xanthan gum, and fortified with riboflavin encapsulated in a whey protein isolate (WPI) nanostructure as a model system. The final formulation was subjected to an *in vitro* static digestion adapted to the general older adult population (>65 years) following the INFOGEST protocol, aiming at determining riboflavin's bioaccessibility and at studying the digestion of formulated food's carbohydrates. Subsequently, riboflavin was quantified, and total sugars were determined throughout different digestion phases using spectroscopic techniques and a total starch assay kit, respectively. It is hypothesized that the carbohydrates present in the carrot influence riboflavin transport up to the absorption phase, which may suggest a synergy between this specific fraction of the food matrix and the WPI nanostructures. This research represents a significant step in understanding the biological fate of bioactive compounds incorporated into nanostructures within foods and the impact of 3D printing on the digestion of functional foods tailored for the elderly community.

References

No references.

Acknowledgments

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Keywords : Functional foods, bioactive compounds, nanostructures, bioaccessibility, elderly,

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(22658) - VISUALIZATION AND ASSESSMENT OF DAMAGE OF DIETARY NUCLEIC ACIDS FROM RAW AND PROCESSED FOOD

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Abstract

Introduction

Dietary nucleic acids (dietNA) once digested and absorbed gain nutritional importance. From the consumer's point of view, the amount of dietNA in the consumed food product, but also distribution in the product structure as well as damage to dietNA caused by food processing may be important. The aim of the presented research was to visualize dietNA in various food samples of animal and plant origin, with varying degrees of processing.

Materials and Methods

Formalin-fixed paraffin-embedded sections and cryosections of selected samples were stained with DNA-specific fluorochromes and used for microscopic imaging of dietNA in food products of plant and animal origin. The impact of grilling, frying, cooking and boiling on nuclear DNA integrity in selected food samples was assessed and visualized by comet assay modified to suit food sample research.

Results

Nuclei containing dietNA visible in the structure of raw meat and plant products are preserved after processing. Microscopic imaging also shows the distribution of dispersed dietNA in dairy products such as cheese. The comet assay results reveal changes in the integrity of the nuclear DNA contained in the food products tested. There are visible differences between the degree of genetic material fragmentation in animal and plant tissues. The degree of fragmentation of nuclear DNA in raw plant products is in the range of 4-18% of DNA in the tail, while in raw meat products reaches approximately 50%. Thermal treatment affects the degree of nuclear DNA fragmentation in meat samples. Cooking, baking and frying reduce the degree of nuclear DNA fragmentation to 10%, while grilling leads to the fragmentation of nuclear DNA into shorter fragments.

Conclusions

Microscopic imaging provides visualization of the abundance and distribution of dietNA in food samples, but is not sufficient to detect dietNA damage, which may be caused by both food processing and enzymatic hydrolysis occurring in the examined tissues. Complementing microscopic observations with comet assay results provides complete data. Thermal processing of meat leads to inactivation of nucleases but when intensive, may also lead to degradation of DNA and changes in the appearance of comet tails.

Keywords : dietary nucleic acids, microscopic imaging, comet assay, thermal processing

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(22659) - DEVELOPMENT OF PLANT-BASED BIGELS FOR CURCUMIN'S DELIVERY

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Abstract

Growing consumer demand for plant-based foods has led to an exponential increase in the development of plant-based foods or analogs with improved functional, physicochemical, nutritional, and sensory properties. Bigels are a type of soft material system consisting of two distinct gelling phases, which have great potential in the food industry as a fat replacer and as a vehicle for both hydrophilic and hydrophobic compounds.

Curcumin is a hydrophobic yellow-orange polyphenol found in the rhizomes of *Curcuma longa L.* and is widely used in the food and pharmaceutical industries. In the food sector, curcumin is used not only as a spice but also as a natural coloring agent in various foods such as yogurt, ice cream, and cheese. Despite curcumin exhibits several health-promoting properties, including antioxidant, anti-inflammatory and anticarcinogenic effects, the main challenges in using curcumin are its low solubility in aqueous solutions, and its sensitivity to alkaline conditions, heat, oxidation, and light. In addition, curcumin has a relatively high rate of metabolic degradation, resulting in the inactivation of metabolic end products and rapid excretion from the body, thus reducing its oral bioavailability and bioactive effects. To overcome these limitations and improve bioactive effects and oral bioavailability, several techniques have been developed, such as encapsulation.

The objective of this study was to develop a plant-based bigel for curcumin's delivery, using a potato protein-based hydrogel and a candelilla wax-based oleogel. The effect of different protein concentrations and hydrogel: oleogel ratios on the structural and rheological properties of the bigel were evaluated, as well as the curcumin's bioaccessibility after *in vitro* digestion.

All samples exhibited a structural arrangement of oleogel within the hydrogel. As the protein concentration increased, the hardness and G* values increased and the structure and consistency of the bigel improved. The increase in oleogel content changed the distribution of oleogel droplets in the hydrogel matrix, which affected the hardness and consistency of the bigel. Overall, increasing the oleogel fraction and protein concentration enabled the formation of bigels with stronger mechanical properties and higher thermal stability. The curcumin's bioaccessibility in bigels was 16.3% and the stability was 43.8%, indicating that this type of structure is promising for the delivery of bioactive compounds in the colon or for the sustained release of bioactive compounds.

Overall, these results show that by changing the protein concentration and the hydrogel: oleogel ratio, the mechanical, rheological and thermal properties of the bigel as well as the bioaccessiblity of bioactive compounds and their delivery targets can be tuned, expanding the application of bigels in different food products.

Acknowledgments
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This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020.

Keywords : Hybrid gels, Textural properties, Rheological properties, In vitro digestion, Bioaccessibility

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(22667) - EFFECT OF DIFFERENT FOOD MATRIX CARRIERS ON THE STABILITY OF ALGINATE HYDROGELS CONTAINING SAFFLOWER OIL

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Abstract

Background: Alginate hydrogel beads are effective means to protect and transport bioactive food ingredients such as probiotics and antioxidants during storage and, upon consumption, during gastrointestinal (GI) transit. They can also be used to deliver intact lipids to the distal part of the small intestine to trigger physiological satiation in humans, a phenomenon that is known as the 'ileal brake'. Food matrix physico-chemical properties, food processing and storage may affect the structure and stability of alginate hydrogels. Therefore, we investigated the effects of different food matrix carriers varying in physical properties (i.e. liquid, semi-solid and solid) on the stability of alginate hydrogel beads containing safflower oil.

Methods: 6 different products, each containing 60g of beads, were developed and prepared, being chocolate slab, semolina, lemon ice cream, cottage cheese, tomato soup and yoghurt. Portions were tested 24, 48, and 72 hours after production. On test days, beads were separated from the food matrix and analysed for bead morphology and size using standard microscopy, oil content extracted from beads using hexane/isopropanol (3:2) and determined by mass balance, and lipid oxidative stability by determining malondialdehyde (MDA) through the thiobarbituric acid reactive substances (TBARS) test. Procedures were repeated each day on beads not incorporated in a food matrix as control. Data were analysed using a two-way ANOVA with statistical cut-off level of $P<0.05$, and post-hoc analyses with Bonferroni correction where appropriate.

Results: Morphology of beads was not affected by the type of food matrix except for ice cream. Microscopy pictures of beads extracted from ice cream showed slight damage in the outer shell, probably due to rupture caused by ice crystals. Ferret diameter of beads fluctuated between 1 and 1.5mm and did not differ from control beads. Oil content per gram of beads in cottage cheese (277.0 ± 3.1 mg/g), semolina (251.5 ± 4.7 mg/g), tomato soup (262.2 ± 4.4 mg/g) and yogurt (222.5 ± 28.1 mg/g) did not differ from control (262.0 ± 2.6 mg/g), while in chocolate (277.0 ± 3.1 mg/g) and ice cream (359.4 ± 5.6 mg/g) oil content was significantly higher than control, possibly due to incorporation of fat from the food matrix into the beads. MDA values in semolina (5.2 ± 0.9 µg/g), cottage cheese (8.5 ± 1.0 µg/g), tomato soup (5.3 ± 0.9 µg/g), and yoghurt (5.1 ± 0.6 µg/g) were not different from control (4.5 ± 0.6 µg/g), while chocolate (8.5 ± 1.0 µg/g) and ice cream (9.2 ± 1.0 µg/g) had significantly higher MDA contents.

Conclusion: Liquid and semi-solid matrices did not significantly affect stability of alginate hydrogels and can be good carriers for alginate hydrogels, except for ice cream which may induce damage through ice crystals formation. Conversely, a solid matrix affected oil content and lipid oxidative stability and is not suitable as carrier for alginate beads.

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Keywords : Food matrix carriers, Lipid oxidative stability, Food processing, Ingredients encapsulation, Alginate hydrogels structure stability

Topic 4: Food structures, digestion and imaging technologies | Poster

(22671) - THE DIGESTIVE FATE OF EMULSIONS FORMULATED WITH PEA AND LUPIN PROTEIN INGREDIENTS: MODULATION OF LIPID DIGESTIBILITY AND BIOAVAILABILITY BY THE INTERFACIAL COMPOSITION?

Keuleyan, Eléna (France)[1]; Laurent, Sophie (France)[1]; Beaumal, Valérie (France)[1]; Riaublanc, Alain (France)[1]; Berton-Carabin, Claire (France)[1,2]; Meynier, Anne (France)[1]

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Abstract

Pea and lupin protein ingredients are suitable sources to promote the transition to more plant proteins in foods, as they display promising nutritional and emulsifying properties. A deep characterization of the composition of pea and lupin protein isolates and concentrates revealed that they contain 4 to 12 wt.% lipids, with over half being phospholipids. A high-pressure homogenization (HPH) treatment was applied to their aqueous suspension. It altered the aggregated state of their proteins and improved protein solubility [1].

Oil-in-water emulsions (10 wt.% oil, 2.5 g proteins/L) were prepared by HPH to obtain droplets around 2.5 µm. Emulsions formulated with protein isolates led to droplet flocs about five times larger than in emulsions prepared with concentrates. These results, supported by microscopic investigations, suggest extensive bridging by protein aggregates present at the droplet surface. The digestive fate of emulsions was examined using *in vitro* static model (pH-Stat) to elucidate how the microstructure and interfacial composition influenced lipid digestibility and bioavailability. Lipolysis was evaluated through lipid class analysis via HPLC.

During the oral phase, the microstructure of the emulsions prepared with protein isolates was notably altered, as the introduction of α-amylase led to the loss of the proteinaceous network bridging the droplets, without inducing coalescence. Comparable values of lipolysis were reached at the end of the gastric phase (from 12.3 to 17.3 % mol/total mol), and after the intestinal phase (from 67.1 to 72.9 % mol/total mol). However, lipid bioavailability varied depending on the protein source, with approximately 85 wt.% of absorbable lipids measured for pea protein-based emulsions, against 49 to 63 wt.% for lupin protein isolate- and concentrate-based ones, respectively. These first results suggest a marked role of endogenous phospholipids, and potentially of other non-proteinaceous components present in plant-based ingredients, on the nutritional fate of the emulsions.

To investigate this hypothesis further, a new series of emulsions (with higher protein-to-oil ratio) were formulated to amplify the competition between proteins and phospholipids for interfacial adsorption. Further characterization was conducted by transmission electron microscopy to gain deeper insights into the interfacial composition of the emulsions. Selected ones were submitted to *in vitro* static digestion (pH-Stat).

This work offers comprehensive insights regarding the incorporation of plant protein ingredients into formulated foods. While food systems lean towards plant-based innovations, understanding how the composition of these ingredients affects their processing and nutritional properties from a perspective of usually overlooked endogenous components can help to rationalize the formulation of sustainable and nutritious foods.

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Acknowledgments

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Keywords : Lipolysis, Plant proteins, Lipid bioavailability, Oil-in-water emulsion

Topic 4: Food structures, digestion and imaging technologies | Poster

(22674) - MONITORING GALACTOLIPID DIGESTION AND SIMULTANEOUS CHANGES IN LIPID-BILE SALT MICELLAR ORGANIZATION BY REAL-TIME NMR SPECTROSCOPY

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Abstract

The use of Nuclear Magnetic Resonance spectroscopy for studying lipid digestion *in vitro* most often consists of quantifying lipolysis products after they have been extracted from the reaction medium using organic solvents. However, the current sensitivity level of NMR spectrometers makes possible to avoid the extraction step and continuously quantify the lipids directly in the reaction medium. We used real-time ^{1}H NMR spectroscopy and guinea pig pancreatic lipase-related protein 2 (GPLRP2) as biocatalyst to monitor *in situ* the lipolysis of monogalactosyl diacylglycerol (MGDG) in the form of mixed micelles with the bile salt sodium taurodeoxycholate (NaTDC). Residual substrate and lipolysis products (monogalactosyl monoacylglycerol (MGMG); monogalactosylglycerol (MGG) and octanoic acid (OA) were simultaneously quantified throughout the reaction thanks to specific proton resonances. Lipolysis was complete with the release of all MGDG fatty acids. These results were confirmed by thin layer chromatography (TLC) and densitometry after lipid extraction at different reaction times. Using diffusion-ordered NMR spectroscopy (DOSY), we could also estimate the diffusion coefficients of all the reaction compounds and deduce the hydrodynamic radius of the lipid aggregates in which they were present. It was shown that MGDG-NaTDC mixed micelles with an initial hydrodynamic radius r_H of 7.3 ± 0.5 nm were changed into smaller micelles of NaTDC-MGDG-MGMG of 2.3 ± 0.5 nm in the course of the lipolysis reaction, and finally into NaTDC-OA mixed micelles (r_H of 2.9 ± 0.5 nm) and water soluble MGG. These results provide a better understanding of the digestion of galactolipids by PLRP2, a process that leads to the complete micellar solubilisation of their fatty acids and renders their intestinal absorption possible.

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Acknowledgments

Keywords : Lipase, galactolipids, digestion, pancreatic lipase related protein 2, NMR spectroscopy, DOSY

Topic 4: Food structures, digestion and imaging technologies | Poster

(22675) - INDUCED VISCOSITY-MILKSHAKE REDUCED THE CALORIE INTAKE WITHOUT A COMPROMISE IN LIPID DIGESTION

Kasprzak, Miroslaw (Poland)[1]; Pająk, Paulina (Poland)[1]; Tullberg, Cecilia (Sweden)[2]; Lett, Aaron (United Kingdom)[3]; Krystyjan, Magdalena (Poland)[1]; Hetherington, Marion M. (United Kingdom)[4]

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Abstract

Introduction. Reformulation of foods by fibre-inducing thickness, acts to promote satiety. However, adding fibre to the food matrix can bind to nutrients hindering delivery of fatty acids, amino acids, vitamins, or minerals.

