9TH INTERNATIONAL CONFERENCE ON

Food DIGESTION

ICFD Gdansk 2026

Gdańsk | Poland

2026

19 - 21 May

BOOK OF ABSTRACTS

GDAŃSK UNIVERSITY OF TECHNOLOGY

INFOGEST

Cellbox Labs

InnoGI technologies

Synutra 优博 瑞可嘉®

Shengyuan Nutrition Food Co., Ltd.

Youbo Ricoga

Lipolytech®

WELCOME ADDRESS

Dear Colleagues and Friends,

On behalf of the Organising and Scientific Committees, it is our pleasure to welcome you to Gdańsk, Poland, for the 9th International Conference on Food Digestion (ICFD2026).

This conference is held under the auspices of INFOGEST, a well-established international network founded in 2011, which today brings together a dynamic community of over 700 researchers from academia and industry, representing more than 200 institutions across nearly 60 countries. Through its collaborative efforts, INFOGEST continues to advance harmonisation of research methodologies and promote innovation in the study of food digestion and its implications for human health.

As a leading forum at the intersection of Food, Nutrition, and Health, ICFD provides an opportunity for scientists and industry professionals to share recent developments, exchange ideas, and strengthen interdisciplinary collaborations. This year's programme will gather participants from around the world to explore key areas of research, including:

- Oral Processing and Digestion
- Food Digestion and its Influence on the Bioaccessibility and Bioavailability of Nutrients and Bioactives
- Advances in Digestion and Absorption Models
- Physicochemical and Imaging Techniques for Characterising Food Digestion
- The Role of Gut Microbiota in Digestion

We are delighted to host this edition of the conference at the Gdańsk University of Technology, an institution with a proud academic heritage and a strong commitment to scientific excellence. Its proximity to the historic centre of Gdańsk offers an inspiring setting for both intellectual exchange and cultural discovery.

We invite you to experience the unique atmosphere of Gdańsk – a city where maritime heritage, Hanseatic architecture, and centuries of history blend with modern vibrancy. From its picturesque streets and waterfront to its rich culinary traditions and renowned hospitality, Gdańsk provides a memorable backdrop for this gathering.

We thank you for joining us and hope that your time at ICFD2026 will be both scientifically rewarding and personally enriching.

With our very best wishes,

Prof. Adam Macierzanka
Chair of the Organizing Committee,
Gdańsk University of Technology

Dr Ilona Kłosowska-Chomiczewska
Co-chair of the Organizing Committee,
Gdańsk University of Technology

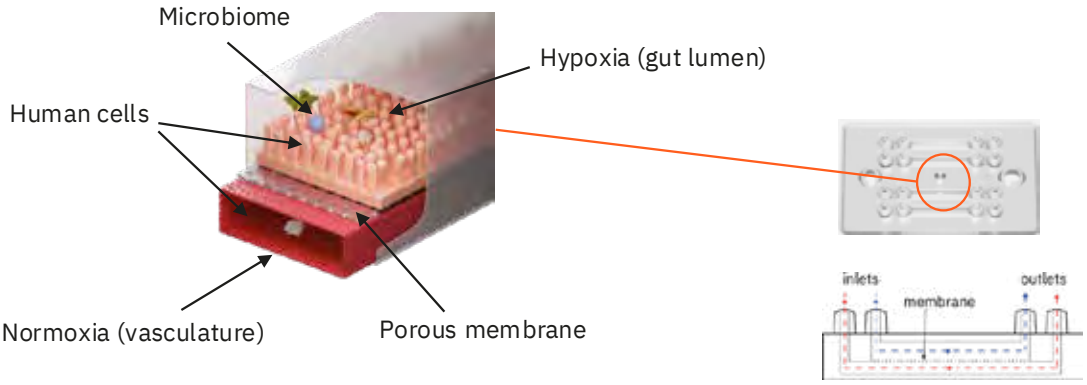
Cellbox Labs

Industrial organ on a chip technology

Cellbox Labs automated all-in-one organ on chip system enables the development of physiologically relevant *in vitro* models for real-time host–microbiome interaction studies, offering flexible experimental design while reducing hands-on time

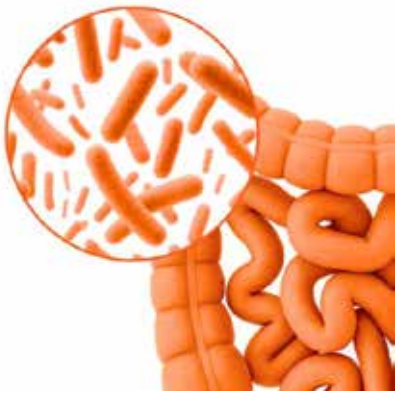
Features

- Co-culture of microbiome with human gut epithelium
- Real-time monitoring of host-microbiome interactions
- Mucus layer and gut-blood barrier
- Compatible with previously obtained microbiome samples



Applications

- Gut barrier integrity and damage assessment
- Molecular transport from gut lumen to blood
- Multi-omics and metabolite analysis
- Probiotic and prebiotic testing
- Disease modelling
- Personalized models using patient specific and iPSC-derived cells with patient-derived microbiome



ICF Gdansk 2026

InnoGI technologies

Leading the way in sustainable oral drug and nutritional R&D by *advancing GI modeling*

InnoGI Technologies is a *renowned contract research organization* (CRO) to service pharmaceutical and food & nutrition companies. We are highly specialized in gastrointestinal (GI) modeling. Our advanced *in vitro* and *in silico* approach (The TIM® Platform) *simulates the entire GI tract*, offering deep, data-driven insights into digestion, bioavailability/ADME, and microbiome interactions.

"*Confident decision making* in oral drug development and nutritional R&D"

Our TIM® Platform includes sophisticated models to *mimick the GI* tract and beyond:

1. TIM *Upper GI*
   Drug release, Dissolution & Digestion
   - *In vitro* stomach and small intestinal models
2. TIM *Colon*
   Microbiome Composition & Activity
   - *In vitro* large intestinal model
3. TIM *Cell*
   Apparent Permeability
   - *In vitro* intestinal epithelial absorption model using TIM® fluids
4. TIM *PK*
   Absorption, Distribution, Metabolism & Excretion
   - *In silico* pharmacokinetics fed by TIM® data

30+ Years Technology Base	*250+* Scientific Publications	*500+* Products Tested	Endorsed by Big Pharma, & FDA

Nothing beats *our gut feeling*

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Lipolytech®

Always provides solution

IN VITRO DIGESTION

ENZYMES FOR

High-quality Products

Gastric Extracts (RGE)
Gastric Lipase (RGL)

High quality Services

Lipids Analysis
Enzymatic Assays
In Vitro Digestion

Lipolytech is an innovative biotech company specialized in enzymes for *in vitro* digestion models.

Lipolytech provides relevant digestive enzymes for simulating the gastric and intestinal phases of digestion, with a special focus on lipolytic enzymes and lipid digestion. We also offer services in the field of *in vitro* digestion using a two-step static model including a gastric phase and a duodenal phase. Our mission is to provide high quality products and premium support that address the needs and demands of today's scientists in the area of in vitro digestion.

LIPOLYTECH is a pioneer in gastric enzymes and gastric extracts (RGE) production. RGE contains the two main gastric enzymes, gastric lipase (GL) and pepsin, involved in the digestion of lipids and proteins, respectively. We offer several RGE preparations with different pepsin to lipase ratios which allows adjusting the respective levels of these enzymes depending on the condition to be simulated in vitro (i.e., infant, adult, elderly). A high purity gastric lipase (RGL) is also available for structure-function and biophysical studies.

LIPOLYTECH provides a two-step static model including all relevant gastric and pancreatic enzymes, as well as the INFOGEST static *in vitro* digestion model recommending the use of RGE as the source of gastric lipase.

LIPOLYTECH also provides lipase assays with the pHstat technique, which allows the quality control of enzyme lots used for *in vitro* digestion based on their activity, as well as quantitative analysis of main lipid classes and their lipolysis products by thin-layer chromatography (TLC) coupled to specific staining and densitometry or FID.

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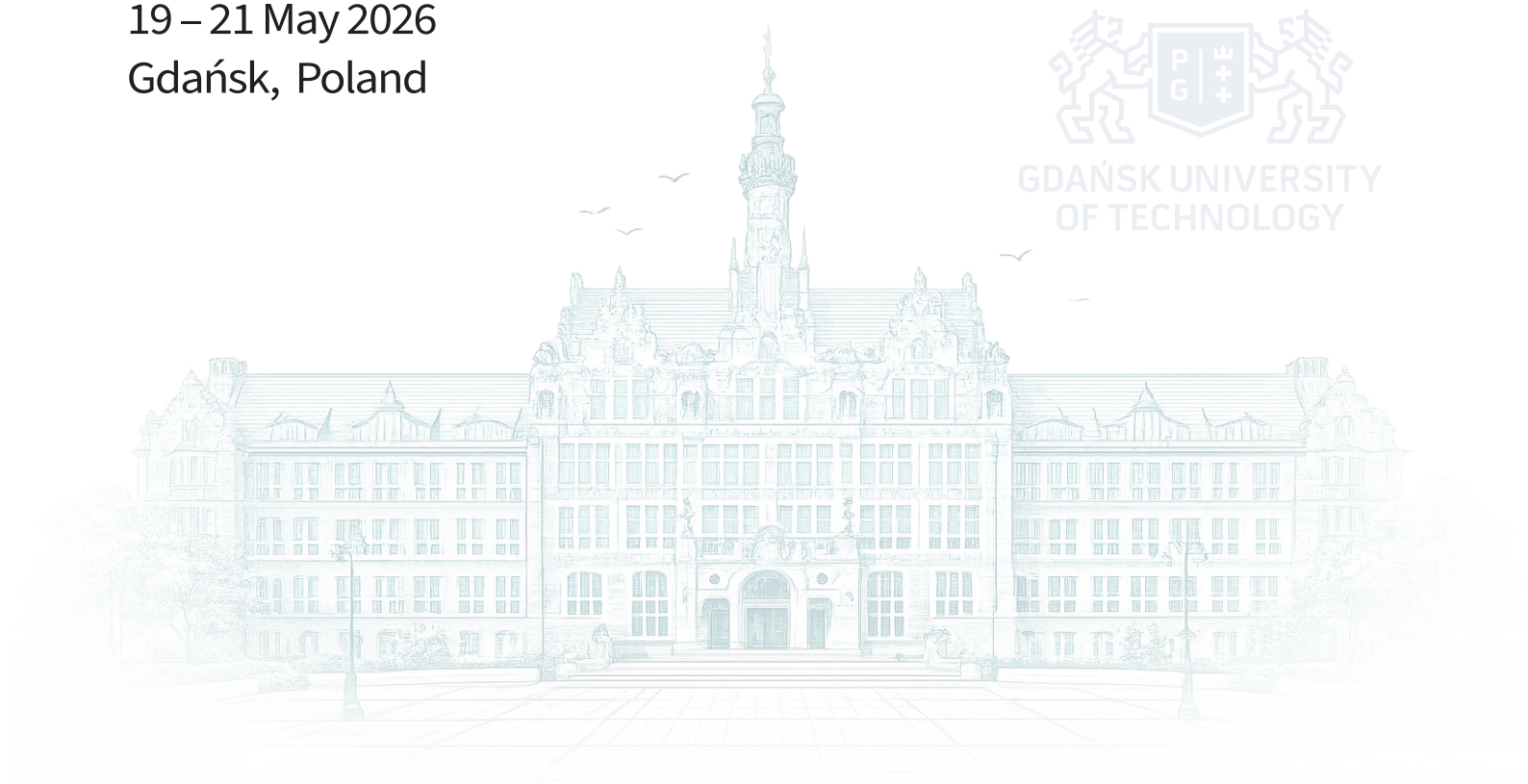
CONFERENCE PROGRAMME

19 – 21 May 2026
Gdańsk, Poland



FULL PROGRAMME

View the complete conference programme online.