This study tested the impact of beta-glucan in a milkshake matrix on satiation (subjective sensory ratings), subsequent food intake (satiety) and an investigation of lipid digestion by *in vitro* method.

Materials. A high internal phase emulsion (milk fat-in-water, 75:25) was prepared using high shear mixing (25 000 rpm/5min) with whey protein (7.5%) added as a lipid source to a milkshake. The control milkshake had no added fibre and the test milkshake had 1% β-glucan (Betaven, Poland), both were produced in a thermomixer and shared the same content of milk fat-based emulsion, cocoa, sugar and whey protein.

Participants were 12 panellists (8 women, 4 men) aged 22 – 28 yr mainly healthy weight (mean BMI = 23.13; range 19.6 – 34.4) who attended the sensory laboratory twice (7 days apart).

Procedure. On each visit, panellists consumed their breakfast at 8am and then rated hunger, appetite and fullness before consuming a milkshake (180g) at 3h, followed by intake of an *ad libitum* test meal of tomato pasta and then banana yoghurt at 4h. Liking and desire to eat ratings were taken before and after the milkshake, for two salty products (cracker and gouda cheese), a small portion of relevant milkshake (10g) and two sweet products (cake and strawberry yoghurt). Ethical approval was obtained from the University of Agriculture ethics committee (Approval No. 136/2023, July 3, 2023).

Assays. The milkshakes were subjected to apparent viscosity, droplet size measurement by image analysis and *in vitro* lipid digestion by INFOGEST (Brodkorb et al 2019).

Characteristics of milkshakes. Both milkshakes exhibited nearly identical droplet sizes (control, $D_{3,2}$= 6.95 ±2.14 μm and test, $D_{3,2}$=6.72 ±2.15 μm). The apparent viscosity was significantly higher for the β-glucan-rich milkshake compared to the control.

Liking. The milkshakes were equally liked, but were liked significantly less than the two salty foods (cracker, cheese) and sweet foods (cake, yoghurt). The liking for the fibre shake was significantly less after intake, but the control shake **did not change in liking** with intake. Liking for the uneaten foods (salty or sweet) remained the same after intake of the shake.

Energy intake. There was a **significant reduction by 50% in energy** intake from dessert when the fibre-rich milkshake was consumed compared to the control. However, there was no difference in intake from tomato pasta meals.

Digestion. The kinetics of *in vitro* lipid digestion for fibre and control milkshakes were not substantially different.

Findings. The viscosity effect reduced intake of dessert without a compromise in lipid digestion as studied *in vitro*.

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Acknowledgments

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Topic 4: Food structures, digestion and imaging technologies | Poster

(22677) - THE IMPACT OF INITIAL FOOD STRUCTURE ON GASTRIC DIGESTION IN SOLID CARBOHYDRATE-BASED FOODS

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Abstract

Structural changes of food during digestion play a crucial role in understanding the overall food breakdown process and subsequent physiological outcomes. This study investigated the relationship between initial food structure and digestion behavior, focusing on microstructural changes, moisture and acid uptake, and softening kinetics in six carbohydrate-based food materials: apple, white bread, chickpea, cantaloupe, and rice flour pellets with two different moisture content levels. The initial microstructure was assessed using micro-computed tomography (micro-CT). Initial hardness was measured with a texture analyzer as the peak force during uniaxial compression of individual particles (apple, cantaloupe, chickpea) or during bulk compression in a 45 mm cup (white bread, rice flour pellets) by a 40 mm diameter cylindrical plunger to 50% strain. To quantify acid and moisture uptake and softening kinetics, static in vitro gastric digestion was conducted in triplicate using a shaking water bath (37 ℃ and 100 rpm). Each individual food material was mixed with simulated saliva (pH 7, 1 mL saliva/g dry matter) for 30 s, simulated gastric juice (pH 1.8, 5 mL gastric/g sample) was added, and the pH was adjusted to 2. Bread, rice flour pellet, apple, and cantaloupe samples were analyzed at 6 digestion time points with a total gastric digestion time of 20, 15, or 180 min, while chickpeas were analyzed at 8 times over 240 min gastric digestion. Different sampling times were needed due to the different softening rates across the food matrices. Hardness, acidity, and moisture content were measured in the solid phase of each gastric digesta sample. Kinetics of normalized hardness during digestion were fit to the Weibull model. From micro-CT images, the rice flour pellet exhibited numerous irregularly shaped pores of varying sizes. White bread contained pores, but they were more uniform compared to those in the rice flour pellet. On the other hand, cantaloupe had fewer pores, and chickpea lacked visible pores. Hardness significantly decreased ($p<0.05$) by the end of the digestion time for each food, while acidity and moisture content increased significantly ($p<0.05$) during digestion for all food materials. For example, the hardness of 27% moisture rice pellet decreased from 231.1 to 3.8 N, acidity increased from 1.9 to 6.2 mg HCl/g DM, and moisture content increased from 0.02 to 4.47 g H_2O/g DM after 15 min in vitro gastric digestion. Half softening time (t50) was significantly different ($p<0.05$) between food materials with chickpea > cantaloupe > apple > bread > 31% moisture rice pellet > 27% moisture rice pellet. It was found that food materials with more pores exhibited shorter half softening time. This study provides a better understanding of the connections between food structure and gastric digestion behavior, which may have implications for optimizing food structures and designing functional foods.

References

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Acknowledgments

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Keywords : In vitro digestion, Moisture and acidity, hardness kinetics, Micro-computed tomography

Topic 4: Food structures, digestion and imaging technologies | Poster

(22678) - CHARACTERIZATION AND IN VITRO DIGESTION OF A CHICKPEA NANOENCAPSULATED PROTEIN HYDROLYSATE

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Abstract

Chickpea protein isolate was hydrolyzed for three different times using a pepsin-pancreatin enzyme sequence; afterwards, the hydrolysate with higher degree of hydrolysis (obtained at 90 min) was nanoencapsulated by freeze-drying, after using Eudragit L30 D-55 (EGLD) as wall material and a high-shear homogenizer (Ultraturrax, TA, EUA) at 13,000 rpm for 30 s to blend the wall material and the hydrolysate. An encapsulation efficiency (EE) of 63.48 ± 0.66% was achieved. The remaining concentration of the hydrolysate inside the nanocapsules subjected to *in vitro* digestion was evaluated.

EE can be influenced by the ratio of core material:wall material, the method used to encapsulate, among other factors (Piacentini, 2016). In the present project, a ratio of 1:40 was used and higher EE was found in comparison with that reported by Tovar-Benitez *et al*. (2016) who used freeze-drying to microencapsulate protein hydrolysates and obtained an EE of 35.95 ± 3.50 % by setting the hydrolysate:EGLD ratio to 1:20

In addition, by scanning electron microscopy (SEM), the nanocapsules reported an average Feret's diameter of 139 ± 4 nm. The submicron-size nanoparticles, in general, show relatively higher intracellular uptake, thus, leading to better bioaccesibility and bioavailability of encapsulated bioactive compounds. However, particle aggregation induced by freeze-drying (Park, 2017) might be observed, as it was actually found in the SEM micrographs. Because of this, the particle size distribution was not corroborated by using a laser scattering equipment.

Through FT-IR spectra, no chemical interaction between the wall material and the hydrolysate was detected.

The well-known digestion protocol Infogest (Minekus *et al*., 2014) was followed to carry out the *in vitro* digestion. Amylase and bile salts were not added given the lack of carbohydrates and lipids in the nanocapsules. After *in vitro* digestion, as compared against the control sample, a percentage of 20.38% of nanocapsule protein content was released in the gastric phase, whereas a bioaccessibility ratio of 70.38 % was calculated from the results of the protein content released by the nanocapsules in the intestinal phase.

In conclusion, the nanoencapsulation process performed was successful to protect the chickpea hydrolysate since most of it was released inside the small intestine-simulated environment in order to promote its bioaccessibility.

References

Minekus *et al*., 2014; Park, 2017; Piacentini, 2016; Tovar-Benitez *et al*., 2016

Acknowledgments

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Keywords : chickpea proteins, protein hydrolysates, nanoencapsulation, freeze-drying, in vitro digestion

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(22688) - ASSESSMENT OF INTRAGASTRIC MILK DIGESTION BY MAGNETIZATION TRANSFER MRI: A FEASIBILITY STUDY IN HUMANS

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Abstract

Background: Understanding the behavior of foods in our digestive system is crucial for developing food products and processing methods that offer optimal health benefits. In our research, we explore the use of Magnetic Resonance Imaging (MRI) to monitor both *in vitro* and *in vivo* digestion of skim milk (SM). Previously, we demonstrated the performance of ^{1}H Magnetization Transfer (MT) MRI as a method for investigating gastric milk protein (MP) coagulation, and the effect of heat treatment on this process during *in vitro* gastric digestion. Here, we conducted an exploratory study to assess the feasibility of using MT MRI for monitoring gastric MP coagulation in humans, in combination with conventional anatomical MRI measurements of the gastric emptying (GE) dynamics using two differently heated milk products.

Methods: Healthy adults (n = 12) were enrolled in this randomized cross-over trial. Participants underwent gastric MRI scans at baseline and after consumption of 300 g of either low-pasteurized SM (LPSM, 3% whey protein denaturation) or High-pasteurized SM (HPSM, 90% whey protein denaturation). We assessed the GE dynamics of the total gastric content (TGC) as well as of the semi-solid and liquid fractions. Additionally, the ^{1}H MT Ratio (^{1}H *MTR*) of the gastric content, a marker of protein mobility and hence coagulum consistency, was used to monitor gastric MP coagulation.

Results: The *MTR* of the gastric content increased with the digestion time ($p<0.001$), indicating a decrease in the protein mobility, and, hence, an increase in the degree of coagulation. For LPSM, this trend was similar to that found during gastric digestion in a semi-dynamic *in vitro* model. There was no effect of heat treatment on the *MTR* with a MD of 16% (95% CI [10-21]) ($p = 0.15$). The TGC volume over time for HPSM was higher than that of LPSM ($p = 0.044$, mean difference (MD) = 40.3 mL 95% CI [25.5 – 55.1]). Furthermore, the AUC of the TGC and liquid volume were also higher for HPSM ($p=0.021$ and $p=0.017$, respectively), and a trend towards a significantly higher AUC was found for the semi-solid volume for HPSM ($p =0.078$). Overall, these results indicate a slower GE of SM upon heat treatment.

Conclusion: Combining conventional anatomical MRI images with MT data enabled the assessment of MP coagulation as well as GE of total, semi-solid and liquid gastric content *in vivo* in humans. This innovative approach opens the way to investigating effects of food processing, composition and structure on intragastric digestion directly in humans. Such data can serve as input for optimizing and validating *in vitro* digestion models.

References

Acknowledgments
Rosalind Vivia Tansy is gratefully acknowledged for her help with the MRI data collection and analysis of GE data.

Keywords : MRI, gastric emptying, coagulation, dairy protein, in vivo

Topic 4: Food structures, digestion and imaging technologies | Poster

(22690) - MICROSTRUCTURAL CHARACTERIZATION OF FREEZE-DRIED ORANGE AND LEMON PEEL POWDERS AND GASTROINTESTINAL STABILITY OF THEIR VOLATILE COMPOUNDS

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Abstract

Orange and lemon peels are a good source of volatile and non-volatile bioactive compounds as functional ingredients. The aims of this study were the microstructural and chemical characterization of freeze-dried orange and lemon peel powders by scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS) as well as the gastrointestinal stability of their volatile compounds using an three-step *in vitro* digestion model (mouth, stomach and duodenum). SEM micrographs of orange and lemon powers showed irregular shapes and smooth surface. Volatile compounds from digesta samples were obtained by headspace solid-phase microextraction (HP-SPME) and their gastrointestinal stability and bioaccessibility were analysed by gas chromatography with mass spectrometry (GC-MS). The main volatile compound in freeze-dried lemon and orange peel powders was D-limonene. Its stability was high during simulated digestion in the mouth, whereas after simulated digestion in the stomach and intestine the stability of D-limonene decreased significantly.

Keywords : in vitro digestion, orange, lemon, peel, freeze-dried, D-limonene, SPME extraction, SEM

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(22705) - HIGHLIGHTING THE ANTIOXIDANT AND HYPOCHOLESTEROLEMIC PROPERTIES OF PEPTIDES FROM SPIRULINA (ARTHROSPIRA PLATENSIS).

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Abstract

Hypercholesterolemia, a pivotal public health concern, propels the collaborative international project CASH (*Chromophores Actifs en Santé Humaine*) to explore natural alternatives to statins for blood cholesterol level reduction, aiming to prevent cardiovascular risks. This project, cofounded by Walloon Region through the WAGRALIM Competitiveness Cluster, is a partnerships between the University of Liège, the University of Louvain-La-Neuve, the University of Lille, Tilman sa and Biores sa (two Wallonia-based compagnies).

Spirulina, a blue-green alga of the Cyanophyceae family, is celebrated for its exceptional nutritional properties and well-established antioxidant, antidiabetic, and anti-inflammatory activities [1, 2]. Spirulina contains the phycocyanin, a chromoprotein used as natural blue pigment in food.

The CASH project unfolds through diverse tasks ranging from: (i) assessing the bioactive potential of peptides from spirulina proteins through an *in silico* approach, (ii) developing bioactive spirulina protein hydrolysates, (iii) evaluating the hypocholesterolemic and antioxidant activities before and after simulated *in vitro* static INFOGEST gastrointestinal digestion [3, 4], and (iv) identifying peptides and chromopeptides responsible for the studied bioactivities through a peptidomic approach.

The project led to the identification of fractions obtained by gel filtration of phycocyanins hydrolysates with high antioxidant activity. This innovative study promises to redefine preventive approaches against cardiovascular diseases by harnessing the bioactive potential of spirulina peptides, paving the way for natural and sustainable solutions to enhance cardiovascular human health.

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Acknowledgments

Keywords : spirulina, antioxidant, hypocholesterolemic, peptidomics, chromopeptide

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(22711) - MASS SPECTROMETRY-BASED QUANTIFICATION OF IMMUNOSTIMULATORY GLIADIN PROTEINS AND PEPTIDES IN COLOURED WHEAT VARIETIES: IMPLICATIONS FOR CELIAC DISEASE

Dias, Ricardo (Portugal)[1]; Da Silva, Sara (Portugal)[1]; Monteiro, Bruna (Portugal)[1]; Guo, Ziqi (Portugal)[1]; Pérez-Gregorio, Rosa (Portugal)[1,2]; Gianfrani, Ricardo (Portugal)[3]; Barone, Maria Vittoria (Portugal)[4]; Martinek, Petr (Portugal)[5]; Freitas, Maria Vittoria (Portugal)[1]

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Abstract

Pigmented wheat varieties (*Triticum aestivum spp.*) are getting increasingly popular in modern nutrition and thoroughly researched for their functional and nutraceutical value. The color of these wheat grains is caused by the expression of natural pigments, including carotenoids and anthocyanins, that can be restricted to either the endosperm, pericarp and/or aleurone layers [1,2]. While contrasts in phytochemical synthesis give rise to variations among purple, blue, dark and yellow grain's antioxidant and radical scavenging capacities, little is known about their influence on gluten proteins expression, digestibility and immunogenic potential in a Celiac Disease (CD) framework. Herein, we provide a comprehensive proteomic-based assessment on the immunogenicity of gliadin proteins and peptides from novel anthocyanin- and carotenoid-rich wheat varieties by (semi)-quantitative high resolution mass spectrometry. It has been found that gliadin expression and peptide release following a simulated gastrointestinal digestion - particularly those containing CD-immunostimulatory -gliadin epitopes - is differential and grain-dependent, and that anthocyanin accumulation, as opposed to carotenoids, correlated with a lower immunogenicity and toxicity of gliadins at both protein and peptide levels. Considering that the amount of immunogenic peptides is the primary factor necessary for the achievement of the threshold for the inflammatory response mediated by T-cells in subjects genetically predisposed to have CD, the consumption of pigmented cereals with a reduced load of gluten epitopes, could be a way to maintain the pathogenic T-cells below the threshold of the inflammatory cascade, thus preventing, or delaying, the detrimental autoimmune response in genetically at-risk individuals.

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Acknowledgments

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Keywords : High-resolution mass spectrometry, Label-free quantification, INFOGEST digestion, Gluten proteins, Celiac Disease

Topic 4: Food structures, digestion and imaging technologies | Poster

(22714) - DEVELOPMENT OF A CAROB SYRUP FOR INCORPORATION INTO FOODS TO PROVIDE FUNCTIONALITY

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Abstract

Background: In recent years, there has been a noticeable rise in the demand for functional foods characterized by reduced sugar content. This trend is also driven by consumers' commitment to a health-conscious lifestyle. The modulation of microbiota equilibrium through prebiotics, such as fructo-oligosaccharides (FOS), has emerged as a promising approach for preventing or treating various diseases of the XX century [1]. Carob pulp has high sucrose content (65–75%) and intrinsic health benefits. Herein, we aimed to develop a FOS syrup using carob pulp as carbon source, for further inclusion in food products. A commercial enzyme complex was evaluated for the catalysis of sugar.

Methodology: Carob pulp extraction conditions were optimized by a central composite design (CCD) to maximize sucrose extraction. Antioxidant, phenolic and flavonoids content of the carob pulp extract were analyzed. A commercial enzymatic complex, Novozym® 960, was evaluated for the synthesis of FOS. The resistance of the FOS syrup to the harsh conditions of the gastro-intestinal digestion was evaluated by applying the standardized INFOGEST protocol.

Results: Optimal carob pulp sucrose extraction conditions were as follows: carob:water ratio of 1:3 (w/v), temperature of 46.1 °C, and extraction time 132 minutes. The obtained extract shown an antioxidant activity of 88.9 ± 0.2 µmol TEq/g DW, by ferric reducing antioxidant power (FRAP) assay, and 114.99 µg/mL by ABTS radical scavenging assay. The total phenolic and flavonoid contents were 8.35 ± 0.01 mg GAEq/g DW and 1.413 ± 0.007 mg CEq/g DW, respectively. Sucrose content was 86 ± 1 g/L. Using the carob pulp extract as substrate, the complex Novozym® 960 produced after 45 minutes of reaction 0.42 ± 0.01 g of FOS per g of carob sucrose, with a purity of 29.6 ± 0.3% (w/w) and a productivity of 49 ± 1 (g_{FOS}/(L×h)).

Conclusions: A prebiotic FOS syrup was successfully produced at a lab scale through enzymatic conversion of carob pulp sucrose content. This substrate proved a promising alternative for pure sucrose in FOS production, offering a cost-effective process coupled with improved nutritional value and functionality. The following step involves assessing the stability and resistance of the prebiotic syrup to the adverse conditions of the gastrointestinal system. Results on *in-vitro* gastrointestinal digestion will be presented at the conference.

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Acknowledgments

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Keywords : Fructo-oligosaccharides, Carob pulp, Novozym® 960, Prebiotics, Antioxidants.

Topic 4: Food structures, digestion and imaging technologies | Poster

(22734) - RELATIONSHIP BETWEEN THE PHYSICO-CHEMICAL PROPERTIES OF NON-STARCH POLYSACCHARIDES AND MACRONUTRIENT DIGESTION

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Abstract

The global rise in obesity and type 2 diabetes has generated significant interest in regulating the glycaemic impact of staple foods. Non-starch polysaccharides (NSP), the main components of plant cell walls (dietary fibre), are known to reduce the rate of starch digestion and attenuate postprandial glycaemia, and thus may provide a potential strategy for the prevention and management of metabolic conditions such as type 2 diabetes. Our previous *in vitro* and *in vivo* studies have demonstrated that the effect of plant cell walls to reduce starch digestibility of bread and improves glycaemia, using a novel intact chickpea cells powder (ICP) to replace refined wheat flour. However, NSP with differing physico-chemical properties can impact starch digestion via different mechanisms. Thus, this study has been designed to develop and test different combinations of fibre-rich plant materials to maximise their capacity to slow down starch digestion. This study utilised a combination of guar gum, a soluble NSP with ICP to investigate *in vitr*o starch digestion kinetics. Three types of bread were studied, including white bread (control), ICP bread (30% replacement of wheat flour with ICP in white bread recipe), and ICP-Guar bread (8% replacement of wheat flour with guar in the ICP bread recipe). Results showed that the addition of guar improved the bread quality when comparing to ICP Bread, including loaf volume, gas cell structure, and bread colour. The moisture content of ICP-Guar bread (50.0%) was significantly higher than the control and ICP breads (37.7% and 40.1%, $p < 0.05$) cause the high water-holding capacity of guar galactomannan. The INFOGEST digestion data, showed that the starch in ICP bread was more digested after gastric phase, but less digested after duodenal digestion when compared to white bread (19.4% vs. 11.9% for gastric and 86.0% vs. 90.5% for duodenal, respectively). Incorporation of guar into ICP bread significantly decreased digestion during the whole process (7.2% after gastric digestion and 82.1% after duodenal digestion). Confocal images also indicated the presence of intact cell walls from ICP and ICP-Guar breads throughout the digestion and evidence of undigested intracellular starch. Logarithm of slope plots of the digestion data during the duodenal phase were analysed for digestion kinetics. The white breads revealed linear plots characterised by a single rate constant, while starch amylolysis of ICP and ICP-Guar breads followed a two-phase process including the rapid and the slower second phase, respectively. Combining fibre types with different physiological actions might be a new, promising way to provide healthier low glycaemic staple foods. However, to understand the influence of cell walls and NSPs on starch digestion, further mechanistic studies need to be carried out, including *in vitro* saliva-gastrointestinal digestion assays, in conjunction with human studies.

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Keywords : INFOGEST, Starch Digestion, Non-starch Polysaccharides, Plant Cell Walls

Topic 4: Food structures, digestion and imaging technologies | Poster

(22745) - ARE PLANT-BASED DRINKS EQUIVALENT TO COW'S MILK IN TERMS OF INGREDIENTS AND PROTEIN QUALITY?

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Abstract

Plant-based drinks are no longer niche products for hipsters and vegans, but are now part of everyday life. What are the nutritional consequences of replacing milk with plant-based alternatives in the daily diet? To address this question, this study (Walther et al, 2022) therefore compared the nutritional composition and protein quality of plant-based drinks with that of cow's milk in order to assess their suitability as an alternative to cow's milk. Twenty-seven plant-based drinks from eight different plant sources, such as almond, cashew, coconut, hemp, oat, rice, soy, and spelt, as well as two whole milk samples were analyzed for their protein, carbohydrate, fat, vitamin and mineral content. Their digestibility was examined in vitro to compare the protein quality. Residue contamination with glyphosate, aminomethylphosphonic acid (AMPA) and arsenic was also taken into account.

Compared to most plant-based drinks, cow's milk had higher levels of energy, fat, carbohydrates, typical vitamins and some minerals. Soy was the only plant-based drink that could compete with cow's milk in terms of protein content. However, most milk alternatives had such a low protein content that they cannot be considered as a protein source. These differences were further amplified when protein quality (in vitro DIAAS) was taken into account. Milk had a higher protein quality than all other sources. The study emphasizes that plant-based drinks are not nutritionally equivalent to milk and can lead to nutrient deficiencies if milk is completely substituted without dietary adjustments.

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Keywords : cow's milk, plant-based drinks, nutrients, protein quality, in vitro DIAAS

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(22750) - IMPORTANCE OF THE COACERVATION PROCESS OF GELATIN WITH GUM ARABIC FOR IN VITRO DIGESTION

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1 - Symrise AG; 2 - Teagasc

Abstract

Spray dried gelatin-gum Arabic coacervates are commonly used for microencapsulation of ingredients to ensure controlled release of bioactive substances. Cross-linking with tannic acid reduces the disintegration of the capsules during gastric digestion, thereby decelerating the release of the encapsulated substance1.

By adjusting the wall thickness and size of the capsules during the coacervation process, the release time interval of the encapsulated material in the gastrointestinal tract can be defined according to the aspired application.

The aim of this study was to characterise the influence of core to wall ratio and particle size of capsules made by complex coacervation on the stability of the capsule walls and the release of the model substance MCT oil in the gastrointestinal tract.

The static digestion of the coacervates was performed according to the INFOGEST 2.0 protocol2 with modifications. The gastric step was performed at pH 2, 3, and 4 to approximate the dynamic digestion. In order to be able to quantify the release of the encapsulated oil in the gastric phase, lipase was not used. The capsules were characterised by LR-NMR, laser diffraction, light and confocal microscopy. Protein hydrolysis was monitored by SDS-PAGE and quantification of amino groups.

It has been shown that the degradation of the coacervate wall begins in the gastric phase through the release of gelatin from the coacervate, followed by protein digestion. As a fibre, gum Arabic is not expected to be digested in the gastric phase and may still provide core protection even though the coacervates have partially broken down.

By reducing the ratio of oil to wall and thus increasing the thickness of the wall, the oil retention in the gastric phase can be increased from 37 - 48 % to 75 - 83 %. During this phase particle size decreases due to thinning of the capsule wall and release of smaller oil droplets incorporated into the coacervate wall, which was detected by confocal microscopy. Decrease of the pH in the gastric phase results in a reduction in particle size which can be explained by higher degradation of the protein.

Unexpectedly, no significant effect of capsule size on oil release was observed.

No capsules were found microscopically in the intestinal phase, although particles with peaks at 0.1 and 10 µm were measured by laser diffraction. This suggests emulsification of the oil by micelle formation and therefore complete release of the core.

On the basis of the results, it can be assumed that coacervates with a thicker wall can be successfully used for the targeted transport of hydrophobic bioactive substances into the intestinal phase with full bioavailability.

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Acknowledgments

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Keywords : Gelatin-gum Arabic coacervates, In vitro digestion, Oil release, Core to wall ratio

Topic 4: Food structures, digestion and imaging technologies | Poster

(22758) - NANOENCAPSULATION OF ANTHOCYANINS BASED ON PECTIN AND LYSOZYME: A NEW TECHNOLOGICAL APPROACH TO INCREASE THE PHYSICOCHEMICAL STABILITY, BIOACCESSIBILITY AND ABSORPTION

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Abstract

Anthocyanins are bioactive compounds with essential properties, such as antioxidant, anti-inflammatory, and anticancer. However, they have high molecular instability, limited bioaccessibility, and bioavailability. The study aimed to develop a new methodology to nano-encapsulate anthocyanins extracted from blackberries (*Rubus* spp.) using nanogels. They were based on citrus pectin associated with lysozyme through molecular self-assembling. Nanoparticles were subjected to evaluation of colloidal stability and behavior on *in vitro* and *in vivo* models. Cyanidin-3-*O*-glycoside was primarily identified as the main anthocyanin (95%), and the nanoencapsulation proportions of 1:2:0.4 (m:m:m) of pectin, lysozyme, and anthocyanin, respectively, were statistically established. The nanoparticles showed a size of 190 nm, Zeta Potential -30 mV, and invariably spherical and homogeneous morphology (polydispersity index of 0.1). Nano-encapsulated anthocyanins were stable at different pH values (2 to 12) and temperatures (4, 25 and 40°C). The INFOGEST 2.0 digestion indicated preserving the anthocyanins' integrity and gradual release. A significant content remained intact in the nanostructure at the end of the intestinal phase. Cytotoxicity was not observed in 2D and 3D models, and nanoparticles were absorbed in both cell systems in a time-dependent way. A new methodology was developed to radiolabel anthocyanins directly with Technetium (^{99m}Tc) and nano-encapsulate them. An *in silico* model was developed to indicate the molecular interaction between the anthocyanin and the radioisotope. Biodistribution in different tissues, Kinect of absorption, and molecular visualization by μSPECT/CT showed that nano-encapsulated ^{99m}Tc-anthocyanins are absorbed differently than free molecules in mice. The molecule in blood, bone, bladder, lung, brain, heart, pancreas, liver, and spleen was significantly higher for nano-encapsulated after 24 hours of oral administration. The *in vitro* and *in vivo* methodologies demonstrated that nano-encapsulation can protect anthocyanins against digestion factors, with a controlled release in the intestine, improving absorption and targeting different organs. These results contribute to knowledge about the bioavailability of nano-encapsulated bioactive compounds and their potential clinical applications for the food and pharmaceutical industries.

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Keywords : Nanoencapsulation, Molecular imaging, Cyanidin-3-O-glycoside, Bioaccessibility, Food colloids

Topic 4: Food structures, digestion and imaging technologies | Poster

(22800) - MEGA-ANALYSIS ON THE RELATION BETWEEN FOOD AND INDIVIDUAL CHARACTERISTICS AND GASTRIC EMPTYING MEASURED WITH MRI

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Abstract

Background: Digestion encompasses a series of complex physiological, mechanical and biochemical processing steps that ultimately facilitate the absorption and utilization of nutrients. Gastric emptying (GE) constitutes a rate-limiting step in the delivery of nutrients to the small intestine for further break-down and is relevant to a range of clinical conditions. It is well established that GE rate is largely determined by the chemical characteristics of food, mainly the macronutrient content, but also by physical characteristics such as the viscosity. However, individual characteristics like age, sex and body mass index (BMI) may also influence GE. To which extent population and food characteristics affect the GE rate has not been comprehensively assessed. Therefore, this mega-analysis aimed to identify the factors that influence GE and to assess to what extent these factors are affecting GE rate. These findings could provide new insights that might be useful in future research on the regulation of food intake, the pathophysiology of gastric-related disorders, and the optimization of postprandial physiological responses.