Scan to view

WI-FI CONNECTION

network: **ICFD2026**

password: **Digestion2026!**

Scan to connect

DAY 1 | 19 MAY

07:30 – 08:30 **Participant registration**

08:30 – 09:00 **Official opening of the Conference**
(Aula of Gdańsk University of Technology)

09:00 – 10:00 ORAL COMMUNICATIONS
Topic: Oral Processing and Digestion
Chairs: Adam Macierzanka and Didier Dupont

- **09:00** **Ciarán Forde** (Wageningen University & Research, the Netherlands), *Understanding digestion in children: How swallowed food bolus properties evolve from ages 6 to 12 compared to adulthood. (ID 67)*
- **09:15** **Esther Staes** (KU Leuven, Belgium), *From bite to metabolite: how in vivo and in vitro oral processing of bean-based wraps with distinct microstructures affect starch digestion. (ID 25)*
- **09:30** **Vincent Mathieu** (INRAE, L'Institut Agro, France), *Investigating the role of oral physiology in inter-individual variability of particle texture perception. (ID 150)*
- **09:45** **Dan Liu** (Wageningen University & Research, the Netherlands), *Does age matter? Oral processing of plant-based foods shows limited differences between young and old adults. (ID 103)*

10:00 – 10:10 **Conference group photo** (in front of the Main Building)

10:10 – 10:50 **Coffee break + Poster session 1** (Main Building, Fahrenheit Courtyard)

10:50 – 12:15 ORAL COMMUNICATIONS
Topic: Oral Processing and Digestion
Chairs: Ciarán Forde and Markus Stieger

- **10:50 – 11:30** PLENARY LECTURE **Miriam Clegg** (University College Cork, Ireland), *Why Mouth Matters: How Oral Processing Shapes Intake Across the Lifespan.*
- **11:30** **Martine Morzel** (INRAE, L'Institut Agro, France), *Saliva modulates the impact of tannins on the mucus layer of intestinal cells. (ID 62)*
- **11:45** **Zhen Liu** (Wageningen University & Research, the Netherlands), *Do inter-individual differences in eating rate influence faecal particle size, gut microbiome composition and functionality in humans? (ID 225)*
- **12:00** **Susana Delgado** (IPLA-CSIC, Spain), *Peptide Profile and Allergenicity Assessment of Simulated Infant Formula Digests. (ID 78)*

12:15 – 13:00 ORAL COMMUNICATIONS
Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives
Chairs: Ciarán Forde and Markus Stieger

- **12:15** **Natalia Perez Moral** (Quadram Institute Bioscience, UK), *Starch bioaccessibility, glycaemia and gut hormone responses: Insights from a human naso-enteric intubation study of chickpeas. (ID 233)*
- **12:30** **Daniela Freitas** (Teagasc Food Research Centre, Ireland), *Perfect matchmaking: food pairing to target gastric starch digestion and modulate postprandial glycaemia in healthy adults under free-living conditions. (ID 290)*
- **12:45** **Camille Dugardin** (Université de Lille, France), *Intestinal sensing of dietary proteins and its impact on glucose absorption. (ID 61)*

13:00 – 14:30 **Lunch break + Poster session 1** (Main Building, Fahrenheit Courtyard)

DAY 1 (continued)

19 MAY

14:30 – 16:40

ORAL COMMUNICATIONS

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

Chairs: Tara Grauwet and Leslie Couedelo

14:30 **Daniëlle W.R. Wessels** (Wageningen University & Research, the Netherlands),
Structural Differentiation of Tuna and Algae Oil and Functional Impact of DHA Carriers on Bioaccessibility, Intestinal Uptake, and Cell Incorporation. (ID 269)

14:45 **Isidra Recio** (CSIC-UAM, Spain),
In silico screening method for discovering novel candidate peptides for CaSR activation in porcine duodenal digests. (ID 197)

15:00 **Marianna Tagliasco** (University of Udine, Italy),
Intact plant tissues in a whole meal reduce nutrient digestibility and colonic fermentation: evidence from an ileostomy study. (ID 198)

15:15 **Sara da Silva** (University of Porto, Portugal),
Anthocyanin-rich pigmented wheat: gastrointestinal digests reveal distinct immune-peptidomic and phenolic profiles with reduced gluten immunogenicity. (ID 144)

15:30 **Rozenn Le Foll** (INRAE, L'Institut Agro, France),
pH variation in the stomach and duodenum affects calcium bioaccessibility: an in vitro study using the 3-compartment DIDGI digestion simulator. (ID 55)

15:45 **Corentin Lannuzel** (Université Paris-Saclay, AgroParisTech, INRAE, France),
Ileal protein digestibility and quality of faba bean extrudate and honey chlorella in healthy humans. (ID 168)

16:00 – 16:40 **PLENARY LECTURE** **Emmanuelle Reboul** (C2VN, INRAE, Inserm, Aix-Marseille University, France),
New insights on factors influencing fat-soluble micronutrient bioavailability.

16:40 – 17:30 **Coffee break + Poster session 1** (Main Building, Fahrenheit Courtyard)

17:30 – 18:45

ORAL COMMUNICATIONS

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

Chairs: Pasquale Ferranti and Jens Brockmeyer

17:30 **Leila Zafra** (CIAL, Spain),
Impact of enzymatic hydrolysis on the in vitro digestibility and insulinotropic activity of plant protein sources. (ID 80)

17:45 **Marzia Giribaldi** (CNR - Institute of Sciences of Food Production, Italy),
Digestion-Resistant Immunity: New Evidence from Human Milk Digestomics. (ID 265)

18:00 **Valérie Micard** (Institut Agro-Montpellier & INRAE Montpellier, France),
Formulation, in vitro digestion, sensory acceptability and nutritional potential of climate-smart gluten-free pasta. (ID 251)

18:15 **Joana Costa** (University of Porto, Portugal),
Modulation of Mollusk Allergenicity by Food Processing and Gastrointestinal Digestion: A Multi-Species Assessment. (ID 204)

18:30 **Madeline Muziot** (PNCA, INRAE, AgroParisTech, Université Paris-Saclay, France),
Impact of a vegetarian diet on plant-protein digestibility and metabolism across lifespan. (ID 174)

19:00 – 21:00 **Welcome Reception at Gdańsk University of Technology**
(Main Building, Hevelius Courtyard)

DAY 2 | 20 MAY

08:00 – 09:00 **Participant registration**

09:00 – 10:00 **ORAL COMMUNICATIONS**

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

Chairs: Beatriz Miralles and Marta Martínez Sanz

09:00 **Isabel Ferreira** (University of Porto, Portugal),
Integrating Digestion Kinetics into Acrylamide Risk Assessment in Cookies via the Semi-Dynamic INFOGEST model. (ID 184)

09:15 **Tanguy Saviard** (INRAE, L'Institut Agro, France),
Goat milk improves intestinal barrier function and lactase expression in a quadricellular model of intestinal epithelium after dynamic in vitro digestion. (ID 22)

09:30 **Yubexi Correa** (CIAL, Spain),
Impact of seaweed-derived polysaccharides on protein digestion in in vivo models. (ID 10)

09:45 **Sébastien Marze** (INRAE, France),
Bioaccessibility and associated concepts: what to use, what to avoid, what's left to define? (ID 59)

10:00 – 10:40 **Coffee break + Poster session 2** (Main Building, Fahrenheit Courtyard)

10:40 – 12:50 **ORAL COMMUNICATIONS**

Topic: Advances in Digestion and Absorption Models

Chairs: Gail Bornhorst and Elena Maria Arranz Gutierrez

10:40 – 11:20 **PLENARY LECTURE** **Stéphanie Blanquet-Diot** (Université Clermont Auvergne, France),
New in vitro models of the small intestine to study microbiota: Bridging the gap between the bench and the human gut.

11:20 **Ana Blanco-Doval** (Agroscope, Switzerland),
Behind the scenes of INFOGEST Quant: insights into the step-by-step protocol for protein digestibility and DIAAS determination. (ID 121)

11:35 **Chloé Beltramo** (Université Clermont Auvergne, France),
Innovative human gastric and small intestinal model simulating differential gastric emptying of real-size food particles and ileal microbiota. (ID 134)

11:50 **Olivia Menard** (INRAE, L'Institut Agro, France),
Towards an international consensus infant in vitro digestion model for different ages: from prematurity to maturity. (ID 123)

12:05 **Pablo Gallego-Lobillo** (University of Valladolid, Spain; Aarhus University, Denmark),
A comparative assessment of rat small intestine extract and purified brush border membrane vesicles from pig to study polyphenol digestion. (ID 283)

12:20 **Gabriel Thomassen** (Danone Research & Innovation, the Netherlands),
A Novel in Vitro Real-Time Digestion, Absorption and Hepatic Utilization Caco-2/HepG2 model Links Infant Milk Lipid Structure to Metabolic Fate. (ID 335)

12:35 **Elva Gonzales-Nieto** (INRAE, L'Institut Agro, France),
Brush Border Membrane Vesicles as a Supplementary Step Process in the In Vitro Gastrointestinal Digestion Model INFOGEST: Consequences on the Proteolysis. (ID 215)

12:50 – 14:00 **Lunch break + Poster session 2** (Main Building, Fahrenheit Courtyard)

DAY 2 (continued)

20 MAY

14:00 – 15:30

ORAL COMMUNICATIONS

Topic: Advances in Digestion and Absorption Models

Chairs: André Brodkorb and Ilona Kłosowska-Chomiczewska

14:00 **Sponsored Presentation I** **Olaf Heckert** (InnoGI Technologies, the Netherlands),
Modeling Human Digestion In Vitro: TIM Upper GI for Nutrition Applications. (ID 77)

14:15 **Lotti Egger** (Agroscope, Switzerland),
ISO DIS 24223 | IDF 253 - In vitro digestion protocol for the determination of protein digestibility and in vitro digestible indispensable amino acid score. (ID 26)

14:30 **Shibo Ma** (The University of Melbourne, Australia),
An in-vitro gastrointestinal model for weaning infants and its performance on protein digestibility under liquid and solid dairy food matrices. (ID 94)

14:45 **Sondos Hejazi** (University of Naples "Federico II", Italy),
The missing link in digestion models: A high-resolution functional blueprint of the Caco-2 human brush border interface. (ID 249)

15:00 **Celien Derboven** (KU Leuven, Belgium),
Population-specific in vitro digestion: how altered digestion conditions relevant for people with obesity and after bariatric surgery impact proteolysis. (ID 53)

15:15 **Steven Le Feunteun** (INRAE, L'Institut Agro, France),
In silico prediction of postprandial gastric emptying half-times in humans, as measured by MRI: Influence of meal properties. (ID 83)

15:30 – 16:10 **Coffee break + Poster session 2** (Main Building, Fahrenheit Courtyard)

16:10 – 18:05

ORAL COMMUNICATIONS

Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

Chairs: Chairs: Paul Smeets and Luca Marciani

16:10 – 16:50 **PLENARY LECTURE** **Werner Weitschies** (University of Greifswald, Germany),
Food-Drug Interactions: Lessons learned from MRI Imaging.