Approach: The study was pre-registered at OSF (https://doi.org/10.17605/OSF.IO/RJ56H). Studies were selected by contacting members of the INFOGEST and UNGAP imaging working group for available data. Additional studies were selected by searching the PubMed database using the following keyword search (all fields): "(gastric AND (emptying OR retention) AND ("Magnetic Resonance Imaging" OR MRI) [full text, clinical trial, human, English]. Inclusion criteria were: 1) data were published in a peer reviewed journal in the last 15 years (in or after 2008), 2) data on gastric content volume were derived from MRI images. Authors of 22 studies provided data on gastric content volumes, food characteristics and participant characteristics. Data from 723 imaging sessions conducted with n=319 individuals from 22 studies were considered eligible for analysis. The studies had a total of 52 different treatments (stimuli). MRI scans were obtained for at least 60 min after the onset of ingestion (range 60 - 375 min). The primary outcome variable was gastric content volume over time. Population characteristics, including age, sex and BMI, and food properties including texture, energy density, macronutrient composition and load volume were included as fixed effects. Subjects, study site, and country were included as random effects. Mixed-model analysis is currently in progress. Results will be reported at the meeting.

Acknowledgments

In alphabetical order the co-authors that contributed are: Jaber Alyami, Guido Camps, Ruoxuan Deng, Daniela Freitas, Dileep N Lobo, Elise van Eijnatten, Steven Le Feunteun, Caroline Hoad, Michael Grimm, Shanthi Krishnasamy, Alan Mackie and Julia Roelofs. We thank all other authors of the contributing studies.

Keywords : MRI, Gastric Emptying, Meta-analysis

Topic 4: Food structures, digestion and imaging technologies | Poster

(22818) - IN VITRO DIGESTIBILITY OF CHICKPEA PROTEIN FRACTIONS: THE ROLE OF THEIR STRUCTURAL AND PHYSICOCHEMICAL CHARACTERISTICS

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Abstract

The nutritive value of legume proteins is largely influenced by their limited susceptibility to digestive proteases, which hinders hydrolysis. The ability of proteolytic enzymes to cleave proteins is influenced by various structural characteristics, including secondary structure, surface hydrophobicity, and disulfide bonds of proteins. Chickpea protein isolates (CPI) contain a high fraction of globulins, representing 80% of the total seed proteins. Globulin can be further subdivided into 11S legumin (CL) and 7S vicilin (CV) subunits. These fractions are responsible for most of the functional and nutritional properties of CPI, but their molecular characteristics are not fully understood. This study aimed to characterize the conformational and structural properties of isolated globulin, legumin, and vicilin fractions from chickpea proteins and to understand the role of these properties in in vitro protein digestibility. Alkaline extraction-isoelectric precipitation and modified salt dissolution-precipitation methods were used to produce the CPI, CL, and CV fractions. The samples were characterized using surface hydrophobicity (fluorescence and ANS methods), sulfhydryl group content (Ellman's method), OPA, and electrophoresis (SDS-PAGE). In addition, their structural properties were characterized by FT-IR and Raman spectroscopy. The protein content in the globulin, legumin, and vicilin fractions was > 90%. The results showed that the fractions had different molecular characteristics. The vicilin fraction had significantly ($p<0.05$) lower surface hydrophobicity, indicating fewer exposed hydrophobic residues or patches, lower free and total SH groups, and disulfide bond content than legumin and globulin. According to the SDS-PAGE results, the vicilin fractions showed subunit bands with lower MW, ranging from 50 to 10 kDa, implying heterogeneous polypeptide subunits. Interestingly, Raman spectroscopy revealed a high content of both α-helix (~46%) and total β-sheet (~38%) secondary structures in CPI. The highest percentage of free amino acids during intestinal digestion was found in vicilin compared with that in the legumin fraction. Lower hydrophobicity and disulfide bonds in vicilin were detected in the Raman spectra, and an increase in the intensity ratio I1360/I1340 was identified, indicating the buriedness of the tryptophan residues. These results confirm that the molecular properties of the vicilin fraction play an important role in influencing the structural characteristics of CPI during in vitro digestion. Characterization of the structural and digestion properties of chickpea protein fractions provided a theoretical basis for understanding their interaction behavior and functional characteristics, and the potential application of these legume proteins for the development of products.

Acknowledgments

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Keywords : Chickpea proteins, Vicilin, Legumin, Protein hydrolysis, Protein secondary structure

Topic 4: Food structures, digestion and imaging technologies | Poster

(24470) - EFFECT OF XANTHAN GUM-MICELLAR CASEIN INTERACTIONS ON THE DIGESTABILITY OF HYBRID POLYSACCHARIDE-PROTEIN SYSTEMS

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Abstract

Polysaccharides are widely used in the food industry as additives in food products. In the particular case of milk products, some polysaccharides have shown the capacity to stabilize the structure of proteins. The interactions between proteins and polysaccharides are not only relevant to control the techno-functional properties of food products, but they also play a crucial role to modulate protein digestibility. By selecting the appropriate polysaccharide and adjusting its concentration, it becomes feasible to regulate protein digestibility and promote the release of biologically active peptides in the distal intestine.

Accordingly, this study aimed to investigate the *in vitro* digestibility of micellar casein in polysaccharide-protein hybrid structures, where xanthan gum (XG) was chosen as the structuring polysaccharide. The study evaluated the impact of the structure type (hydrogels vs. aerogels) and the XG:casein ratio. Gastrointestinal digestions were performed following the INFOGEST protocol, and the resulting digestion products were characterized to determine the degree of proteolysis and microstructural changes. The results revealed that the addition of XG significantly delayed the digestion process, particularly during the gastric phase. Following intestinal digestion, it was observed that a fraction of casein was strongly bound to XG, remaining in undigested granules, as evidenced by CLSM micrographs. This phenomenon was more pronounced in the case of the aerogels as compared to hydrogels. However, the overall degree of hydrolysis was more influenced by the XG concentration than the type of structure, with higher XG concentrations leading to a lower degree of protein digestion.

Thus, by adjusting the of XG and the developed structure types, it is possible to modify the digestibility of micellar casein, thereby promoting the release of biologically active peptides in the intestine.

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Keywords : aerogel, protein, structure

ICFD 2024

TOPIC 5

IMPACT OF DIET ON GUT MICROBIOTA

Portugal | April 9-11th, 2024

Topic 5: Impact of diet on gut microbiota | Poster

(21397) - PROBIOTIC YEASTS FROM DAIRY PRODUCTS FOR FUNCTIONAL FOODS USEFUL FOR THE GUT MICROBIOTA

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Abstract

The microbiota of spontaneously fermented dairy products represents an important source for isolation of yeast strains with metabolic traits essential for the development of food flavor and texture and for the enrichment of its nutritional properties.

The present study studies the antimicrobial and probiotic characteristics of five yeasts strains isolated from spontaneously fermented dairy products: *Pichia kudriavzevii* CMGB-L3S, *P. kudriavzevii* CMGB-SM3, *Ogataea polymorpha* CMGB-P50, *C. parapsilosis* CMGB-DA1 and *Saccharomyces cerevisiae* CMGB 234.

The anti-bacterial activity was tested against frequently encountered bacterial food pathogenics: *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* CMGB333, *Bacillus cereus* CMGB 53-100, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 14028. The optimal growth media for both yeasts and bacterial pathogens was selected and semi-quantitative antimicrobial activity was determined by an adapted diffusion assay. Best results were obtained on slightly acidic growth media (YGSA). All the yeast strains inhibited the growth of *P. aeruginosa* ATCC27853.

The anti-*Candida* potential was tested against *C. parapsilosis* CBS 604, *C. paraspsilosis* CMGB 79, *C. albicans* ATCC 10231, *C. tropicalis* CMGB 115, *C. tropicalis* CMGB 165 and *C. krusei* CMGB 94 (reference strains), respectively, against *C. parapsilosis* CMGB-Y3, *C. krusei* CMGB-Y8, *C. catenulata* CMGB-Y7 and *C. albicans* CMGB-Y12 (human pathogenic strains). The mechanism of anti-*Candida* activity involved production of killer toxins, the strains *O. polymorpha* CMGB-P50, *S. cerevisiae* CMGB 234 and *C. parapsilosis* CMGB-DA1 inhibiting the growth of *C. albicans* ATCC10231, *C. krusei* CMGB-Y8 and *C. parapsilosis* CMGB-Y3 when cultivated at low pH. However, no significant inhibitory effect was observed against microbial strains with probiotic potential (*Streptococcus salivarius ssp thermophylus* ATCC 19258, *Lactobacillus acidophilus* ATCC 4356, *Saccharomyces boulardii* CMGB-S, *Kluyveromyces. lactis* CMGB 112, *Kluyveromyces marxianus* CMGB 159). Esterase production was also determined, followed by determination of butyric acid release, a prebiotic compound involved in regulation of basal metabolism genes, reducing oxidative stress and modulating the immune response. All strains secreted thermostable esterase (20°C, 28°C, 37°C), suggesting their possible use as basis for the development of functional foods.

In conclusion, the present work allowed characterization of five new yeast strains with important antimicrobial and prebiotic abilities, as promising microbial resources for food industry.

Acknowledgments

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Keywords : functional foods, antimicrobial activity, probiotic yeasts, spontaneously fermented dairy products

Topic 5: Impact of diet on gut microbiota | Poster

(21431) - TRADITIONAL FERMENTED FOOD EXERT POSTBIOTIC EFFECT ON IN VITRO GUT MICROBIOTA OF CHILDREN

Nyati, Murambiwa (Zambia)[6,7]; Van Mastrigt, Oscar (Netherlands)[1]; Talsma, Elise F (Netherlands)[2]; Bakker, Sibbe (Netherlands)[3]; Shindano, John (Zambia)[4]; Zwaan, Bas (Netherlands)[5]; Schoustra, Sijmen (Netherlands)[3]

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Abstract

Traditional fermented foods (TFF) are reported to exert a beneficial effect on the human gut microbiota. However, most indigenous TFF lack compelling evidence to support their beneficial claims. In this study, we investigated a popular Zambian dairy TFF known as Mabisi. Our aim was to determine its potential to modulate the in vitro gut microbiota of children aged 6 to 12 months. Additionally, we studied its potential to prevent the proliferation of known bacterial pathogen strains by deliberately contaminating the in vitro gut microbiota of small children. We expected that the presence of Mabisi digest in the in vitro colon would positively promote beneficial bacteria and the production of short-chain fatty acids (SCFA) at the expense of the proliferation of added pathogens.

The INFOGEST digestion and fermentation protocols addressed the study hypothesis, with treatments including in vitro colon units exposed to Mabisi digest. To comprehensively address the question of whether Mabisi has a beneficial effect, we also prepared sterile Mabisi by heating, Fructooligosaccharides (FOS) as positive and negative controls (without Mabisi). Both treatments and controls were digested and then incubated for 24 hours.

Our primary outcome was an increase in beneficial bacteria genera, including *Lactobacillus, Pediococcus, Bifidobacterium, Sarcia,* and *Collinsella* in the Mabisi, sterile Mabisi, and FOS treatment groups. Fructooligosaccharides induced growth in a more diverse bacterial community compared to Mabisi, sterile Mabisi, and the negative control. Treatment with Mabisi and sterile Mabisi resulted in a higher production of Acetate, Isobutyrate, Propionate, Lactate, Formate, and Succinate compared to the positive and negative control. We find Mabisi to have prebiotic or postbiotic effect, as opposed to a probiotic effect. The added bacterial pathogens (*Listeria innocua* and *Escherichia coli*) did not establish in this experiment. This study contributes to the evidence of the beneficial role of traditional fermented food for the in vitro gut microbiota of small children.

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Keywords : Fermented, Gut, Microbiota, Postbiotic, Prebiotic

Topic 5: Impact of diet on gut microbiota | Poster

(21438) - DIGESTION AND FECAL INOCULA FERMENTATION OF PINE NUT SKIN CARBOHYDRATES AND PHENOLICS

Silva, Soraia P. (Portugal)[1]; Gonzalez, Abigail (Portugal)[2]; Roupar, Dalila (Portugal)[2]; Salvador, Andreia F. (Portugal)[2,3]; Nobre, Clarisse (Portugal)[2,3]; Coimbra, Manuel A. (Portugal)[1]; Coelho, Elisabete (Portugal)[1]

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Abstract

Pine nut skin (PNS) is a by-product recovered during pine nut processing, which is usually discarded for heat production. The exploitation of by-products as sources of valuable compounds agrees with the current demand for the reduction of waste, and a transition to a more sustainable production and consumption [1]. Therefore, PNS was extracted and the gastrointestinal digestibility and fermentability of the recovered valuable compounds was assessed.

PNS subcritical water extraction allowed to obtain extracts rich in phenolic compounds and mono- and oligosaccharides (low-molecular weight fraction) and rich in pectic polysaccharides and xyloglucans (high-molecular weight fraction). These fractions were separately digested using the INFOGEST protocol to evaluate the impact of gastrointestinal conditions on the different compounds. Then, human fecal inocula were used to evaluate the impact of each fraction on the microbiota composition, analysed by 16S rRNA gene sequencing, and on their production of short-chain fatty acids (SCFAs).

After digestion simulation, oligosaccharides remained unmodified, while phenolic compounds suffered a 20 % (gallic acid equivalents) decrease in the intestinal phase. The polysaccharides found in the high-molecular weight fractions demonstrated stability throughout the digestion process, releasing 0.6 % of free sugars. This indicates that polysaccharides have remained intact and reach the colon without significant changes. Thus, it is proposed that the extracted carbohydrates from the low- and high-molecular weight fractions reach the large gut intact, where they can be fermented by the microbiota.

The fermentation of mono- and oligosaccharides and phenolic compounds did not cause a significant microbial shift upon fermentation, nonetheless it stimulated significantly ($p < 0.05$) the growth of the prebiotic *Bifidobacterium adolescentis* and the SCFAs production, specifically acetate and propionate.

Pectic polysaccharides and xyloglucans had an impact on the microbiota composition similar ($p < 0.05$) to that of the positive control (fructooligosaccharides), also increasing the abundance of *B. adolescentis*. The core microbiome upon these polysaccharides' fermentation was composed of multiple butyrate producers, which was reproduced in the increase ($p < 0.05$) of butyrate concentration. Besides, it was observed a microbial utilization selectivity towards xyloglucans in detriment to rhamnose-containing pectic polysaccharides and type II arabinogalactans.

Pine nut skin compounds recovered with subcritical water extraction were observed to be mostly stable throughout digestion, able to be selectively utilized by human gut microbiota. The polysaccharide-rich extract presents a high potential as a prebiotic food ingredient.