16:50 **Jean-Baptiste Cavin** (Nestlé Institute of Health Sciences, Nestlé Research, Switzerland),
Rethinking Lactose Malabsorption: Digestive Response to a Low-Lactose, High Galacto-Oligosaccharides Milk Assessed by MRI in Healthy Chinese Adults. (ID 89)

17:05 **Maja Musse** (INRAE, France),
Probing digestion of bread and fruit at the scale of a food piece using MRI. (ID 64)

17:20 **Roseanne Minderhoud** (Wageningen University & Research, the Netherlands),
Measurements of redox balance and pH along the gut using a miniaturized ingestible sensor. (ID 98)

17:35 **Maria de las Nieves Siles Sanchez** (Aarhus University, Denmark),
Unravelling the speciation and structural dynamics of heme iron during digestion by x-ray absorption spectroscopy. (ID 69)

17:50 **Vincent Mathieu** (INRAE, L'Institut Agro, France),
When milk meets cocoa, coffee and tea: hindered coagulation and favored gastric emptying of milk proteins in a biomimetic in vitro digestion system (NERDT). (ID 151)

20:00 – 24:00 **Gala Dinner at Gdańsk Old Town**
(the Baltic Philharmonic, Address: Ołowianka 1, 80-751 Gdańsk)

DAY 3 | 21 MAY

09:00 – 10:55

ORAL COMMUNICATIONS

Topic: Role of Gut Microbiota in Digestion

Chairs: Isidra Recio and Myriam Grundy

09:00 **Edoardo Capuano** (Wageningen University & Research, the Netherlands),
Production of Colonic Microbial Metabolites from Different Protein Sources Using Human Ileal Digesta and a Dynamic Model of Colon Fermentation. (ID 142)

09:15 **Thomas Van Hecke** (Ghent University, Belgium),
Mycoprotein inclusion in hybrid meat products attenuates oxidative reactions during digestion and modulates colonic microbial activity in rats. (ID 314)

09:30 **Jonna Koper** (Lesaffre Institute of Science and Technology, France),
Targeting the gut-bone axis: In vitro effects of probiotic S. cerevisiae CNCM I-3856 on the microbiota of patients with osteoporosis and healthy controls. (ID 149)

09:45 **Brunette Katsandegwaza** (University of Liège, 4000 Liège, Belgium),
In-vitro dynamic gastrointestinal models for the prediction of complex gut microbiome-product interactions in microbial, food and pharmaceutical research. (ID 112)

10:00 **Sponsored Presentation I** **Arturs Ābols** (Cellbox Labs Ltd., Latvia),
Modeling microbiota – host interactions using anaerobic gut-on-chip system. (ID 340)

10:15 – 10:55 **PLENARY LECTURE** **Kieran Tuohy** (University of Leeds, United Kingdom),
Role of Gut Microbiota in Digestion and its potential to influence "Nutri-Kinetics".

10:55 – 11:30 **Coffee break** (Main Building, Fahrenheit Courtyard)

11:30 – 12:45

ORAL COMMUNICATIONS

Topic: Role of Gut Microbiota in Digestion

Chairs: Steven Le Feunteun and Lotti Egger

11:30 **Cost Action Presentation I** **Stéphanie Blanquet-Diot** (Université Clermont Auvergne, France),
Advancing in vitro colon model for understanding gut microbiota-host interactions: the INFOGUT cost action CA23110. (ID 176)

11:45 **Xiaona Tian** (Ghent University, Belgium),
Glycation of meat during processing and gastrointestinal digestion modulates digestibility and gut microbial composition, fermentation and immune responses. (ID 192)

12:00 **Giulia Di Filippo** (University of Udine, Italy),
Enzymatic hydrolysis extent of pea proteins shapes gut microbiota metabolism and short-chain fatty acid production after simulated digestion. (ID 312)

12:15 **Roseanne Minderhoud** (Wageningen University & Research, the Netherlands),
Protein fermentation biomarkers in plasma and urine vary between protein sources during a randomized fully controlled dietary intervention. (ID 244)

12:30 **Paulo Berni** (Federal University of the State of Rio de Janeiro, Brazil),
Human Microbial Biotransformation of Brazilian Berry Anthocyanins under Obese and Eutrophic Conditions: An INFOGEST-Based MS/MS Metabolomic Approach. (ID 156)

12:45 – 13:45 **Official summary & Closing, Awards presentation**

13:45 – 14:45 **Lunch break** (Main Building, Fahrenheit Courtyard)

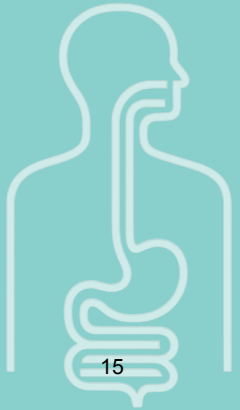
15:00 – 18:00 **Infogest Scientific Network – Working group (WG) meetings**

15:00 – 16:30
WG1 *(Conference Room)* **WG4** *(Main building, Room 300)* **WG5** *(Chemistry A building, Room 112)* **WG7** *(Chemistry C building, Room 1.4)*

16:30 – 18:00
WG2 *(Conference Room)* **WG3** *(Main building, Room 300)* **WG6** *(Chemistry A building, Room 112)* **WG8** *(Chemistry C building, Room 1.4)*

SESSION

1 Oral Processing and Digestion



Miriam Clegg

Senior Lecturer in Human Nutrition,
Programme Director BSc Nutritional Sciences (Hons)

School of Food and Nutritional Sciences, University College Cork, Ireland

Dr Miriam Clegg is a Senior Lecturer in Human Nutrition and programme lead for the BSc Nutritional Sciences at University College Cork. She is a Registered Nutritionist and member of the Council of Trustees with the UK Association for Nutrition and is on the Editorial Board for the British Journal of Nutrition. At present, her work focuses strongly on exploring nutrient intakes across the lifespan, and the impact that more sustainable diets and foods can have on nutrient intakes and health. She is particularly focused on investigating mechanisms for improving foods, diets and appetite control, and increasing protein intake in older adults. Miriam's current research is funded by funding obtained from the Irish Health Research Board, UK Research and Innovation (UKRI), Biotechnology and Biological Sciences Research Council (BBSRC) and Medical Research Council (MRC). Miriam became a Senior Fellow of the Higher Education Academy in 2020, and recently, as a member of the UK Quality Assurance Agency (QAA) Advisory Group, has developed the Subject Benchmark Statement for Higher Education in Agriculture, Rural Environmental Sciences, Animal Studies, Consumer Science, Forestry, Food, Horticulture and Human Nutrition.

ABSTRACT

Why Mouth Matters: How Oral Processing Shapes Intake Across the Lifespan

Oral processing is the first critical step in digestion, shaping not only how food is mechanically broken down, but also how it is perceived, enjoyed, and ultimately utilized by the body. Across the lifespan, oral function evolves in response to development, ageing, health status, and environmental influences, with important consequences for dietary intake, nutritional status, and overall health.

In early life, the development of oral motor and sensory skills establishes the foundations of eating behaviour, with sucking rates and bottle emptying in early life being associated with increased weight in childhood. In the transition from sucking to chewing, exposure to a variety of textures and flavours supports the acquisition of feeding skills and the formation of food preferences that can persist into later life. As oral processing capabilities mature through childhood, individual patterns of eating behaviour begin to emerge, including differences in eating rate, bite size, and chewing efficiency, which can influence energy intake and dietary quality.

During adulthood, oral processing remains sensitive to the structure and texture of foods. The extent and duration of oral exposure influence satiation, while the degree of food breakdown and saliva incorporation affect gastric emptying, nutrient bioaccessibility, and metabolic responses. In this way, oral processing acts as a key mediator linking food structure to digestion and appetite regulation.

In older age, declines in oral function become increasingly evident. Changes in dentition, reduced bite force, diminished saliva production, and alterations in sensory perception can impair bolus formation and swallowing safety. These changes often lead to modified food choices, reduced dietary variety, and lower energy intake, increasing the risk of malnutrition and associated health complications. However, recent evidence has shown that food manipulation can offer potential solutions to offset some of the challenges associated with diminished oral function in ageing.

Taken together, oral processing represents a dynamic and modifiable component of the eating process that underpins nutrition from infancy to older adulthood. Understanding how oral function changes across the lifespan provides important opportunities to support healthy eating through targeted interventions, including early-life feeding practices, food structure design, and strategies to maintain oral function and dietary intake in ageing populations.

ORAL PRESENTATIONS

Topic: Oral Processing and Digestion

FROM BITE TO METABOLITE: HOW IN VIVO AND IN VITRO ORAL PROCESSING OF BEAN-BASED WRAPS WITH DISTINCT MICROSTRUCTURES AFFECT STARCH DIGESTION

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Abstract

Despite environmental and nutritional benefits, pulses are only limitedly consumed in the Western world. To increase consumption, they can be used as flours in various products. In this context, flours containing intact cells can be produced by disintegrating hydrothermally treated pulses. In such cellular flours, nutrients are bioencapsulated by intact cell walls, forming a barrier for diffusion of digestion enzymes, hence slowing digestion. In contrast, traditional raw-milled flours show more rapid digestibility due to the release of intracellular nutrients. Only few studies have investigated the impact of flour microstructure on the digestion of real food products, predominantly focusing on gastric and small intestinal digestion. While increasing evidence highlights the importance of the oral phase for solid starch-rich foods, it remains understudied, especially for foods with innovative pulse flours.

Therefore, this work investigated the impact of the oral phase on starch digestion in novel bean-based wraps with different microstructures. Three wrap types were studied, consisting of 100% traditional bean flour, 80% traditional and 20% cellular bean flour, or 80% wheat and 20% cellular bean flour. An in vivo mastication study was performed with 38 participants. Chewing time, number of chews and bolus weight were recorded, and bolus microstructure and amylolysis level were assessed. As in vivo oral processing is not always feasible and large individual variations complicate standardization, we also tested the impact of different in vitro oral phase simulation approaches: (i) different salivary amylase activities to span the variability observed in vivo, and (ii) Bacillus sp. amylase as a cost-efficient alternative to human saliva.