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Acknowledgments

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Keywords : Pectic polysaccharides, xyloglucans, prebiotic food ingredient, microbiota, Pine nut skin

Topic 5: Impact of diet on gut microbiota | Poster

(21451) - EFFECT OF FOOD ADDITIVES MONOSODIUM GLUTAMATE AND ALLURE RED ON GUT MICROBIOTA AND HOST

Vázquez, Xenia (Spain)[1]; Sabater, Carlos (Spain)[1]; Granja, Adela (Spain)[1]; Hevia, Arancha (Spain)[1]; Garrido-Romero, Manuel (Spain)[2]; Muñoz-Labrador, Ana (Spain)[2]; Moreno, Javier (Spain)[2]; Ruiz, Lorena (Spain)[1]; Ruas-Madiedo, Patricia (Spain)[1]

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2 - Instituto de Investigación en Ciencias de la Alimentación (CIAL-CSIC-UAM)

Abstract

Classical food safety risk assessment approaches have not considered the contribution of the gut microbiome to the metabolization of non-absorbable dietary compounds, and the potential impact of this microbial biotransformation on human health, although this have begun to be explored in recent years. In this work, we have used a faecal batch fermentation model, in combination with targeted metabolomics, as well as an intestinal cell line model, to *in vitro* investigate the potential impact of two food additives on gut microbiota and epithelium. After the approval of the Ethics Committee, faecal samples were provided by 6 young healthy donors (paired by gender) and used to inoculate five vessels containing a basal fermentation media (BFM) without additives (control), supplemented with two different doses of monosodium glutamate (MSG; E621: 0.08 mM and 0.8 mM) or with two different doses of allura red (AR; E129: 0.07 and 0.7 mM). A basal sample (0 h) was collected from the control vessel before incubation at 37ºC, for 24 h in an anaerobic cabinet. Microbial pellets were collected for DNA isolation and fecal supernatants were used to quantify SCFA (short chain fatty acids by GC/FID) and metabolites derived from the microbial metabolization of the food additives (by HPLC), as well for toxicity upon HT29 (by the RTCA-DP xCELLigence).

After fermentation, some variations were observed in some genera respect to the basal sample and, concomitantly, the production of SCFAs was also higher. In general, no significant variations in the relative abundance of the taxa, nor in SCFAs, were detected due to the presence of the additives. MSG and its derived metabolite GABA, showed a decrease after 24 h of fermentation in all the conditions tested, indicating consumption or transformation into alternative metabolites through microbial activity. Similarly, AR was also efficiently bio-transformed in metabolites resulting from azo-reduction. Finally, no cytotoxic effect of the faecal supernatants was observed upon HT29 monolayer. In conclusion, our study suggests MSG and AR, under our experimental conditions, do not appear to have significant effects directly on the human gut microbiota, nor on the host intestinal cell.

References

Acknowledgments

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Disclaimer: The conclusions, findings, and opinions expressed in this communication reflect only the view of the authors and are not the official position of EFSA.

Keywords : faecal microbiota, food additives, biodegradation, cytotoxicity, HT29

Topic 5: Impact of diet on gut microbiota | Poster

(21459) - TRADITIONAL FERMENTED FOOD EXERTS PREBIOTIC EFFECT ON SMALL CHILDREN GUT MICROBIOTA

Nyati, Murambiwa (Netherlands)[1]; Van Mastrigt, Oscar (Netherlands)[2]; Talsma, Elise (Netherlands)[3]; Bakker, Sibbe (Netherlands)[1]; Shindano, John (Zambia)[4]; Zwaan, Bas (Netherlands)[1]; Schoustra, Sijmen (Netherlands)[1,4]

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Abstract

Traditional fermented foods (TFF) are reported to exert a beneficial effect on the human gut microbiota. However, for most TFF, compelling evidence to support their beneficial claims is missing. In this study, we focus on a popular Zambian dairy TFF known as Mabisi. Our aim was to determine Mabisi's potential to modulate the gut microbiota of children aged 6 to 12 months and further assess its potential to suppress known bacterial pathogens by deliberately contaminating the gut microbiota of small children. We expected that Mabisi would promote beneficial bacteria and the production of short-chain fatty acids (SCFA) and suppress pathogens.

We used an in vitro experimental approach to expose gut microbiota of small children to Mabisi digest. The INFOGEST digestion and fermentation protocols addressed the study hypothesis, with treatments including in vitro colon units exposed to Mabisi, sterile Mabisi and positive (Fructooligosaccharides) controls and negative (water) controls.

Our primary outcome was an increase in beneficial bacteria genera, including Lactobacillus, Pediococcus, Bifidobacterium, Sarcia, and Collinsella in the Mabisi, sterile Mabisi, and positive control groups. FOS induced development of a more diverse bacterial community compared to Mabisi, sterile Mabisi, and the negative control. Treatment with Mabisi and sterile Mabisi resulted in a higher production of Acetate, Isobutyrate, Propionate, Lactate, Formate, and Succinate compared to the positive and negative control. This demonstrated that Mabisi harbors a prebiotic, as opposed to a probiotic effect. The added bacterial pathogens (Listeria innocua and Escherichia coli) did not establish in this experiment. This study contributes to the evidence of the beneficial role of traditional fermented foods for the gut microbiota of small children.

Keywords : Fermentation, Gut, Microbiota, Prebiotic, Traditional

Topic 5: Impact of diet on gut microbiota | Poster

(21465) - 'FROM CHEW TO POO': DOES CHEWING BEHAVIOR OF CONSUMERS IMPACT GUT MICROBIOME METABOLITES?

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Abstract

The metabolic activity of the microbiome in the gut is considered an important part of food digestion, providing energy and the production of important metabolites that can influence health. Many factors can influence colonic fermentation and metabolite production including lifestyle, antibiotics, immunological response, and habitual diet. Differences in food texture and oral processing behaviours have been shown to directly impact the kinetics of metabolic responses to ingested nutrients, largely driven by differences in bolus surface area and saliva uptake during the oral phase of digestion. Differences in oral processing behavior and food structure could influence the substrate surface area available for colonic fermentation and could consequently potentially impact microbiome metabolite production. The current study sought to investigate how differences in food oral breakdown impact bolus surface area, gut microbiome fermentation and metabolite production (GMMs) during carbohydrate digestion. Using an *in vitro* model with a fixed portion of Basmati rice our preliminary findings show that differences in bolus surface area resulting from 5, 10, 20 and 40 chews during the oral phase of digestion are retained when the food reaches the colon. Colonic fermentation activity was compared using biological microbiome samples to reveal significant differences in short-chain-fatty-acid (SCFA) production that correlated with observed differences in bolus surface area. Significant differences in bolus surface area were associated with different concentrations of acetic, propionic, and butyric acid production during colonic fermentation. These findings provide preliminary support for a link between habitual oral processing behaviors and colonic metabolite production by the fecal microbiome. Further research is needed to establish whether observed differences are clinically relevant. Our results aim to broaden our understanding of the habitual diet and lifestyle factors that influence microbiome metabolite production, to create new opportunities to modulate gut health through both behavioral and dietary strategies.

Acknowledgments

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Keywords : Oral processing behavior, bolus properties, dietary fiber, gut fermentation and metabolites, health

Topic 5: Impact of diet on gut microbiota | Poster

(21482) - FUNCTIONAL PROPERTIES OF FERMENTED PLANT-BASED BEVERAGES PRE- AND POST IN VITRO DIGESTION

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Abstract

Introduction:

Rice, tiger nut and carob are typical Mediterranean products with valuable nutritional and organoleptic properties for the development of new foods, such as fermented beverages[1]. Fermented foods are becoming increasingly important in the human diet due to their potential health benefits. One of the main beneficial effects of fermented foods is their ability to promote the growth of beneficial bacteria in the human gut microbiota. However, many fermented foods have not been tested to confirm their functional properties [2].

Aim:

The objective is to evaluate the fermentation kinetics, probiotic and antioxidant potential of three fermented vegetable drinks based on rice, tiger nut and carob, prior and after gastrointestinal digestion.

Method:

Three vegetable extracts of rice, tiger nut and carob were fermented employing four commercial probiotic lactic acid bacteria (LAB) consortia (VEG022, VEG033, VEG053 and VEG061) at two temperatures (30°C and 37°C) and with the addition of three sugar levels (0%, 7.5-15% and 15-30%). Once the optimal fermentation parameters were selected (37°C and intermediate sugar levels), survival of probiotic microorganisms were determined in the final product and changes in antioxidant capacity were evaluated at different fermentation stages using diverse methods (TEAC, ORAC and total polyphenol content). Finally, the consortium selected (VEG061) for its superior antioxidant activity was subjected to simulated *in vitro* digestion (INFOGEST 2.0) to evaluate the antioxidant activity and the survival of the probiotic microorganisms after digestion.

Results:

The results showed that all consortia adequately fermented the vegetable beverages, as verified by a decrease in pH. In addition, microbial counts of at least 10^7 cfu/mL were obtained in nearly all samples. Depending on the matrix, some consortia increased antioxidant levels in the beverages, particularly total polyphenols and ORAC in tiger nut and carob beverages. Furthermore, after simulated *in vitro* digestion, the VEG061 LAB consortium maintained part of its viability with a decrease of 1 log unit for rice beverages and 4 log units for carob and tiger nut beverages. In addition, a noticeable amount of antioxidants was maintained or even increased in fermented beverages following digestion.

Conclusion:

These results demonstrate that fermented plant-based beverages with commercial LAB consortia can be a source of beneficial antioxidants and probiotics for health in the gastrointestinal tract.

References

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Acknowledgments

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Keywords : plant-based beverages, fermentation, probiotics, antioxidants, digestion

Topic 5: Impact of diet on gut microbiota | Poster

(21499) - HMO COMPLEX FOR INFANT NUTRITION AND ITS IMPACT ON INTESTINAL AND MICROBIOME MATURATION

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Abstract

Humanization of infant milk formulation (IMF) with next generation oligosaccharides, proteins, peptides, and lipids has been led by biotechnological advances. Supplementation of IMF with the most abundant human milk oligosaccharide (HMO), 2′-fucosyllactose (2′-FL), has demonstrated clinical benefits for immune health and establishing a healthy microbiota in infants. A complex of HMOs, providing a closer representation of the HMO profile of human milk, is hypothesised to advance IMF function, however, further studies are required.

In this study, we performed an infant-adapted digestion (INFOGEST) protocol of IMFs containing 2′-FL or 5 HMO plus 2′-FL before assessing their effects on infant intestinal barrier function (epithelial Caco-2 cells) and immunomodulation (Caco-2 cells and THP-1 macrophages). Resulting digestas were dialysed prior to infant faecal fermentation (n=6 samples from breastfed infants) with SIFR® technology to assess impact on gut microbiota establishment. Organic acid production, pH shift and 16S rRNA gene profiling were used to monitor fermentation.

The complex of 6 HMO structures demonstrated superior immunomodulatory activity and supported intestinal barrier function when compared with the no-HMO and 2′-FL controls. Similarly, the presence of 6 HMOs resulted in a significant shift in the infant microbiome profile suggesting the unique microbiome-modulating functions of HMO complexes.

Keywords : human milk oligosaccharides, infant milk formula, faecal fermentation, immunomodulation, microbiome

Topic 5: Impact of diet on gut microbiota | Poster

(21500) - EFFECT OF INFOGEST PROTOCOL ON GUT MICROBIOTA INVITRO FERMENTATIONS OF CHILDREN'S WITH ASD

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1 - Universidad de La Sabana

Abstract

The delivery of probiotics and biocompounds that enhance the nutritional profile and exert health benefits for the host has been widely studied in the last few years. For probiotics and biocompounds to arrive at the site of action, they have to pass through the gastrointestinal tract, and finally, the not-absorbed digesta is going to interact with the different microbiota of the intestine sites[1]. On the other hand, the design of appropriate carriers (nano and macro) guarantees the correct delivery of probiotics and bioactives to the site at which they are going to exert their activity[2]. Also, the interaction between microbiota and these capsules has been studied; however, most of the in vitro studies reported that the compounds and probiotics were subjected directly to the gut microbiota without performing first the digestion process that occurs in the live systems[3].

Thus, in the research group, we developed controlled release systems for colonic delivery. The aim of this project was to evaluate the effect of the INFOGEST 1.0 protocol over in vitro fermentation assays on the gut microbiota of children with and without autism spectrum disorder. Microcapsules of *Limosilactobacillus ferementum* K73 and oils rich in oleic acid were encapsulated in a double emulsion pellet/oil/water, whose formulation was optimized by an RSM experimental design and finally sealed by spray drying technique. Later, the viability of the probiotics was measured in each part of the gastrointestinal tract, and the effect of the INFOGEST protocol was confirmed on the microbiota modulation study. For this assay, microbial change and the production of short-chain fatty acids were measured.

The experimental design let us obtain a formulation of a microcapsule that survives the drying process and passes through the gastrointestinal tract at 7.6 x 106 and 9.8 x 105 cell concentrations, respectively. This work demonstrated that the use of the INFOGEST protocol is a necessary requirement to evaluate the performance of probiotics and compounds in modulating the gut microbiota. The in vitro digestion process did not have a negative effect on the gut microbiota of the two samples; however, it did impact the production of short-chain fatty acids. The results showed that in the two groups, biotics exerted a change in the microbial structure, especially native probiotic species, which were enriched in treatments containing probiotics. Also, there was a significant difference between samples with and without ASD.

References

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3 10.1039/D2FO02723B

Acknowledgments

The authors are thankful to the Universidad de La Sabana for the funding of the project ING-261-2020 in which this study was developed. Also, author Katherine Bauer Estrada is thankful to the Engineering department for the scholarship Carlos Jordana and MinCiencias scholarshp with which she is developing her doctorate studies.

Keywords : in vitro fermentations, gut microbiota, INFOGEST 1.0 protocol, ASD, SCFAs

Topic 5: Impact of diet on gut microbiota | Poster

(21556) - INFLUENCE OF RS3 ON HUMAN GUT MICROBIOTA AND IMMUNE RESPONSE USING IN VITRO COMPLEMENTARY APPROACHES

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1 - MEDIS; 2 - UNH; 3 - LIMAGRAIN INGREDIENTS

Abstract

Resistant starches type 3 (RS3) are prebiotic insoluble fibers mainly found in cooked and cooled starchy food. RS3 can be provided by High Amylose Wheat (HAW) for which gut microbiota modulation has been documented but without being linked to immune responses so far. The aim of this study was to evaluate the antioxidant and anti-inflammatory properties of RS3 from HAW, compared with inulin, a soluble prebiotic fiber, as mediated by gut metabolites by using complementary *in vitro* human colon model and leukocytes isolated from human blood.