In vivo mastication parameters varied largely across individuals, but no effect of wrap type was observed. However, wrap type clearly impacted bolus microstructure, with minimal impact of individual chewing behavior. Boli of wraps with cellular flour showed intact cells, proving they are retained upon wrap preparation and mastication. The presence of cells significantly decreased the level of oral amylolysis from 10 to 7%, but large individual differences were observed again. This variability probably prevented clear correlations between mastication parameters and starch digestion.

In vitro oral simulation resulted in similar microstructures as in vivo, with amylolysis levels decreased by intact cell presence, although differences were not significant. Amylase activity majorly impacted oral amylolysis, within the in vivo range. Similar levels of oral amylolysis were reached for human versus Bacillus amylase, indicating the latter could be an appropriate alternative.

This study shows how food microstructure can substantially affect oral digestion with large inter-individual variation. Moreover, our work highlights the importance of considering and developing relevant oral simulations for in vitro digestion studies.

Topic: Oral Processing and Digestion

SALIVA MODULATES THE IMPACT OF TANNINS ON THE MUCUS LAYER OF INTESTINAL CELLS

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Abstract

Dietary tannins may interact with host proteins such as salivary proteins (including mucins) or digestive enzymes, but less is known about their interaction with intestinal mucins. The present study aimed at testing the hypotheses that tannins reaching the intestine affect the mucin layer lining the epithelial cells, and that previous complexation of tannins with salivary proteins modify this impact. A polyphenol extract was obtained from “Dous Möen” cider apples: it contained 730mg/g of total polyphenols, of which 322 mg/g were condensed tannins. This extract, in absence or presence of saliva at a ratio of 0.08 g of polyphenols per g of protein, was digested following a slightly modified version of the static INFOGEST 2.0 protocol. Digests were applied for 2 hours to Caco-2/HT29-MTX co-cultured cells, where mucins are secreted. Cultures were fixed and double-stained for F-actin using rhodamine-phalloidin (as an indicator of the cytoskeleton), and for sialic acid using WGA-Alexa488 (as an indicator of mucins). The structure of the mucous layer was explored by confocal microscopy. Ten to fifteen 3-D images (consisting of around 80 stacked images on average) were acquired per experimental condition. The open-source software ilastik was used to segment images: pixels were manually annotated from 20 images as part of bright mucin staining, weak mucin staining or background and a classifier was trained. Images were then segmented with this classifier. Quantitative data were extracted: average thickness, volume and proportion of dense (brightly stained) mucus.

Confocal microscopy evidenced that cultures showed a clear topographic pattern with domes (pseudo villi) whose height was around 70 µm on average. Brightly-stained mucin clusters were visible in the extracellular apical space particularly on top of the previously mentioned domes, which suggests that the mucin-secreting HT29-MTX cells are concentrated in such regions. Overall, digested tannins (whether in presence of saliva or not) increased significantly the height of the pseudo villi observed on the cell culture (65.5 ± 12.8 vs 74.2 ± 12.1 µm). Tannins also reduced the volume and significantly the proportion of the dense mucus on cells compared to the blank (cells exposed to digestive medium without tannins). Interestingly, this effect was not retained when tannins had been mixed with saliva prior to digestion. This suggests that an in-mouth event, namely interaction of polyphenols with saliva, may have digestive consequences as far as in the intestine.

Keywords

saliva, polyphenols, tannins, intestinal cellular model, mucus, image analysis, machine learning

Acknowledgements

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Topic: Oral Processing and Digestion

UNDERSTANDING DIGESTION IN CHILDREN: HOW SWALLOWED FOOD BOLUS PROPERTIES EVOLVE FROM AGES 6 TO 12 COMPARED TO ADULTHOOD

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Abstract

Understanding the mechanisms of food digestion is of paramount importance to determine the effect foods have on human health. Along with nutritional needs, the performance of the oro-gastrointestinal tract evolves throughout the lifespan, and collecting physiological data becomes essential for conceptualizing in vitro digestion models adapted to each period of life.

The oral phase of digestion is primarily governed by the structural properties of the consumed food and by the oral physiology (mastication and salivation) of the population studied. Previous collection of oral physiological data in older people enabled the INFOGEST community to recently propose a consensus in vitro model adapted to this population, complementing existing models for infants and adults. However, physiological data are still needed to develop a comparable model for childhood.

Supported by physiological development of the oral cavity and esophagus, the childhood period is characterized by a period of dental transition, improved capacity to break down harder foods while simultaneously swallowing larger particles. How these developing capacities impact the physical properties of the swallowed bolus remain unclear. It is also unknown to which extent swallowed boluses differ between the children and adults, and how these differences contribute to differences in food-digestion.

The present study investigated the childhood period between 6 to 12 years old, which is marked by the transition between the primary and the permanent dentition leading to maturation of the masticatory functions. We assessed the evolution of food bolus properties at the point of swallowing for different model foods in children, and compared these with boluses from an adult population.

Participants (N=30) with healthy dentition are recruited across four age groups (6–8, 8–10, 10–12 and 25–35 years), and had their oral physiology (dentition, tongue strength, chewing efficiency) characterized. Natural oral processing behaviour for four products varying in textural properties (carrot, rice, cheese, biscuit) was assessed from video analysis and food boluses were collected at the point of swallowing and characterized for particles distribution, saliva uptake and mechanical firmness.

Preliminary data comparison (30 children and 30 adults) shows that tongue strength and chewing efficiency increases between the age of 6-8, 8-10, 10-12 and 25-35 years old. Chewing rate (g/min) is shorter for softer foods (rice and cheese) when compared to harder ones (carrot and biscuit), and tend to increase with age, but only for harder foods. Bolus saliva uptake remained stable across ages, whereas bolus hardness depended on both age and food type.

These data, together with on-going particle size analysis will provide relevant physiological information to characterize in vitro modeling of food oral processing and digestion during childhood.

Keywords

food oral processing, children, bolus properties, dentition

Topic: Oral Processing and Digestion

PEPTIDE PROFILE AND ALLERGENICITY ASSESSMENT OF SIMULATED INFANT FORMULA DIGESTS

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Abstract

Cow's milk protein allergy (CMPA) is one of the most common food allergies in infants, particularly in non-IgE-mediated cases where the specific allergens are not identified. Extensively hydrolysed formulae (eHFs) are commonly used to minimise allergenic exposure. This study uses an integrated INFOGEST-based in vitro digestion, peptidomics, and computational modelling approach to assess peptide release and allergenic potential in infant formulas.

The next formulae were subjected to simulated infant gastrointestinal digestion following an INFOGEST protocol adapted to infant physiological conditions: a control standard formula (CSF), an eHF and a whey protein-supplemented hydrolysed formula (seHF). The peptides generated during the intestinal phase were analysed using high-resolution UHPLC–MS/MS and identified via peptidomic workflows. Comparative bioinformatic analyses revealed 32 peptides that were uniquely present in the seHF. These peptides were then analysed further using machine-learning models (Random Forest and XGBoost), which were trained using physicochemical descriptors. This analysis identified hydrophobicity, net charge and isoelectric point as the most informative variables for peptide discrimination.

Allergenicity prediction was performed using a consensus of AllerTOP, AlgPred and AllergenOnline results. This was followed by molecular stratification based on weight (≥1.2 kDa) to prioritise peptides retaining potential IgE-binding capacity. This process identified five high-risk peptides, all of which were derived from β-lactoglobulin. Sequence mapping revealed their localisation within digestion-resistant regions that overlap with previously reported allergenic epitopes. We further explored structural relevance through peptide–protein molecular docking against immune receptors implicated in allergic sensitisation. Docking analyses revealed moderate to strong binding affinities, supported by stabilising electrostatic and hydrogen-bonding interactions.

Allergenicity prediction using in vitro and in silico approaches allows early identification of potential allergenic peptides derived from milk reducing the use of animal models, optimizing the biological validation with further in vivo experimentation.

Keywords

Cow's milk protein allergy, infant formulae, in vitro digestion, peptidomics, allergenicity prediction

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Topic: Oral Processing and Digestion

DOES AGE MATTER? ORAL PROCESSING OF PLANT-BASED FOODS SHOWS LIMITED DIFFERENCES BETWEEN YOUNG AND OLD ADULTS

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Abstract

For old adults, maintaining muscle mass and function is challenging due to reduced appetite and potentially reduced protein digestibility. With the increasing popularity of plant-based diets, it is important to understand whether plant-based foods can meet the nutritional needs of old individuals. Aging may impact food oral processing behavior, which may limit oral structural breakdown and subsequent macronutrient digestibility in the gastrointestinal tract. This study aimed to compare the oral behavior, bolus properties and digestibility of black beans, sorghum and plant-based meat analogues patties between young and old adults. Young (n=14, 29±3 yrs, gait-speed=1.07±0.10 m/s) and old (n=14, 87±6 yrs, gait-speed=0.77±0.22 m/s classifying the old adults as frail) adults were asked to take three bites of each food, chew them naturally and expectorate the bolus before swallowing. Bite size, eating rate, chewing duration, number of chews per bite and chewing rate were annotated from video recordings. Bolus particle size distribution was determined using wet sieving and bolus saliva content was measured gravimetrically. Compared to young adults, old adults showed 25-44 % smaller bite sizes and 35-54 % slower eating rates depending on food type, with the largest difference for sorghum (p 0.001). Old adults tended to chew 14-35 % more and 30-56 % longer, while significant difference in number of chews and chewing duration were only observed for patties ($p \leq 0.05$). Chewing rate did not differ significantly between young and old adults across foods. The boli of old adults contained 41 % and 28 % less saliva than those of young adults for sorghum ($p = 0.02$) and patties ($p = 0.04$), respectively, whereas no significant differences were observed for black beans. The boli of old adults contained 47-99 % more large bolus particles (> 2 mm, p 0.01), with the largest difference for black beans. No significant difference between age groups was observed for small particles (2 mm). For all measurements, there were differences between the three foods (p 0.001). Preliminary in vitro digestion results showed that for boli generated after young adult chewing, the amount of released free amino groups per g protein was 34% lower under old compared to young adult digestion conditions. Digestion measurements of the boli produced after old adult chewing are ongoing. In conclusion, the degree to which aging affects the oral processing behavior of plant-based foods strongly depends on food type. Despite a threefold age difference (87 yrs vs. 29 yrs), only limited differences in oral behaviors and bolus properties were observed between young and old adults. Oral processing was mainly affected by food type in both young and old adults. Subsequent digestion studies are ongoing to investigate the combined effects of oral processing and adapted digestive conditions of old adults on the nutrient digestibility of plant-based foods.