The ARtificial COLon (ARCOL) was used to reproduce the main nutritional, physicochemical and microbial parameters of the colonic environment of healthy human adults. Three bioreactors were run in parallel, one used as control and two daily supplemented with 15g/L of RS3 or inulin. Fermentations were performed in triplicate, using stool samples from three healthy adult donors. Microbial activities were evaluated through gas and main short chain fatty acid (SCFA) measurement. Bacterial composition was assessed by 16S Metabarcoding and qPCR analysis on targeted populations. Supernatants from ARCOL fermentations were incubated with leukocytes isolated from human blood to measure reactive oxygen species (ROS) by DHR123 fluorescent detection. Pro-inflammatory cytokines were also quantified after cell stimulation with lipopolysaccharides.

Supplementation with RS3 or inulin led to significant increases in main SCFA concentrations (mainly acetate and butyrate) and gas production, with donor-dependent effects on profiles. The impact of RS3 and inulin on microbiota composition was also donor-dependent. However, prebiotic supplementation led in both cases to an increase in *Ruminococcus* abundance. Interestingly, *Blautia* was more prevalent with all donors when fermentative media was supplemented in RS3. Supernatants from ARCOL bioreactors led to decreases in ROS production and in some pro-inflammatory cytokines by human leukocytes. However, no difference was observed between the control and treated (both RS3 and inulin) conditions.

RS3 and inulin induced donor-dependent beneficial modulation of microbiota composition, with no obvious effect on the antioxidant and anti-inflammatory pathways studied. Other experiments are ongoing to assess the effect of these prebiotics on leucocytes pro-inflammatory gene expression.

References

Acknowledgments

Keywords : Resistant starch, Human gut microbiota, In vitro colon model, Human leukocytes, Inflammation

Topic 5: Impact of diet on gut microbiota | Poster

(22569) - PERSONALIZED FIBER INTAKE INCREASES EXERCISE PERFORMANCE IN HEALTHY INDIVIDUALS

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Abstract

Gut microbiota can affect human health via its' impact on nutrient metabolism and immune function, and the production of metabolites that modulate the host's physiological processes. The high interindividual variability in gut microbiota composition highlights the need for personalized approaches in the development of dietary interventions. Optimizing the composition and activity of gut microbiota via targeted approaches, including prebiotic supplementation, may enhance skeletal muscle function and, hence, exercise performance. This study explores whether modulation of the gut microbiome by personalized prebiotic supplementation can improve endurance exercise performance.

In a randomized, controlled trial in 34 healthy (BMI 18.5–25 $kg \cdot m^{-2}$, 18-40y) recreationally active participants, endurance exercise performance was determined before and after a six-week personalized prebiotic supplementation intervention, by a time-to-exhaustion (TTE) cycling test. Prebiotics selection, 15 g/day, was based on the individuals' fecal microbiome composition. The selection of prebiotics was determined by evaluating the relative abundance of the genera *Lactobacillus*, *Bifidobacterium*, *Akkermansia*, and *Faecalibacterium* in comparison with an in-house reference set. At baseline and after the intervention, fecal samples were analyzed using 16S rRNA gene sequencing analysis, while fasted blood samples were collected to measure short chain fatty acids, insulin, glucose, and cytokine levels.

Following six weeks of personalized prebiotic supplementation, a significant increase of 22.7 ± 9.1% in TTE was observed in the prebiotics group compared to the control group ($p=0.002$), whereas the control group showed no significant change after six weeks ($p=0.543$).

The intervention demonstrated marginal effects on the overall shift in microbiota composition (Bray-Curtis; $R^2=0.006$; $p=0.021$; Amplicon Sequence Variant (ASV) taxonomic level). Nevertheless, significant changes in the abundance of several taxa were observed. *Bifidobacterium*, *Collinsella*, and *Lachnospiracae* abundance increased within the prebiotic group following the 6-week intervention. Blood parameters were not affected in both the prebiotic group and control group following 6 weeks of intervention.

Personalized prebiotic intake significantly improved endurance exercise performance in healthy individuals, concomitant with an increase in the relative abundance of specific taxa. This study showed that skeletal muscle function can be improved by targeting an individual's gut microbiome and may contribute to the development of new strategies to improve muscle health in relevant target populations, such as muscle associated clinical conditions and endurance athletes.

Keywords : Endurance exercise, gut microbiota, prebiotics

Topic 5: Impact of diet on gut microbiota | Poster

(22584) - COOKING AND PROCESSING OF SEAWEED IMPACT ON PROTEIN DIGESTIBILITY AND GUT MICROBIOME

Oh, Geraldine (Singapore)[1]; Cheong, Eleanor (Singapore)[4]; Bowie, Dale (New Zealand)[2]; Deb-Choudhury, Santanu (New Zealand)[3]; Haines, Stephen (New Zealand)[3]; Ross, Alastair (New Zealand)[3]; Teo, Jonathan (Singapore)[1]; Rettedal, Elizabeth (New Zealand)[3]; Samuelsson, Linda (New Zealand)[3]; Chan, James Chun Yip (Singapore)[1]

1 - Agency for Science, Technology and Research; 2 - Ideas 2 Plate; 3 - AgResearch; 4 - Procter and Gamble Innovation Center

Abstract

Seaweed is a source of alternative protein which does not require arable land or fresh water. However, the tough seaweed polysaccharide cell wall restricts the access of digestive enzymes to various nutrients in the seaweed. We hypothesize that in contrast to raw-rehydrated seaweed, cooking increases the bio-accessibility of seaweed nutrients (proteins in particular) while preserving its whole food nature. Additionally, the indigestible and unabsorbed components of seaweed may be fermented by the gut microbiota into beneficial bioactive metabolites. To investigate these hypotheses, *Undaria pinnatifida* from New Zealand and *Ulva* sp. seaweed from Singapore and New Zealand were characterized for nutritional and safety profiles, then prepared into two palatable food formats (dumpling and pesto-pasta) by a professional chef. Control food formats without seaweed were also prepared. Here, we outline the strategy to investigate the digestibility of these forms of seaweed. A semi-dynamic simulator of the human intestinal microbial ecosystem (SHIME®) model representing the stomach, duodenum and jejunum is used to reproduce the upper gastrointestinal digestive process. Intestinal absorption was simulated through the use of 3 kDa cut-off dialysis carriers in the jejunum. Digestates will be analyzed using a multi-omics strategy by monitoring the generation of peptides, amino acids and metabolites at various timepoints during digestion. Controls without seaweed provide a baseline to correct for the bio-accessible fraction from seaweed. By comparing the nutritional profiles of digestates from cooked vs raw-hydrated seaweed, the impact of cooking on seaweed digestibility can be assessed. Finally, a model of the proximal and distal colon using Mucin-SHIME® (M-SHIME®) will be inoculated with New Zealand and Singaporean microbiota using human stool, then treated with various unabsorbed digestates from the earlier experiment. Metagenomic, proteomic and metabolomic analyses will be performed to investigate the longitudinal impact of seaweed consumption on the microbiome of both populations.

Acknowledgments
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Keywords : Seaweed, Gut microbiome, Digestibility, Processing, Protein

Topic 5: Impact of diet on gut microbiota | Poster

(22603) - A COMPARATIVE IN VITRO EVALUATION OF COLONIC FERMENTATION OF NEW FOOD BASED ON FERMENTED LEGUMES AND PSEUDO-CEREALS IN OLDER ADULT CONDITIONS.

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Abstract

Aim: Ageing leads to changes in the gut microbiota, such as a reduction in microbial diversity or dysbiosis. Therefore, targeted dietary or supplementation strategies are needed to tackle this challenge. Developing products made of fermented legumes and pseudo-cereals (vegetable protein) could be a source of high dietary fibre related to the prebiotic potential to improve the colonic microbiota. This study evaluates the impact of new concepts of foods based on fermented legumes and pseudo-cereals on the elderly's microbiota through a static *in vitro* colonic fermentation model.

Method: Ten new prototypes based on fungal fermented or unfermented quinoa and/or lentil were developed as gel-like or muffin-like products. The INFOGEST protocol for simulating gastrointestinal digestion in older adult conditions was applied for prototypes gastrointestinal digestion, and the undigested fraction was used for colonic fermentation using a "faecal pool" of 4 elderly donors. Bacterial DNA extraction was performed using the Norgen Biotek Corp® (Thorold, ON, Canada) faecal DNA isolation kit, following the manufacturer's protocol and recommendations. Microbiological analysis was performed by amplification with specific primers of the V3-V4 regions of the 16S rRNA using Illumina.

Results: Data collection is ongoing, and study results will be available by the time of the Congress. The expected results will allow to evaluate the differences in relative abundances at phylum and genera levels after in vitro digestion and colonic fermentation of each prototype. Also, changes in the relative abundance of each bacterial genera will be compared considering the prototypes' processing parameters (fermented/unfermented).

Conclusion: This approach contributes to offering a new dietary option for the older adult population that meets the characteristic problems of aging by providing the prebiotic potential.

Acknowledgments
The project is co-funded by the Investigo 2022 Program and My Best Elderly Food-MyBEEF) together with funds from the FOOD&HEALTH group of the Institute of Food Engineering for Development of the Universitat Politècnica de València.

Keywords : Ageing, protein, microbiota, fermentation, legumes

Topic 5: Impact of diet on gut microbiota | Poster

(22612) - IMPACT OF A MORE REALISTIC FERMENTATION MEDIUM ON MICROBIOTA COMPOSITION AND METABOLIC ACTIVITIES IN TWO WELL-VALIDATED IN VITRO HUMAN COLON MODELS

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Abstract

Background: In recent decades, numerous studies have shown the key role of gut microbiota in human nutrition and health. Dietary intake and nutritional patterns greatly influence the gut microbiota composition and metabolic functions. To perform mechanistic studies on human gut microbes, *in vitro* systems that mimic human colonic environment provides a valuable alternative to *in vivo* studies. However, the fermentation medium remains a gap between the genuine colonic status and execution by colonic simulator systems. This study aimed to develop a new fermentation medium that relies on current nutritional intake in western countries (WDM), and evaluate it in two different *in vitro* systems simulating a human proximal colon habitat, by comparison to the commonly used MacFarlane medium (MFM)[1].

Methods: WDM was designed to more accurately simulate the undigested food molecules reaching the human colon based on recent food surveys, i.e. with less starch and inulin and more pectin and xylan than in MFM. The impact of WDM and MFM media on the composition, diversity and metabolic activity of gut microbes was investigated daily for two weeks in two different colon models set-up under human adult conditions: the Polyfermentor Intestinal Model (PolyFermS)[2] and the Mucosal Artificial Colon (M-ARCOL)[3]. The experiments were replicated using stools samples from two healthy donors (one woman and one man). Gut microbiota composition was assessed by 16S metabarcoding and microbial activities were evaluated through gas and short chain fatty acid (SCFA) measurement.

Results: Microbial α-diversity was significantly higher with WDW (Shannon's $p \leq 0.01$ and Simpson's $p \leq 0.001$) compared to MFM in the PolyFermS, while no significant difference was observed between the two media in the M-ARCOL. PERMANOVA tests based on the Bray-Curtis distance showed large variations between the microbiomes supplied with WDM and MFM ($p \leq 0.01$). Change in microbiota composition were observed, in particular in the PolyFermS. WDM significantly promoted fiber-degrading bacteria and SCFAs producers such as *Butyricicoccus* or Lachnospiraceae species. This can be associated with a higher SCFA production in the M-ARCOL, significant for acetate ($p=0.0006$) and butyrate ($p=0.02$). Also, WDM generated less intestinal gases than MFM in the M-ARCOL.

Conclusions: Even if the impact of WDW and MFM media on gut microbes were model-dependent, our results suggest that the more realistic WDM fermentation medium possesses the potential to cultivate gut microbes in two well-validated *in vitro* colon models, capturing more bacterial diversity from the initial stool samples.

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Acknowledgments

We are thankful to Dr. Ayman Elsayed for his technical support. This study was supported by a Strategic Research Grant from the University of Ottawa

Keywords : In vitro colon models, Gut microbiome, Fermentation medium, Fibers, SCFAs

Topic 5: Impact of diet on gut microbiota | Poster

(22614) - PROJECT GUT2BRAIN: EXPLORING THE MICROBIOTA-GUT-BRAIN AXIS IN OBESITY

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Abstract

Obesity and associated comorbidities represent a major concern for modern societies and a substantial financial burden for healthcare systems. However, its underlying bio-behavioral mechanisms remain largely unknown. Increasing evidence suggests that gut-derived signals, such as gut-microbiota neuromodulatory metabolites and appetite hormones, can influence pathways in the brain implicated in food intake and, ultimately, in the pathophysiology of obesity [1]. Furthermore, obesity has been associated with unbalanced microbiota composition (dysbiosis) [2]. Dietary prebiotics, may restore microbiota composition and modulate eating behavior, acting in the microbiota-gut-brain axis (MGBA).

The project Gut2Brain aims to generate evidence on how gut microbiota and its modulation by prebiotics affect appetite hormones, brain function, and, ultimately, eating behavior in obesity. Complementary research, both *in vivo* and *in vitro*, has allowed an in-depth investigation of the different MGBA mechanisms in this disorder.

The study investigates the impact of prebiotics in reverting dysbiosis and the specific alterations observed in obesity, through a Randomized Clinical Trial. Individuals with obesity are receiving a prebiotic/placebo for 12 weeks. Psycho-behavioral functioning, functional connectivity (rs-fMRI), appetitive hormones, and microbiota are being assessed before and after the intervention.

The biological mechanisms linking modulation of the MGBA in obesity by prebiotics are being studied *in vitro* using a unique gut model developed by the group, allowing bi-directional interaction between microbiota and human intestinal cells, with segmental mixing simulating peristaltic movement. The microbiota from the participants of the clinical trial is being used in the *in vitro* trials. Results will help to achieve the proof of concept of the gut model.

This project aims for an impactful understanding of the MGBA and its relationship with obesity, opening new perspectives and opportunities for novel treatments of eating-related disorders.

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Keywords : Gut model, Microbiota-gut-brain axis, Obesity, Prebiotics, Randomized clinical trial

Topic 5: Impact of diet on gut microbiota | Poster

(22650) - COLONIC HEALTH BENEFITS OF DRIED APPLE BAGASSE AS A POTENTIAL PREBIOTIC

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Abstract

Apple processing can result in a significant quantity of food residues, harming the environment and leading to waste functional ingredients, mostly dietary fibre (DF) and bioactive compounds. However, apple by-products could be introduced into the food chain, as dried apple bagasse, becoming a functional food prebiotic ingredient. Thus, the aim of the present study was to evaluate the composition of dried apple bagasse and its impact on colon health as a prebiotic.

For this purpose, proximate composition analysis was performed using standard AOAC methods. Additionally, gastrointestinal digestion of the dried bagasse was conducted according to the standardized INFOGEST protocol, followed by *in vitro* colonic digestion over 48h, using human faecal microbiota. Pectin digestion was considered as the positive control, while digestion without a carbon source served as the negative control. Following the colonic phase, short chain fatty acids (SCFAs) concentration was quantified by GC-FID and gut microbiota was analysed by qPCR.

The main components in the dried apple bagasse were carbohydrates (71.8 g/100g dry weight (dw)) and DF (18.3 g/100g dw), being 9.6 g/100g dw of insoluble fraction and 8.8 g/100g dw of soluble fraction. After 24h of colonic digestion, dried bagasse increased *Lactobacillus* spp. (1398.2%) compared to both controls as well as the *Bifidobacterium* (265.8%) population *vs* the negative control, being statistically equal to the positive control. However, at 48h, the relative abundance of *Bifidobacterium* levels in colonic digested dried apple bagasse was 8.6-fold higher than the positive control. This enhancement can be attributed to its balanced factions of soluble and insoluble DF of dried apple bagasse. Regarding the SCFA production profile, propionic acid levels did not differ between dried apple bagasse and the controls at 24 h of colonic digestions. However, by 48h, the propionic acid concentration was statistically equal at the colonic digested apple bagasse (7.9 mM) and the positive control (6.5 mM), and both were higher in comparison to the negative control. At the same time, the acetic acid concentration produced was higher by the dried apple bagasse (35.8 mM) than for the negative control (13.3 mM), and similar to the levels produced by the positive control (31 mM). Moreover, butyric acid concentration at 48h was significantly higher for the colonic digested apple bagasse (10 mM) than for the controls. These increases in SCFA concentrations at 48h could be attributed to the proliferation of probiotic gut bacteria promoted by dried apple bagasse.

These findings indicate that apple bagasse promoted the selective growth of probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* stimulating their metabolic activity, thus, metabolising DF into key SCFA. Therefore, dried apple bagasse could be considered as a potential prebiotic and nutraceutical ingredient.

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(22669) - EVALUATING THE METABOLIC EFFECTS OF REPLACING RED MEAT WITH PULSES IN THE DIET: DE LEGUMINIBUS STUDY

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Abstract

Although nutritional guidelines worldwide recommend increasing the consumption of pulses as a source of protein, the consumption of meat and meat products is still prevalent. While meat consumption could be associated with mortality and the development of Chronic Non-Communicable Diseases, such as cardiovascular diseases, much evidence indicates that the consumption of plant-based foods provides metabolic benefits. The physiological mechanisms underpinning such benefits and the implication of gut microbiota modulation are unclear.

This study aims to evaluate the effect of replacing fresh and processed red meat with pulses on cardiovascular risk markers in healthy subjects with a high consumption of red meat and meat products and a low level of physical activity.

The study consists in a two-month randomized controlled trial. Eighty-four subjects will be randomly assigned to one of three intervention groups: one group (28) will consume the habitual diet (HabD, control), the other groups will receive personalized diets replacing the habitual intake of proteins from red meat with pulses (pulses diet, PulD, 28) or with a combination of pulses-based food products (plant protein diet, PPD, 28). At the beginning of the intervention and after 4 and 8 weeks, participants will attend a visit for the collection of anthropometric variables, body composition, blood pressure, fasting blood samples, urine and feces as well as a series of questionnaires to evaluate diet composition, physical activity, appetite sensations, quality of life and sleep over the week prior the visit.

After Ethic committee approval of the protocol, selection of subjects started by using an online questionnaire. At the moment, 343 people filled out the selection questionnaire (110 M /233 F, 55,4% in the age range 18-30 years old, average BMI of 24.42±4.6 kg/m^2). The majority of participants (n=314) consume fresh red meat: 51.6% consume it once a week, 32.7% 2-3 times a week, and 8.5% do not consume it at all. Among consumers, 97.1% consume 100-200 g of meat per serving. Almost all respondents (n=289) consume processed red meat at least once a week: 55.7% consume at least one portion a week, 21.9% twice a week and, 15.7% do not consume it at all. Among consumers, 94.8% of the people consume 50/100 g of meat per serving.

Only 35.4% of the participants consume pulses at least 3 times a week. Based on the information collected only 26 (10 M/16 F, average BMI of 24.33±3.8 kg/m^2) are eligible to participate in the protocol.

The detailed study design and preliminary results related to the diet characteristics of the people who responded to the questionnaires and will be enrolled in the protocol as well as the preliminary results of the intervention will be presented.

References

Acknowledgments
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Keywords : chronic disease, habitual diet, pulses, proteins, red meat

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(22670) - IN-DEPTH ANALYSIS OF THE IN VITRO DIGESTIBILITY AND MICROBIAL ACTIVITY OF EDIBLE YEAST-BASED PROTEINS COMPARED TO MILK REFERENCE

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Abstract

Background

The global protein demand is in constant increase requiring sustainable and healthier alternative proteins for animal and human nutrition. Yeast-based proteins (YBP) represent a non-negligible environmental-friendly fermentation-based solutions with high nutritional quality and bioavailability. Although *in vitro* studies cannot reflect the full complexity of *in vivo* digestion, it is considered as useful alternatives to animal models assessing protein digestibility.

Methods

A 5h TIM-1 digestion model (n=3, TNO Gastro-Intestinal Model) was used to assess the digestibility profile and amino acid bio-accessibility of YBP (3 production batches) compared to milk-based reference (MBP). Every hour, luminal and dialysate samples were collected. Total nitrogen and free amino acid (FAA) were quantified. To assess the microbial impact, YBP digestate was subjected to 48h colon-on-a-plate batch fermentation (n=12), after which the microbial composition by shotgun sequencing and microbial activity by SCFAs, BCFAs (GC-MS) and untargeted metabolomics (LA-REIMS) was analyzed.

Results

YBP were as good as the MBP in terms of digestibility and small intestinal absorption reached up to 60% total bio-accessible protein after 5h. Microbial activity through SCFA quantification was significantly increased for both YBP and MBP compared to blank. Interestingly, YBP showed no significant difference in BCFAs compared to the blank, in contradiction to MBP suggesting less proteolytic microbial activity. Besides, YBP showed a different metabolite profile compared to blank and MBP.

Conclusions

Altogether, our results suggest that YBP could be a nutritionally relevant animal protein alternative. Finally, human trials are warranted to confirm YBP as a relevant protein alternative for sustainable human nutrition.

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Acknowledgments

We would like to acknowledge Pauline Spolaore and Rudy Menin (BioSpringer), Eric Oriol (Procelys), François Machuron (Lesaffre International) and ProDigest for their helpful contributions.

Keywords : Yeast-based protein, Digestibility, Microbiota, in vitro, bio-accessibility

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(22682) - PROTEIN BIO-ACCESSIBILITY OF MYCOPROTEIN ELUCIDATED USING IN VITRO GASTROINTESTINAL DIGESTION

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Abstract

Background: A fungal biomass consisting of proteins, fiber, carbohydrates and lipids was used as an alternative protein ingredient in meat analogues. Digestion of this ingredient and the final products could be affected by the presence of anti-nutritional factors or cell wall structures, which may result in a lower protein availability. To test this, the fungal ingredient, meat alternatives and animal-based counterparts were digested in-vitro. During digestion, some fibers and non-digestible carbohydrates are not absorbed in the small intestine but will enter the colon intact. In the colon, these compounds might be utilized as a substrate by the microbiota. For this reason, both the meat analogues and their animal-derived counterparts were subjected to gut microbiota fermentation after digestion.

Methods: The fungal ingredient, meat analogues and animal-derived counterparts were digested according to the INFOGEST protocol (1). After digestion, the absorbable and non-absorbable protein fractions were separated by methanol precipitation (2). The amino-acid compositions were determined and the total ileal digestibility (TID) of the absorbable protein fraction was calculated (3). The digested meat analogues were fermented by SHIME stabilized human gut microbiota, derived from the proximal (PC) and distal (DC) colon. The pH, pressure and production of short-chain fatty acid (scFA) were followed in time.

Results: The fungal ingredient was shown to have a TID of 80%, whereas the TID of the meat analogues ranged from 76-97%. During fermentation, differences in scFA production by the PC and DC microbiota were found. For the PC, both meat analogues and their animal-derived counterparts showed similar scFA production, whereas fermentation by DC microbiota resulted in a higher scFA production for some meat analogues.

Discussion: The digestion resulted in a TID of 80%, which is high for an alternative protein. Colonic fermentation showed no major differences between meat analogues and their animal-derived counterparts.

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Acknowledgments

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Keywords : Mycoprotein, gut microbiota, fermentation, INFOGEST digestion

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(22702) - EVALUATION OF THE PREBIOTIC AND ANTIOXIDANT ACTIVITIES OF FORMULATED CHICKPEA BISCUITS AFTER GASTROINTESTINAL DIGESTION AND FECAL FERMENTATION

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Abstract

Biscuit products undergo continuous reformulation to meet consumer demands, substituting traditional cereal flours with healthier alternatives[1]. Legume flours are becoming popular because they contain phenolic compounds, oligosaccharides resistant to digestion and fiber, contributing to their beneficial effects in gut health.[2] In the present research, four functional gluten-free biscuits enriched with different chickpea flours (30%) were designed and compared with a traditional shortbread biscuit. Their antioxidant and prebiotic properties were investigated after gastrointestinal digestion and fecal fermentation. The anaerobic fermentation was carried out using a pool of fecal slurries from 10 healthy adults with which the non-bioaccessible fractions of digested biscuits were incubated (anaerobiosis, 37°C, 20h) to subsequently study the evolution of the total phenolic content (TPC), antioxidant activity (ABTS and FRAP) and the bacterial populations of the fecal microbiota, thus assessing their possible prebiotic action. *In vitro* gastrointestinal digestion and fecal fermentation greatly increased the antioxidant profile of biscuits compared with the undigested food. The fecal fermentation step seemed to release compounds with marked antioxidant properties. The effects were more prominent in those biscuits enriched with chickpea landraces flours compared to commercial chickpea flours. Metagenomics data revealed Firmicutes and Actinobacteriota as the most abundant phylum. After fermentation of digested biscuits, an increase in the phylum Actinobacteriota and its family Bifidobacteriaceae was observed; at genus level, *Blautia*, *Collinsella* and *Bifidobacterium* lead the relative abundances. Among *Bifidobacterium* species, *B. adolescentis* was the most abundant, especially in fermentations with control biscuits, followed by those from commercial chickpea flours. The inclusion of chickpea flours in the biscuit recipe improved the TPC and antioxidant profile after gastrointestinal digestion and fermentation, especially when flours derived from Spanish chickpea landraces were included. In summary, the use of chickpea flours in biscuit formulations favored the growth of bifidobacteria, which remarks the prebiotic potential of some of the tested chickpea seeds used as functional ingredients.

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Acknowledgments

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Keywords : chickpea, gastrointestinal digestion, fecal fermentation, prebiotic activity, antioxidant action

Topic 5: Impact of diet on gut microbiota | Poster

(22751) - EFFECT OF PHYTOCHEMICAL-RICH BEETROOT EXTRACTS IN THE MODULATION OF GUT MICROBIOTA IN VITRO

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Abstract

Polyphenols, which comprise a large group of plant secondary metabolites, exhibit a range of biological actions, including antioxidant and specific anti-bacterial activities. They consequently hold the potential to significantly modulate gut microbiota, shape metabolite profiles and thereby benefit gut health. The aim of this work was to evaluate the effect of phytochemical-rich beetroot extracts (PRBEX) on the modulation of gut microbiota activities and metabolite formation.

PRBEX was tested at concentrations of 1%, 2% and 4% in an *in vitro* batch fermentation model under anaerobic conditions. Incubations were performed at 39ºC for 24 h, using pig faecal slurry as inoculum and feed as substrate, as well as the inclusion of zinc oxide as antimicrobial reference.

Gas pressure was measured every two hours, and samples were taken for pH, polyphenol content (PP), antioxidant capacity (TEAC and FRAP), and microbiology analyses at 0, 12, and 24 h.

The results indicate a positive effect of PRBEX to modulate microbiota activities in a dose-response manner, increasing the amount of microorganisms by 4.2, 10.0 and 19.5%, at inclusion of 1, 2 and 4%, respectively, in comparison with the control. These increases, correlated with the rise in gas production, are suggesting that the inclusion of PRBEX has the potential to enhance the richness of gut microbiota and to stimulate the production of organic acids. Further, the polyphenol content increased dose-dependently during the fermentation, with PRBEX at 4% exhibiting a 46.6% increase compared to the control.

Current findings indicate the potential of PRBEX to be included as a supplement in functional food and feed solutions benefitting gut symbiosis and gut health.

Acknowledgments
This project is supported by Innovate UK

Keywords : beetroot, phytochemicals, phenolic compounds, gut fermentation, gut microbiome

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(22780) - STUDY OF THE IMPACT OF EDIBLE MUSHROOM BIOMASS OBTAINED FROM BY-PRODUCTS UPCYCLING ON THE HUMAN INTESTINAL MICROBIOTA

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Abstract

In recent years, there has been a rise in the consumption of mushrooms, leading to an increase in the production of related by-products. Given their nutritional profile, mushrooms have the potential to represent a novel source of dietary fiber, which has been linked to prebiotic benefits.

Through the utilization of a human feces' fermentation model, this study aimed to assess the possible prebiotic effect of by-product-derived mushroom biomass. To accomplish this, by-products from 2 types of mushrooms, namely *Pleurotus ostreatus* and *Pleurotus eryngii* (attained after different extraction procedures - M1 and M2), were nutritionally characterized, with a particular emphasis on the fiber, structural carbohydrates, and glucans content (components known to be used as a substrate by microbiota microorganisms and for their prebiotic potential). The results showed that *P. ostreatus* possessed a marginal advantage over *P. eryngii* in terms of fiber content (*P. ostreatus*: 36.39 – 41.01% DW; *P. eryngii*: 35.19 – 39.69 % DW) and higher levels of β-glucans (*P. ostreatus*: 50.92 - 52.29 % DW; *P. eryngii*: 41.96 - 43.64 % DW). When comparing the flours, it was found that the M2 extraction process produced flour with higher quantities of structural carbohydrates and fiber. Therefore, the 2 *P. ostreatus*' flours were selected and submitted to a simulated digestive process and tested in an *in vitro* human fecal fermentation model considering fluctuations in the microbiota (*Firmicutes*, *Bacteroidetes*, *Lactobacillus*, *Bifidobacterium*, and *Clostridium*) profile, and the formation of short-chain fatty acids (SCFAs) and other relevant organic acids. Overall, the impact of mushrooms flours on gut microbiota led to a Firmicutes to Bacteroidetes ratio consistently close to 1, which supports their prebiotic potential. The SCFA (acetate, butyrate, and propionate) production also revealed a positive modulation of the microbiota metabolism. There was an increase in all metabolites (except for lactic acid) compared to the controls, indicating the flour's prebiotic potential, even though the results of bacterial communities were not directly correlated with the metabolites produced.