Keywords

aging, oral behavior, plant-based foods, bolus properties

Acknowledgements

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Topic: Oral Processing and Digestion

INVESTIGATING THE ROLE OF ORAL PHYSIOLOGY IN INTER-INDIVIDUAL VARIABILITY OF PARTICLE TEXTURE PERCEPTION

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Abstract

Food texture perception is a multidimensional and dynamic process that depends on the interaction between food properties and individual oral physiology. In particulate foods, texture perception is strongly influenced by particle size, shape, rigidity, and volume fraction, as well as by the viscosity and composition of the continuous phase. However, particle perception shows substantial inter-individual variability, the underlying physiological factors of which remain poorly understood. This study aimed to identify the physiological mechanisms involved in the perception of particles in plant-based semisolid foods.

Smooth chickpea purées containing roasted chickpea particles with sizes ranging from 100 to 800 µm were developed for sensory evaluation with thirty-two healthy adults (59% women, 41% men; aged 21–61 years, mean age 44 years). Participants were divided into two groups based on their individual particle perception thresholds, determined using a series of Three-Alternative Forced-Choice tests. The mean best estimated threshold was 217 ± 140 µm for the high-sensitivity group (n = 16) and 766 ± 143 µm for the low-sensitivity group (n = 16).

Lingual tactile function was assessed using three complementary tests, each representing specific aspects of tactile dimensions: light touch sensitivity (Von Frey monofilaments, one-point pressure test), spatial perception (dynamic two-points discrimination test), and roughness sensitivity (using paper coupons with varying grit sizes). Salivary parameters were also measured, including unstimulated salivary flow rate, fresh saliva viscosity, and salivary film thickness. Active tongue performance was evaluated by measuring maximal tongue pressure during contraction with the Iowa Oral Performance Instrument, whereas passive tongue stiffness at rest was assessed using shear wave elastography (ultrasound imaging). The study protocol was approved by the Ethics Committee of Université Bourgogne Franche-Comté (CER UBFC-2025-02-03-010).

Particle perception thresholds were significantly correlated with spatial acuity, roughness sensitivity, and active tongue performance. Compared with the low-sensitivity group, individuals in the high-sensitivity group exhibited better spatial discrimination (1.4 ± 0.8 mm vs. 2.5 ± 1.6 mm), higher roughness sensitivity (4.1 ± 3.0 µm vs. 6.5 ± 2.9 µm), and greater active tongue performance (54.6 ± 9.1 kPa vs. 40.8 ± 8.5 kPa). No significant differences were observed for light-touch sensitivity, salivary flow, saliva viscosity, salivary film thickness, or tongue stiffness at rest.

These results confirm the existence of marked inter-individual variability in particle perception, which appears to be related to the physiology of the tongue. As particle perception may represent a barrier to the acceptance of plant-based semisolid foods, a better understanding of these sensory mechanisms is essential to guide product formulation and improve consumer acceptability.

Keywords

Plant-based food, Particles, Sensory analysis, Texture perception, Oral physiology

Acknowledgements

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Topic: Oral Processing and Digestion

DO INTER-INDIVIDUAL DIFFERENCES IN EATING RATE INFLUENCE FAECAL PARTICLE SIZE, GUT MICROBIOME COMPOSITION AND FUNCTIONALITY IN HUMANS?

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Abstract

Differences in eating rate could influence the substrate size and area available for gut microbiomes and could potentially impact microbiome metabolic activity and metabolite production.

The current study aimed to determine whether inter-individual differences in eating rate (ER) are associated with fecal particle size, gut microbiome composition, and functions in humans, and whether these associations are mediated by diet ER. Individual ER (g/min), oral processing behaviour, and bolus properties of participants (n=41, mean ± SD age: 27 ± 5, mean ± SD BMI: 23.9 ± 1.9) were assessed based on average consumption of three replicate fixed-portion meals (202 g rice-based porridge). Participants were grouped into Slower-eaters (mean ± SEM ER: 30. 5 ± 0.3 g/min) and Faster-eaters (mean ± SEM ER: 61.9 ± 1.3 g/min) based on their individual ER. All participants completed two two-week texture-based interventions that promoted either faster eating (Fast-diet) or slower eating (Slow-diet). Faecal samples were collected at baseline and after each week of the dietary intervention, and faecal particle sizes were determined by laser diffraction and image analysis. Gut transit time was measured using a test food containing a blue dye. Results showed that a slower eating rate, characterised by more extensive chewing and smaller bolus particle size, was associated with smaller faecal particle sizes and longer gut transit time. Accordingly, Slower-eaters exhibited significantly smaller (p0.05) average faecal particle size (mm2) compared to Faster-eaters, with more pronounced differences at baseline (21.5% smaller) and under the Slow-diet (26.2%) than the Fast-diet (14.4%). Slower-eaters had on average 10 hours longer ($F(1, 38)=10.45$; $p=0.003$) gut transit time than Faster-eaters across different periods, whereas this difference did not reach statistical significance during the Fast- ($\Delta=3.8$ hours, $p=0.38$) and Slow- ($\Delta=7.5$ hours, $p=0.09$) diets. These results suggest that eating rate and oral processing could influence the substrate size available for the gut microbiomes and the time that microbiomes interact with the substrate. Shotgun metagenomics and targeted SCFAs analysis were employed to test whether oral processing affects gut microbiome composition and functions. Faecal propionate was lower (24-41%) in the Slower-eaters compared to the Faster-eaters during both diets. A smaller median faecal particle size (D50) was associated with higher faecal total branched-chain fatty acids (BCFAs), lower propionate and acetate concentrations. These findings from faecal particle size, gut transit time, and SCFAs provide preliminary support for a link between oral processing behaviours and gut microbiome. Data on gut microbiome composition and metabolic functions from the intervention will be presented to further explore these relationships.

Keywords

Eating rate, oral processing behaviour, bolus particle size, faecal particle size, gut microbiomes, SCFAs

POSTER PRESENTATIONS

Topic: Oral Processing and Digestion

UNDERSTANDING THE CONTROLLED ORAL BREAK-DOWN OF MEAT AND THEIR VEGETARIAN ANALOGUES – AN IN-VITRO APPROACH

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Abstract

Over the past decade, many products have been introduced as plant-based substitutes for traditional meat. These products typically fall into two structural categories: isotropic systems, whose material properties are not direction-dependent (e.g., sausages), and anisotropic systems, which exhibit direction-dependent properties (e.g., chicken chunks or plant-protein extrudates). Despite advancements in structuring and formulation technologies, plant-based products are still often perceived as texturally different from real meat. Understanding these differences requires examining their step-by-step oral breakdown.

In this study, an artificial masticator capable of applying controlled mastication sequences (variation of force, shear, and saliva flow) was used to evaluate two product groups: chunks (chicken and vegetarian- Heüra™ chunks) and sausages (chicken and vegetarian-Herta Knacki™). To mimic real saliva, an in-house artificial saliva formulation was prepared consisting of mucin, α-amylase, and a buffer containing mineral salts. Various number of mastication cycles (5, 10, 15, 20, 25) and two saliva flow rates (1.36 ml/min and 2.72 ml/min) were tested. These parameters were determined after an initial in-vivo study with an internal panel.

Initial results showed that bolus firmness changed little under mechanical mastication without saliva. However, adding artificial saliva led to a gradual decrease in firmness with continued mastication. Particle-size distributions, with and without saliva, showed a progressive reduction in particle size with increasing mastication, resulting in a higher number of particles. However, chicken chunks exhibited more pronounced bolus agglomeration. Confocal microscopy revealed a gradual release of fat during mastication for chicken samples, whereas vegetarian analogues displayed readily available fat from the beginning. This controlled breakdown study provides insight into the structural deconstruction of plant-based products and highlights mechanisms that may explain differences in dynamic and overall texture perception.

Topic: Oral Processing and Digestion

DEVELOPMENT OF PREBIOTIC BREAKFAST CEREALS USING SUSTAINABLE INGREDIENTS: TECHNOLOGICAL, SENSORY, AND DIGESTIVE INSIGHTS

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Abstract

Reducing added sugars while enhancing nutritional functionality is a key challenge in the development of breakfast cereals. This study aimed to produce extruded chocolate cereals in which sugars were replaced by sustainable, prebiotic-rich ingredients, including carob pulp flour, yacon flours (Morado and Hualqui), and enzymatically synthesized fructo-oligosaccharides (FOS), to support digestive health.

Prebiotic ingredients were incorporated into cereal formulations to achieve 1.8–3 g FOS per 100 g of product. Extrusion parameters were optimized to maintain expansion, texture, and crunchiness. Carbohydrate composition was characterized by HPLC and LC-MS. Sensory evaluation involved 100 consumers using projective mapping and a 9-point hedonic scale. Gastrointestinal stability and potential prebiotic functionality are being assessed using the standardized INFOGEST 2.0 in vitro digestion protocol.

All prebiotic ingredients were successfully integrated without compromising product structure. Extruded cereals exhibited desirable crunchiness and aeration. Formulations with yacon flours achieved the highest overall liking (5.9–6.0), with Morado yacon cereals described as balanced in flavor and particularly crunchy, while carob-based cereals were softer with moderate crunch. Preliminary digestion studies indicate good gastrointestinal stability, supporting potential prebiotic activity.

Replacing added sugars with prebiotic ingredients in chocolate breakfast cereals is technologically feasible and well accepted by consumers. The use of carob and yacon flours adds bioactive polyphenols and enhances sustainability. These findings support the development of low-sugar, functional breakfast cereals with potential digestive health benefits, aligning with clean-label and sustainable food trends.

Keywords

Prebiotics, Extrusion processing, Yacon, Carob pulp, Breakfast cereals, Low-sugar functional foods

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Topic: Oral Processing and Digestion

MODULATION OF TRYPSIN ACTIVITY IN THE PRESENCE OF POLYPHENOLS AND/OR SALIVARY PROTEIN-POLYPHENOL COMPLEXES

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Abstract

Polyphenols are specialized metabolites produced by plants with a well-documented overall health effect. They are also described in the literature to interact with proteins with possible impacts on protein functionality. In the context of digestion, polyphenols can interact with host proteins in the mouth for example salivary proline rich proteins (PRPs), or in the digestive tract, for example digestive enzymes such as trypsin or pepsin. PRPs have long been considered as protective against the detrimental effect of tannins on digestive proteolysis by binding tannins before they can interact with digestive enzymes. In this study, we tested this hypothesis: we examined the proteolytic activity of trypsin by using whey protein isolate (WPI) as substrate in the presence of epigallocatechin gallate (EGCG) or EGCG and PRP.

Fresh saliva was collected from a donor at least one hour after any meal or dental hygiene procedure followed by a clarification step by centrifugation. Higher molecular weight proteins were removed using Vivaspin concentrators equipped with 30 kDa molecular weight cut-off membranes. SDS-PAGE electrophoretic profiles confirmed that the ultrafiltrate was depleted from high molecular weight proteins, and that bands most likely corresponding to PRPs were present. The ultrafiltrate was further concentrated by drying: the protein content of the extract was 7.2 mg/ml, approximately ten times more than in fresh saliva. Trypsin activity measurement was adapted from Borgonovi et al. (2025). The final concentration for proteins (WPI or WPI+salivary proteins), trypsin and EGCG were set at 20 g/L, 5 mg/L and 1 mM, respectively. After stopping trypsin activity after 10, 20 and 30 min, samples were centrifuged to precipitate higher molecular weight proteins. Released peptides were quantified by reading optical density at 280 nm in the supernatant.

In the presence of EGCG, we observed a ~24% inhibition of trypsin proteolytic activity. When salivary proteins were added, a ~78% reduction in trypsin activity was observed. Our preliminary results suggest that EGCG impacts negatively trypsin activity, and contrarily to what is usually admitted, that the presence of low molecular weight salivary proteins enhanced this inhibition. A thorough description of the formation of binary or ternary complexes between EGCG, WPI and PRPs would provide useful information on the mechanisms at the origin of trypsin inhibition.

Because polyphenol structure is very described in literature as an important factor influencing protein polyphenol interactions, further work is currently on-going on other polyphenols with different structural feature and higher degree of polymerization. Assays on pepsin activity will also be conducted. This work adds knowledge on the role of saliva in bioaccessibility of food proteins and/or polyphenols.

Keywords

Salivary proteins, polyphenols, trypsin activity,

Topic: Oral Processing and Digestion

NUTRALYS® FAVA S900M: A HIGHLY DIGESTIBLE PLANT PROTEIN WITH AN INTERMEDIATE SLOW DIGESTION PROFILE

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Abstract

Objectives: This work aimed at evaluating in vitro the nutritional quality of fava bean protein isolate through its Protein Digestibility-Corrected Amino Acid Score (PDCAAS) and to characterize its digestion pattern by measuring its apparent viscosity under simulated gastric conditions.

Methods: Protein digestibility was assessed using an internationally recognized in vitro digestion method that simulates, sequentially, gastric and intestinal phases (1). The protein was exposed to digestive enzymes, and the undigested fraction was quantified to determine overall digestibility. In a complementary study, gastric digestion was modeled using the NIZO SIMPHYD system to compare the respective digestion kinetics of NUTRALYS® Fava S900M, whey and casein, by monitoring viscosity changes over time and identifying “fast” (like whey) versus “slow” (like casein) digestion profiles.

Results: Fava bean protein isolate shows strong concentrations of arginine, lysine, glutamic acid, and branched-chain amino acids, providing a solid and well balanced essential amino acid profile, despite a relative deficiency in sulfur amino acids. The in vitro protein digestibility reached 98%, resulting in PDCAAS values of 69 (FAO/WHO 2007 reference, commonly used in Europe for adults) and 60 (FAO/WHO 1991 reference, used in the U.S. for all age groups except infants). During simulated gastric digestion, the protein exhibited a marked increase in viscosity during acidification, followed by a sharp decrease upon enzyme addition. A second viscosity peak appeared after approximately 2 hours, revealing a digestion pattern closer to that of casein than that of whey, consistent with an intermediate-slow digesting protein profile.

Conclusions: The fava bean protein isolate demonstrates a good nutritional quality supported by an excellent in vitro digestibility and a slow digestion profile. These characteristics make it particularly suitable for applications targeting satiety and weight management, sustained aminoacid delivery in sports nutrition, and metabolic health. Combining this fava bean protein isolate with other protein sources, such as cereals, can help improve the amino acid score. Overall, the findings support the use of high-quality plant-based proteins such as fava bean protein isolate in the development of nutritious protein rich foods and beverages.

(1) Based on Minekus et al. 2014 and optimised by ROQUETTE R&D department. A standardised static in vitro digestion method suitable for food – an international consensus. DOI:10.1039/C3FO60702J

Topic: Oral Processing and Digestion

PLANT-MARINE BLENDS: ASSESSING THE IMPACT OF FOOD PROCESSING ON IN VITRO PROTEIN DIGESTIBILITY.

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Abstract

1. Introduction

The increasing demand for sustainable, nutrient-dense plant-based foods has accelerated the development of novel protein ingredients derived from legumes and microalgae. However, these matrices often contain anti-nutritional factors (ANFs) such as phytic acid, phenolics, and a rigid cell wall, which can limit protein digestibility and mineral bioaccessibility. This study investigated how processing methods, specifically, blending and high-moisture extrusion (HME), modify the nutritional quality of fava bean protein isolate (Fpi) formulated with three microalgae species (Spirulina, Chlorella, Tetraselmis). Using the standardised INFOGEST static in vitro digestion model, we assessed in vitro protein digestibility (Amino Acid and Total Nitrogen analysis). In addition, total mineral, phytic acid, and phenolic analyses were conducted for nutritional and anti-nutritional composition of the food matrices.

2. Method overview

For analysis, in vitro digestion followed the INFOGEST standardised static digestion protocol with an analytical workflow (modified) that allows the assessment of in vitro protein digestibility. Unhydrolysed samples (supernatant) derived straight after methanol precipitation were used to determine the degree of protein hydrolysis (DH) using the o-Phthalaldehyde (OPA) assay (terminal primary amine groups). Hydrolysed samples, both pellets, and supernatants (after 6M HCl hydrolysis for the release of free amino acids) were used to determine in vitro protein digestibility using High-Performance Liquid Chromatography (HPLC), and Total nitrogen (TN) analysis. Mineral analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was performed using Microwave Digestion Method (HNO3 + H2O2) for elemental analysis. Crude polyphenol analysis was measured using the Folin-Ciocalteu (F-C) method, and Phytic acid (IP6) analysis was measured using the MEGAZYME Phytic Acid Assay Kit as prescribed.

3. Interpretation of results

Results showed that blending moderately improved protein digestibility by mixing ingredients and diluting ANFs. High-moisture extrusion significantly enhanced protein digestibility across all formulations, driven by increasing enzyme accessibility, protein denaturation, cell matrix disruption, and partial inactivation of ANFs. Furthermore, for total mineral analysis (Ca, Fe, Zn), no significant differences were observed between the blended and extruded samples, but significant differences were observed within the unprocessed ingredients group and between the formulations. Indicating that, mineral content responses were heterogeneous due to algae species differences. For total phenolics, significant differences were observed between the unprocessed, blended, and extruded matrices. Finally, for phytic acid content, significant differences were observed between the blended, extruded, and unprocessed groups, but not between the unprocessed and extruded samples.

Keywords

Digestibility; Indispensable amino acids; Protein; Anti-nutrients; Food processing; INFOGEST

Acknowledgements

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Topic: Oral Processing and Digestion

IMPACT OF DEGREE OF FOOD PROCESSING ON THE IN VITRO ORAL BOLUS TRANSFORMATION AND GLUCOSE RELEASE OF SWEET POTATO, CASSAVA, AND GRAPE POMACE EXTRUDATES

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Abstract

Introduction: The demand for functional snacks has led to the development of healthy snacks such as puffed extrudates. The degree of processing, defined here by the specific mechanical energy (SME) applied during extrusion, significantly modifies the physical matrix of extrudates, influencing the breakdown of foods in the early phases of digestion. Understanding the correlation between SME and bolus' structural integrity is important for formulating snacks with tailored digestion profiles. This study assessed the effects of varying SME levels of extrudates on glucose release and textural changes during simulated in vitro oral digestion.

Methods: Extrudates containing sweet potato, cassava, and grape pomace produced at four SME levels (600, 1000, 1500, and 2000 kJ/kg) were compared to a non-extruded raw flour control. Simulated salivary fluid was used to digest samples in vitro using the modified INFOGEST protocol. To simulate varying mastication degrees, two forms were examined: (1) powdered samples (5 g, 2 mm) representing complete mastication and (2) whole pieces (0.5 g, ~0.5 cm) representing incomplete mastication. After digestion, D-glucose was quantified via the Glucose Oxidase/Peroxidase assay. Whole-piece samples underwent texture analysis immediately post-digestion to assess changes in hardness (g) and adhesiveness (g). Experiments were performed in triplicate on a dry weight basis (ANOVA, p 0.05).

Results: SME levels generated varying trends in glucose release across the two physical forms. In powder, 600 SME exhibited maximum glucose release (0.84 ± 0.13 g/100 g), while 2000 SME showed a significant decline (0.52 ± 0.05 g/100 g), comparable to the raw control. This reduction is due to high-shear-induced amylose-lipid complexes limiting α-amylase activity. In contrast, the whole-piece samples, simulating incomplete chewing, showed peak glucose release in the 2000 SME sample (3.47 ± 0.21 g/100 g). This is attributed to the high expansion and porosity, which increased the surface area for salivary penetration. Textural data supported this, with hardness decreasing significantly from 94.26 ± 12.71 g (600 SME) to 9.76 ± 0.98 g (2000 SME) and adhesiveness increasing significantly from -19.29 ± 0.77 g to -6.69 ± 0.13 g.

Conclusion: These findings demonstrate that the degree of processing, or SME, is a significant factor in modulating oral digestion. While lower SME increases starch susceptibility in homogenized forms, higher SME promotes the rapid physical breakdown of intact structures. This may lead to unexpectedly high glucose release in consumers exhibiting incomplete mastication, providing a basis for tailoring glycemic indices through mechanical energy regulation.

Note: Future studies are underway to investigate the long-term effects of SME on digestibility in the gastric and small intestine phases.

Keywords

Degree of Processing; extrusion; oral digestion; glucose release; texture analysis; bolus formation

Topic: Oral Processing and Digestion

NON-TARGETED HS-GC-IMS VOLATILE FINGERPRINTING AND CHEMOMETRIC ANALYSIS FOR ENTOMOLOGICAL AUTHENTICATION OF HONEY

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Abstract

Food digestion research presupposes that the ingested food matrix is authentic, compositionally defined, and reproducible. For natural foods such as honey, variability arising from botanical origin and bee species can result in pronounced matrix differences, which may influence sensory perception and digestion-related outcomes if food identity is not rigorously established. Consequently, reliable food authentication is essential for ensuring the compositional integrity and traceability of honey. However, while most existing authenticity approaches focus on botanical or geographical discrimination, robust analytical strategies for entomological authentication of honey remain limited.

In this study, a rapid, non-targeted volatile fingerprinting strategy based on headspace gas chromatography–ion mobility spectrometry (HS-GC-IMS) combined with chemometric analysis of ion mobility sum spectra (IMSS) was developed to discriminate stingless bee honey from conventional Apis mellifera honey. Headspace extraction conditions were optimized using a Box–Behnken experimental design, identifying incubation temperature and salt concentration as the primary factors governing volatile signal intensity. The optimized method demonstrated good analytical performance, with repeatability and intermediate precision expressed as coefficients of variation below 3% and 10%, respectively.

Unsupervised multivariate analysis of normalized IMSS data resulted in consistent separation between stingless bee and honeybee honeys, indicating distinct and reproducible matrix-level volatile fingerprints. This discrimination was further confirmed using supervised classification models, including random forest and support vector machine algorithms, which achieved complete classification accuracy. Importantly, classification was achieved without reliance on individual volatile identification, highlighting the suitability of IMSS fingerprints as a peak-agnostic and high-throughput authentication strategy.