It is essential to note that the process of valuing mushroom by-products of mushroom in a zero-waste approach permitted the production of added-value extracts and the use of the residual biomasses as a functional prebiotic ingredient that might be used in new functional foods.

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Acknowledgments

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The author, André Cima, would like to acknowledge FCT for the individual PhD grant (PRT/BD/154693/2023).

Keywords : Mushrooms by-products, Prebiotic, Dietary fiber, Microbiota, INFOGEST

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(22798) - EXTRA VIRGIN OLIVE OIL EFFECT ON HEALTH: LESSONS FROM A DIETARY INTERVENTION ON METABOLISM, INFLAMMATION AND MICROBIOTA

Correia, Marta (Portugal)[1]; Peixoto, Ana Gomes (Portugal)[2]; Moreia, Ines (Portugal)[1]; Jane El Maghariki, Jane (Portugal)[1]; Magalhães, Tânia (Portugal)[3]; Alves, Paulo (Portugal)[3]; Rosa, Nuno (Portugal)[2]; Mendes, Karina (Portugal)[2]; Correia, Maria José (Portugal)[2]; Gomes, Ana Maria (Portugal)[1]

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Abstract

Extra virgin olive oil (EVOO), and particularly high-polyphenol EVOO, is known to significantly decrease the risk of cardiovascular disease through the modulation of LDL-cholesterol levels and amelioration of oxidative-stress-related outcomes. Additionally, Portugal is known for its high-quality EVOO. In the scope of a previous project ("Bio-n2-value"), it was demonstrated that EVOOs from different locations revealed different lipid and polyphenolic compositions at concentrations that may support EVOO's biological functionality. This study aimed to evaluate the impact of the consumption of a Northern Portuguese EVOO on various important clinical parameters of healthy adult volunteers (19<age<55y). This was a quasi-experimental intervention study in which the impact of the intake of EVOO for a period of 100 days was assessed. Serum total cholesterol, HbA1c, HDL-c, LDL-c, CRP, anthropometric measures and hand grip strength, were assessed and food logs were analyzed, as well as stool and saliva samples for microbiota profiling. Serum HbA1c (5.1±0.32%; 4.9±0.24,p=0.000) and LDL-c (96.5±28.6mg/dL; 87.4±31.4mg/dL,p=0.017) significantly decreased following EVOO intake. Daily energy significantly increased, but no changes in other dietary parameters, or anthropometry, were observed. Adherence to the Mediterranean diet did not explain the differences found in individuals regarding serum lipid profile and HbA1c, reinforcing the role of EVOO's. Additionally, multiplex Immunoassay technology allowed the simultaneous quantification of pro- and anti-inflammatory proteins present in participants saliva samples: anti-inflammatory proteins (INFy, IL-4) and the pro-inflammatory TNFalpha were not changed from basal levels after taking EVOO. However, after EVOO consumption the pro-inflammatory IL-1βsignificantly decrease (ANOVA, p<0.05). Quantification of the Bacteroidota and Bacillota were performed in stool and saliva samples. Although no significant differences were achieved in the relative abundance of Bacillota in stool samples, Bacteroidota content increased upon EVOO consumption (p = 0.0001). In both cases, it is possible to observe an increase in the relative abundance of Bacteroidota (p = 0.0456) and Bacillota (p = 0.0018) after 100 days of EVOO consumption. As a conclusion, EVOO lowered the serum levels of LDL-c and HbA1c, providing clues on the effect of EVOO-putative health benefits. These results pave the way for a deeper exploration of EVOO as a functional food.

References

Acknowledgments

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Keywords : Extra virgin olive oil, Microbiota, inflammation, Diet, Metabolism

Topic 5: Impact of diet on gut microbiota | Poster

(22801) - FERMENTATION OF ARABINOGALACTAN-RICH FRACTIONS AND REGULATION OF METABOLITES PRODUCED BY GUT MICROBIOTA

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Abstract

The colon is a key component of the digestive system, hosting numerous bacteria, which play an important role in the prevention of metabolic disorders, thus affecting cardiovascular health (Rinninella et al. 2019). One of the primary activities of the gut microbiota is the fermentation of dietary fiber, which includes non-digestible polysaccharides. Short-chain fatty acids (SCFA), mostly acetate, propionate and butyrate are the main end products of this saccharolytic fermentation. The balance between cholesterol synthesis in the liver, given by acetate proportions, and inhibition, promoted by propionate, renders the acetate:propionate ratio an important biomarker for the regulation of cholesterol homeostasis (Wong et al. 2006). Another metabolite produced by the microbiota are the secondary bile salts, originated from the dihydroxylation of primary bile salts, which confers the former a higher hydrophobicity. This change in the bile salts enhances cholesterol bioaccessibility, contributing to hypercholesterolemia (Machado et al. 2023).

In this work, in vitro fermentations (48 h) by human gut bacteria were carried out in the absence (control) and presence of two different coffee arabinogalactan-rich fractions, one insoluble (Et75) and another soluble (EtSn) in 75 % aqueous ethanol solutions. The effect of these polysaccharide rich fractions on microbiota population, SCFA and secondary bile salt production was evaluated. Both fractions were able to stimulate the growth of bacteria considered probiotic, namely *Bifidobacterium*, *Lactobacillus* and lactic acid bacteria. Acetate:propionate ratio was lower in Et75 fraction (3.6) than EtSn (4.3), suggesting that the former may condition cholesterol synthesis in the liver in a higher extent. On the other hand, in the presence of EtSn fraction, the conversion of primary to secondary bile salt was lower, resulting in 0.16 mg/mL of secondary bile salts, when compared to Et75 (0.27 mg/mL). The lower amount of these compounds promoted by the presence of EtSn may confer this fraction a hypocholesterolemic potential.

These findings highlight the potential of coffee arabinogalactan-rich fractions to modulate gut microbiota and its metabolic activities, thereby influencing cholesterol homeostasis and potentially contributing to cardiovascular health. Further in vivo studies are needed to validate these effects.

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Acknowledgments

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Keywords : Fermentation, Short-chain fatty acids, Bile salts, Cholesterol, Coffee polysaccharides

Topic 5: Impact of diet on gut microbiota | Poster

(22808) - EXTRA VIRGIN OLIVE OIL NUTRITIONAL PROPERTIES AND MICROBIOTA MODULATION

Correia, Marta (Portugal)[1]; Moreira, Inês (Portugal)[1]; El Maghariki, Jane (Portugal)[1]; Barbosa, Joana C. (Portugal)[1]; Moreira, Ivone (Portugal)[1]; Machado, Manuela (Portugal)[1]; Salsinha, Ana Sofia (Portugal)[1]; Alves, Paulo (Portugal)[2]; Magalhães, Tânia (Portugal)[2]; Gomes, Ana M. (Portugal)[1]

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Abstract

Extra-virgin olive oil (EVOO), a major fat source of the Mediterranean dietary pattern, is composed of many health-promoting nutrients and bioactives including monounsaturated fatty acids and phenolic compounds, known for preventing non-communicable chronic diseases (NCDs). The health of the intestinal environment and associated gut microbiota play an important role in the development of NCDs and how EVOO may impact on such indicators is less studied. Hence, the objective of this study was to assess the impact of EVOO on gut microbiota modulation and production of microbially-derived metabolites.

The EVOO was first characterized by examining its lipid and phenolic profiles and its antioxidant capacity, after which its nutritional value was determined. Then, a quasi-experimental intervention study was designed to assess the impact of the intake of a Northern Portuguese polyphenol-rich EVOO (PR-EVOO) on various clinical parameters of healthy adult volunteers (19<age<55y) over a 100-day period. Faecal samples were duly collected at baseline and upon 100 d intervention and analysed in terms of microbial communities and metabolic dynamics (short chain fatty acids (SCFA)). Samples were collected and frozen at −20 °C until microbial DNA isolation. The overall microbiome composition of the faecal samples, to species level, were obtained by amplification of the 16S rRNA gene and sequencing of the PCR amplicons on the Illumina paired-end platform. The SCFA analysis involved direct injection of extracted SCFA (without prior derivatization) by gas chromatography coupled to flame ionization detection (GC-FID), with a run time of 13 min.

The studied PR-EVOO was composed mainly of monounsaturated fatty acids (74.48%, mainly Oleic acid C18:1 c9), and showed a total phenolic content of 224.9 µg GAE/g with an antioxidant capacity of 235.49 ± 4.42 µmol of Trolox equivalents/mL of PR-EVOO. Following DNA extraction and dilution, real-time PCR showed an increase in *Akkermansia muciniphila* species. The impact of the intervention increased the structural heterogeneity when comparing with the basal condition. Overall, an increase in *Bifidobacterium*, *Faecalibacterium*, *Bacteroides*, and *Lactobacillus* genera and a decrease of *Blautia* and *Enterococcus*, among others, was noted. Moreover, microbially produced SCFA, like acetic, butyric, and propionic acids, increased post PR-EVOO consumption. These results reveal an important role for PR-EVOO on positive outcomes for intestinal health.

Acknowledgments

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Keywords : extra virgin olive oil, polyphenol-rich, gut microbiota modulation, short chain fatty acids

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(22858) - IMPACT OF NOVEL CLEAN LABEL HAM FORMULATIONS ON THE HUMAN GUT MICROBIOTA

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Abstract

The influence of diet on the human gut microbiota has been a topic of much discussion, particularly concerning innovative products that are still considered novel. Dietary habits have a fundamental impact on the human gut microbiota, which explains the variations observed between individuals and over the course of a lifetime (Carlström et al., 2020). This study aimed to assess the impact of four clean-label ham formulations (without sodium nitrite and with natural nitrate sources combined with the addition of nitrated-reducing cultures) on the human gut microbiota of potential consumers after *in vitro* digestion and colonic fermentation following the INFOGEST protocol. The impact of each novel ham formulation on the gut microbiota communities was assessed by quantitative Next Generation Sequencing. The suggested clean-label approach yielded encouraging results, with no significant differences in microbial populations detected between the new formulations and conventional ham during colonic fermentation. Bacterial diversity and richness were similar in all samples, including the control traditional ham. *Bacillota* and *Pseudomonadota*, the most abundant phyla in the human gut, were the phyla found in higher relative abundance after colonic fermentation in all the samples.

These findings show that the use of natural nitrate sources combined with nitrate-reducing cultures can be a clean-label solution to replace added sodium nitrite in cooked ham, demonstrating the promising use of this technology in everyday foods while maintaining food safety and human health.

References

Carlström et al., 2020 (https://doi.org/10.1016/j.freeradbiomed.2020.10.025)

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Keywords : Gut microbiota, Infogest, Bacterial communities

Topic 5: Impact of diet on gut microbiota | Poster

(22942) - ASSESSMENT OF THE SELECTED BEETROOT VARIETIES ON THE SENSORY AND FUNCTIONAL PROPERTIES OF PROBIOTIC ICE CREAM ENRICHED WITH THE SACCHAROMYCES CEREVISIAE VAR. BOULARDII

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Abstract

Loss of firmness of vegetables during storage and limited ability to sell them is a common problem. Currently, its usage is impossible, and the farmer is unable to predict the shelf life of commercial quality vegetables. Therefore, food industry is looking for innovative products with high quality, safety and optimal nutritional properties to effectively use each part of such vegetables, fruits etc. On the other hand, consumers focus on functional foods and probiotic food constitutes a growing range of this group.

The aim of the work was to develop a production technology of new ice cream by using beetroots, which can constitute an attractive base for the final product and will constitute a matrix stabilizing the number and viability of microorganisms both at the production stage and after storage. Moreover, an important step was to assess the suitability of selected fruits as additives affecting the sensory quality of the developed products. As a result of the work, the composition of the beetroot base was optimized (2 variants of common beetroot) and a probiotic strain was selected - *Saccharomyces cerevisiae* var. *boulardii*. The utilization of other fruits -red currant (*Ribes rubrum*), black currant (*R. nigrum*), plum-leaf chokeberry (*Aronia Prunifolia*), strawberry (*Fragaria ananasa*), common gooseberry (*Ribes grossularia*) – as ice cream flavor additives was also assessed. Conducted studies showed that probiotic ice cream variants based on off-the-shelf beetroot with *S. cerevisiae* var. *boulardii* met the criterion of high sensory quality. Moreover, from a microbiological point of view, most of the samples did not detect the presence of saprophytic food microorganisms. The share of probiotic microorganisms *S. boulardii* was on average 103–104 CFU/g.

Studies confirmed that off-the-shelf beetroot is adequate component of frozen vegetable products together with proposed probiotics, which can contribute to improving the profitability of beetroot production and increasing the competitiveness of farms.

Acknowledgments

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Topic 5: Impact of diet on gut microbiota | Poster

(23199) - INTESTINAL FATE OF CEREAL PROTEINS AND FIBERS DURING IN VITRO DIGESTION AND GUT FERMENTATION

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Abstract

The green transition involves a higher intake of proteins from plants. However, the plant proteins might suffer from lower nutritional quality than proteins of animal origin, such as less bioavailable due to lower digestibility. The undigested plant proteins will instead reach the large intestine and provide a higher protein supply to the gut microbiota, with consequences we know little about. One concern is that higher degree of protein fermentation might result in more harmful metabolites in the gut. In the project GutFeedingNow[1] we aim to explore these knowledge gaps by investigating the intestinal fate of proteins of different plant sources. In this study, we have investigated digestibility of different processed plant ingredients derived from cereals (oat, wheat) and pulses (faba bean, pea) using the INFOGEST digestion model. A variable degree of digestibility was observed, with the highest digestibility of proteins derived from protein isolates compared to protein concentrates. The two protein ingredients with highest digestibility (wheat gluten) and lowest (oat protein) were further selected for in vitro gut fermentation with human feces as inoculum. A simplified INFOGEST protocol was used to prepare the representative undigestible proteins for gut fermentation, which resulted in comparable amounts of proteins resistant to digestion as the INFOGEST model. The fermentability of the cereal derived proteins were followed for 24 hours and compared to cereal derived fibers (arabinoxylan, beta-glucan). Interestingly, both cereal proteins were fermented earlier than the cereal fibers, confirmed by both formation of short chain fatty acids (SCFAs) and gas production. Measurement of the temporal changes on the gut microbiota are in preparation, as well as more characterization of protein and fiber turnover.

References

1. GutFeedingNow: https://nofima.no

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Keywords : plant protein, digestibility, fermentation, gut microbiota

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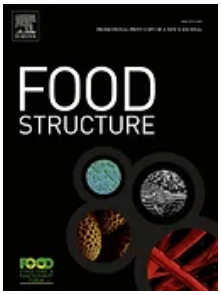

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