Beyond authentication, such volatile fingerprints provide a holistic description of aroma-related matrix properties that are relevant to sensory perception during food consumption and oral processing. By enabling non-targeted, matrix-level characterization without reliance on compound-specific identification, HS-GC-IMS coupled with IMSS-based chemometrics supports food digestion research by ensuring food identity and capturing perception-relevant properties. Overall, this approach represents a practical and robust tool for entomological honey authentication, quality control, and traceability.

Keywords

HS-GC-IMS, Volatile fingerprinting, Chemometrics, Ion mobility sum spectra (IMSS), Entomological authentication

Topic: Oral Processing and Digestion

IMPACT OF FOOD STRUCTURE AND LUBRICATION ON CHEWING BEHAVIOR AND SALIVARY UPTAKE DURING IN-VIVO MASTICATION

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Abstract

Food structure and lubrication influence oral processing by regulating chewing behavior and saliva-food interactions. This study examined the effect of food structure and lubrication on chewing time, number of chews, chewing rate and salivary uptake during chewing in healthy adults. It was hypothesized that foods with higher initial moisture content would provide intrinsic lubrication and require less chewing and saliva incorporation than foods with lower moisture content that relied primarily on salivary lubrication. An in vivo study was conducted with eighteen healthy subjects that chewed individual bites (8g/bite) of five foods or food combinations designed to vary in their structure and lubrication characteristics: cooked pasta, halloumi cheese, halloumi cheese-cooked pasta, halloumi cheese-cooked semolina porridge, and cooked pasta in cheese sauce. The five meals differed in physical structure and initial moisture content. Halloumi cheese-cooked semolina porridge and pasta with cheese sauce were formulated to have comparable moisture levels (~0.64-0.67 wet basis), allowing the effects of food matrix and lubrication mechanisms to be distinguished. Subjects completed three experimental sessions in which all five foods were consumed. Chewing time and number of chews were quantified from video recordings. Across all sessions, subjects expectorated boluses at their ready-to-swallow point, and salivary uptake was determined gravimetrically by oven drying (18h, 105C). Unstimulated saliva flow rates were measured by pooled saliva collection over a 5 min period. Chewing time and number of chews differed significantly between foods (p 0.001). Foods with lower moisture content and more solid structures, including cooked pasta, halloumi cheese, and halloumi cheese-cooked pasta required longer chewing times (range:15-45s; median 22s) and higher number of chews (range: 25-70 chews; median 33), while higher-moisture semi-solid foods showed shorter chewing durations (range; 6-25s; median: 16s) and required fewer chews (range: 8-36 chews; median: 21.5). In contrast, chewing rate did not significantly differ between foods (p=0.29) and had less variability between food types and individuals (range: 1.09-1.90 chews s-1). Food structure and moisture content influenced chewing duration and number of chews, but not the chewing rate. Salivary uptake also varied significantly between foods (p 0.001), ranging between 0.04-1.36 g/g dry matter (median: 0.55 g/g dry matter). The highest salivary uptake was observed for halloumi-cooked pasta and pasta in cheese sauce, suggesting that saliva incorporation depended on food matrix characteristics. The findings provide quantitative in vivo insights into how food structure and lubrication strategies modulate chewing behavior and salivary uptake, advancing understanding of bolus properties relevant to subsequent gastric digestion, and informing food design and development of in-vitro oral processing models.

Keywords

Food structure, Mastication, Lubrication, Bolus properties, In-vivo

Topic: Oral Processing and Digestion

COMPARATIVE ALLERGENIC PROFILING OF HEATED AND UNHEATED LUPIN FLOURS FOLLOWING IN VITRO SIMULATED DIGESTION

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Abstract

Eight flours obtained from different lupin cultivars of blue lupin seeds (L. angustifolius) both heated (90 °C,15 min) and unheated were subjected to simulated gastrointestinal digestion using the harmonized INFOGEST protocol (Brodkorb et al., 2019). Proteins extracted from heated and unheated flours before digestion, and digested samples at gastric and intestinal phases were separated by LDS-PAGE, and allergenicity was evaluated by immunoblotting with pooled sera from five lupin-allergic patients. Immunoreactivity was detected in all the flours and in four of eight gastric-phase digests, whereas no reactive bands were observed after the intestinal phase. Immunoreactive proteins were subsequently identified by HPLC-HRMS/MS.

The main allergen detected was Lup an 1 (β-conglutin), a vicilin-type seed storage protein composed of polypeptides ranging from 20 to 80 kDa linked by disulfide bonds. No immunoreactivity was observed at molecular weights corresponding to Lup a 1 (profilin) or Lup an 3 (non-specific lipid transfer protein). In conclusion, Lup an 1 appeared to be the predominant allergen, with allergenic activity abolished after complete in vitro digestion.

Brodkorb A, Egger L, Alminger M, Alvito P, Assunção R, Ballance S, Bohn T, Bourlieu-Lacanal C, Boutrou R, Carrière F, Clemente A, Corredig M, Dupont D, Dufour C, Edwards C, Golding M, Karakaya S, Kirkhus B, Le Feunteun S, Lesmes U, Macierzanka A, Mackie AR, Martins C, Marze S, McClements DJ, Ménard O, Minekus M, Portmann R, Santos CN, Souchon I, Singh RP, Vegarud GE, Wickham MSJ, Weitschies W and Recio I.
INFOGEST static in vitro simulation of gastrointestinal food digestion. Nature Protocols, volume 14, pages991–1014 (2019)

Keywords

lupin, food allergy, INFOGEST protocol, Lup an1

Topic: Oral Processing and Digestion

TIME-DEPENDENCY IN SIMULATING GASTRIC DIGESTION BY IN-SITU AND EX-SITU BULK RHEOLOGY WITH VARYING CHEMICAL CONDITIONS

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Abstract

Background and motivation: In aging societies, the demand for enteral nutrition is increasing, and even younger patients may require tube feeding. A typical indication for tube feeding is dysphagia (1) which brings challenges with it such as vomiting and aspiration (2). The motivation of this study is to develop and evaluate different rheological approaches for studying structural changes of tube feed during gastric digestion which serves as a basis for understanding and optimizing the structure of tube feed. The aims addressed in this study are:
1. Transfer physiological conditions during gastric digestion to bulk rheological characterization on a shear rheometer, e.g. in terms of shear, pH, temperature and time.
2. Compare two different ex-situ approaches with the Rheodialysis in-situ approach and discuss their applicability for gastric digestion simulation considering the above-mentioned factors.

Materials and methods: The nature of gastric digestion includes time-dependent gastric motility and gastric secrections (3), yielding a mechanical, enzymatic and acid break-down of the food (4). As a consequence, also the structural changes at the food-gastric-juice-interface vary over time (5). In this study, we transfer acid- and mechanically induced changes during gastric digestion to the food to shear rheological testing of a commercial tube feeding product containing fat, milk and soy proteins as well as banana juice. We compare two ex-situ approaches (multi-blade stirring on a cup geometry and titration-based digestion) with the Rheodialysis in-situ approach which allows for changing the chemical environment of the food sample during the rheological measurement and hence, describing the time behaviour continuously.

Results and discussion: Results from the three approaches are shown in the form of frequency and multiple time frequency sweeps for Rheodialysis measurements. We discuss the findings from the respective approaches regarding changes in sample structure by adding acid and digestive solutions, observable effects of dilution and energy input due to shear. In addition, we discuss the applicability of the investigated rheological approaches for the characterization of time-dependent structural break-down of food during gastric digestion.

References:
1 Culkin, A. and Gabe, S.M. et al. Nutritional support: Indications and techniques. Clinical Medicine, 2002.
2 Blumenstein, I. Shastri, Y.M. and Stein, J. Gastroenteric tube feeding: Techniques, problems and solutions. World Journal of Gastroenterology, 2014.
3 Bornhorst, G.M. Gastric mixing during food digestion: mechanisms and applications. The Annual Review of Food Science and Technology, 2017.
4 Bornhorst, G.M. and Singh, R.P. Gastric digestion in vivo and in vitro: How the structural aspects of food influence the digestion process. The Annual Review of Food Science and Technology, 2014.
5 Scheuble, N. Controlling satiety by tailored interfaces. PhD Thesis, ETH Zurich, 2016.

Keywords

Rheology, Dysphagia, Tube feed, Gastric digestion, Rheodialysis

Topic: Oral Processing and Digestion

SOUS-VIDE COOKING AS A POTENTIAL ALTERNATIVE TO BOILING FOR PULSES: ASSESSING ANTINUTRIENTS REDUCTION AND PROTEIN DIGESTIBILITY

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Abstract

The global shift toward plant-based diets necessitates processing methods that maximize both the nutritional and sensory quality of pulses. Sous-vide cooking, characterized by long-term, low-temperature heating in a vacuum-sealed system, is regarded as a possible strategy to avoid the loss of nutrients, including essential amino acids, vitamins, and minerals. Sous-vide cooking is expected to induce less protein denaturation and/or aggregation compared to boiling, thereby leading to higher protein digestibility. However, the digestibility of cooked pulses depends on both preservation of protein conformation and reduction of antinutrients. Antinutrients such as trypsin inhibitors, phytic acid, and lectin are known to impair protein digestibility by hindering the accessibility of digestive enzymes to dietary protein. The impact of Sous-vide processing on pulse protein digestibility and antinutrient reduction remains unexplored.

In this study, pea, lentil, and faba bean were sous-vide cooked in three different media (water, vinegar, and oil) at varying temperatures: 75/85 °C for pea and faba bean and 65/75 °C for lentil for 24 h. Their antinutrient levels, protein characteristics, and digestibility were compared with raw and boiled pulses (Pea and faba bean: soaked 24 h and boiled 40 min, lentil: boiled 25 min). Our results suggest that both sous-vide cooking and boiling reduced the trypsin inhibitor activity (TIA) and lectin significantly. Compared with boiling, Sous-vide cooking resulted in significantly lower phytic acid content in pea and faba bean. SDS-PAGE and LC-MS/MS results together suggested less protein denaturation in low-temperature sous-vide cooked pulses compared to boiling. Although the high temperature (85 °C for pea and faba bean, 75 °C for lentil) used for sous-vide cooking in this study was lower than the denaturation temperature of pulse proteins, the long-term heating still induced more intense protein denaturation and degradation than boiling. The lower degree of protein denaturation in low temperature (75 °C for pea and faba bean, 65 °C for lentil) sous-vide cooked pulses, combined with low levels of TIA and lectin, resulted in higher protein digestibility than high temperature sous-vide cooking and boiling in pea and lentil. Therefore, these data illustrate that low-temperature sous-vide cooking could be an alternative to conventional boiling, as it can reduce antinutrients effectively while minimizing excessive protein structural damage, thereby preserving optimal protein digestibility.

Keywords

sous-vide cooking, pulses, protein digestibility, antinutrients

Topic: Oral Processing and Digestion

SWALLOW READY PROPERTIES OF LAYERED FOOD SYSTEM

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Abstract

In vitro digestion models are highly standardised and limited to a narrow set of conditions. In general, a small amount of a food sample is comminuted, mixed with water and enzymes, and then processed in a simulated gastric and/or intestinal environment to yield digesta products of interest (e.g. glucose, amino acids). The oral processing step is commonly highly simplified. However, the outcome of oral processing (the swallowed bolus) affects the way subsequent digestion takes place (Kim et al. 2022). In vivo oral processing is highly variable between people, and the size, structure and composition of a swallowable bolus varies over a large range. Bolus properties of single foods are often determined and related to digestion outcomes. However, extrapolating these results to a meal is complex as foods with multiple components are not processed simply in an additive manner. To explore this relationship experiments with a model hamburger were performed. The model hamburger consisted of layered bread, meat, cheese and mayonnaise. Eighteen participants had their chewing parameters (chew time, bite size, salivary flow) determined for each individual layer and combinations of the different layers. Each sample was masticated for each subject's natural chew time, or half that time, and then expectorated. Boluses were collected and characterised for moisture content, particle size, and slip extrusion (Ng et al. 2017). The results demonstrated the effects of chewing style parameters (such as chewing rate and bite size) on the bolus moisture content, particle size and swallow properties for single layers and layered composite food. These data gave new insights into the effect of mastication on digestion and provided guidance to bolus preparation for in vitro digestion studies.

Kim, E.H., Wilson, A.J., Motoi, L., Mishra, S., Monro, J., Parkar, S.G., Rosendale, D., Stoklosinski, H.M., Jobsis, C.M.H., Wadamori, Y., Hedderley, D.I., Morgenstern, M.P., 2022. Chewing differences in consumers affect the digestion and colonic fermentation outcomes: in vitro studies. Food Function 13, 9355–9371.

Ng, G.C.F., Gray-Stuart, E.M., Morgenstern, M.P., Jones, J.R., Grigg, N.P., Bronlund, J.E., 2017. The slip extrusion test: A novel method to characterise bolus properties. Journal of Texture Studies 48, 294–301.

Keywords

Mastication, chewing, saliva addition, digestion, hamburger, bolus

Topic: Oral Processing and Digestion

EFFECT OF COMMERCIAL ENOLOGICAL ADDITIVES ON THE ORAL BEHAVIOR OF WINE AROMA COMPOUNDS

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Abstract

Commercial enological additives are widely used in winemaking to improve the technological properties of wines. Beyond their technological role, a preliminary investigation from our research group showed that these additives might modulate aroma persistence after wine tasting, particularly in white wines[1]. This effect may be closely related to the influence of enological additives on the oral behavior of aroma compounds during consumption. However, this preliminary study was conducted with a limited number of additives, and a more comprehensive evaluation is required to confirm and expand these findings.

Therefore, the aim of this work was to evaluate the effect of ten commercial enological additives belonging to three product categories (polyphenol-based additives, polysaccharides, and mannoproteins), added to a white Malvar wine, on the oral aroma behavior of esters. The volatile profile of the control wine was characterized by liquid-liquid extraction (LLE) and analyzed by gas chromatography–mass spectrometry (GC-MS). Then, a control white wine and ten wines containing additives were prepared. Thirteen participants (mean age = 26 y/o) were recruited for the study and performed standardized wine rinses following the SOOM procedure. Volatile compounds were extracted from the expectorated samples by LLE-GC-MS. The wines prior to rinsing were also analyzed. The oral behavior of the aroma compounds after the rinses was evaluated by comparing the amount of aroma extracted from the expectorated samples with the amount originally present in the wines. Afterwards, the differences between the oral behavior observed for the control wine and the wines supplemented with enological additives were assessed. The results of this study show that the oral behavior of wine aroma molecules can indeed be modulated through the use of commercial enological additives, which in turn may influence aroma perception.

References:

1. Velázquez-Martínez, R. I., Criado, C., Muñoz-González, C., Crespo, J., & Pozo-Bayón, M. Á. (2023). Evaluation of the Long-Lasting Flavour Perception after the Consumption of Wines Treated with Different Types of Oenological Additives Considering Individual 6-n-Propylthiouracil Taster Status. Foods, 12(15), 2835.

Keywords

Wine oral processing, Spit-Off Odorant Measurement (SOOM), Aroma persistence, Polyphenols, Mannoproteins

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Topic: Oral Processing and Digestion

RAPID METABOLISM OF FRUITY ESTERS DURING ORAL PROCESSING IS LINKED TO BODY MASS INDEX, SALIVARY ESTERASE ACTIVITY, AND THE ORAL MICROBIOME

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Abstract

Flavor perception is strongly influenced by events occurring during oral processing (such as dilution, retention and metabolism), yet the metabolic fate of aroma compounds in the mouth remains poorly understood. Carboxylic esters are key contributors to fruity notes in many foods, and although their metabolism has been demonstrated ex vivo, in vivo evidence has been lacking due to methodological challenges. This study investigated, for the first time, the in vivo metabolism of fruity carboxylic esters in the human oral cavity and examined its relationships with individual characteristics (age, sex, body mass index (BMI)), salivary parameters and the oral microbiome.

A total of 101 participants performed 30 s mouth rinses with either water (control) or a solution containing four esters (ethyl butanoate, ethyl hexanoate, allyl hexanoate and ethyl octanoate). Original and expectorated solutions were analysed by GC–MS to evaluate esters and their corresponding carboxylic acids. Unstimulated saliva was collected to determine total protein content (TPC) and salivary esterase activity towards carboxylic esters (SEAC). Shotgun metagenomics was employed to characterise the taxonomic and functional profiles of the oral microbiome.

Results showed that three of the four esters (ethyl hexanoate, allyl hexanoate and ethyl octanoate) underwent oral hydrolysis within seconds, with significant ester loss and a concomitant increase in their acids (p 0.05). Oral ester recovery was highly individual-dependent and negatively associated with BMI (p 0.05), suggesting that oral metabolism may contribute to inter-individual differences in flavour perception and, ultimately, food preferences and eating behaviour. Ester recovery was compound-specific and inversely related to key physicochemical properties (molecular weight, $\log P$ and boiling point) ($r = -0.98$; p 0.05). Significant negative correlations between ester recovery and SEAC, but not TPC, support a predominant enzymatic contribution, while not excluding simultaneous retention mechanisms. SEAC was further associated with both the taxonomic composition and functional features of the salivary microbiome. Higher SEAC levels co-occurred with increased abundance of genera linked to dysbiosis and caries (Actinomyces, Prevotella, Veillonella), as well as with genes encoding carboxylic ester hydrolases (e.g., arylesterase, monoterpene ε-lactone hydrolase, unspecific carboxylesterases), though not with canonical carboxylesterase (EC 3.1.1.1).

Together, these findings provide compelling evidence that the oral cavity acts as an active site of food-component digestion with potential implications for both upstream (flavour perception) and downstream phenomena (detoxification, nutrient metabolism). This work opens new avenues for personalised nutrition based on oral enzymatic phenotypes.

Keywords

in vivo oral ester metabolism; fruity carboxylic esters; salivary esterase activity (SEAC); oral microbiome; BMI

Acknowledgements

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Topic: Oral Processing and Digestion

COMPARATIVE ALLERGENIC PROFILING OF HEATED AND UNHEATED LUPIN FLOURS FOLLOWING IN VITRO SIMULATED DIGESTION

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Abstract

Eight flours obtained from different lupin cultivars of blue lupin seeds (L. angustifolius) both heated (90 °C,15 min) and unheated were subjected to simulated gastrointestinal digestion using the harmonized INFOGEST protocol (Brodkorb et al., 2019). Proteins extracted from heated and unheated flours before digestion, and digested samples at gastric and intestinal phases were separated by LDS-PAGE, and allergenicity was evaluated by immunoblotting with pooled sera from five lupin-allergic patients. Immunoreactivity was detected in all the flours and in four of eight gastric-phase digests, whereas no reactive bands were observed after the intestinal phase. Immunoreactive proteins were subsequently identified by HPLC-HRMS/MS.

The main allergen detected was Lup an 1 (β-conglutin), a vicilin-type seed storage protein composed of polypeptides ranging from 20 to 80 kDa linked by disulfide bonds. No immunoreactivity was observed at molecular weights corresponding to Lup a 1 (profilin) or Lup an 3 (non-specific lipid transfer protein). In conclusion, Lup an 1 appeared to be the predominant allergen, with allergenic activity abolished after complete in vitro digestion.

A. Brodkorb et al., INFOGEST static in vitro simulation of gastrointestinal food digestion. Nature Protocols, volume 14, 991–1014 (2019)

Keywords

lupin, food allergy, INFOGEST protocol, Lup an1

Topic: Oral Processing and Digestion

DIGESTION AND ABSORPTION OF DIETARY NUCLEIC ACIDS; THE UNDERSTUDIED AREA CONCERNING AN ABUNDANT FOOD COMPONENT WITH MULTITUDE OF NUTRITIONAL EFFECTS

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Abstract

The nutritional role of dietary nucleic acids (dietNA) has been for long overshadowed by the genetic role of these biomolecules. Accordingly, currently these food components are mostly perceived as molecular tools to monitor food microbiological contamination or to track food adulteration. However, although sketchy and scattered, scientific literature suggests that DNA/RNA found in foods can play a multitude of important nutritional functions that deserve to be studied in more depth. The digestion of dietNA in human organism is believed to begin in mouth, though the enzymatic activities involved are not clear. This stage of digestion is indirectly confirmed by the sensitivity of umami taste receptors to purines. The most recent dedicated studies demonstrated that exogenous DNA digestion may be continued in stomach. The best known are enzymatic activities present in the duodenum and small intestine. The pancreatic juice contains ribonucleases and deoxyribonucleases, while in small intestine are present enzymes that degrade oligonuleotides to nucleotides and the latter finally to nucleobases, sugars and phosphate residues. The absorption of the products of dietNA digestion is realized by several mechanisms involving two transporter families: Concentrative Nucleoside Transporters (CNTs; SLC28 family) and Equilibrative Nucleoside Transporters (ENTs; SLC29 family).
The presentation will summarize current state of knowledge on dietary NA abundance in food, their digestion and absorption from alimentary tract as well as will refer to the nutritional roles and the potential genotoxic risks associated with the consumption of modified dietNA abundant in processed foodstuffs.

1. Ding, T., Song, G., Liu, X., Xu M., Li Y., Nucleotides as optimal candidates for essential nutrients in living organisms: A review. J. Functional Foods, 82: 104498, 2021.
2. Liu, Y., Zhang, Y., Dong, P., An, R., Sue, C., Ge, Y., Wei, L., Liang, X., Digestion of nucleic acids starts in the stomach. Scientific Reports, 5: 11936, 2015.
3. Cieślewicz J., Koziara Z., Ćwiklińska W., Bartoszek A., The toolbox of methods for multidirectional characterization of dietary nucleic acids; verification for raw and processed food products, Food Analitycal Methods, 14: 1482-1497, 2021
4. Pastor-Anglada, M., Urtasun, N., Pérez-Torras, S., Intestinal nucleoside transporters: Function, expression, and regulation. Comprehensive Physiology, 8: 1003–1017, 2018
5. Du X., Chen Q., Zhuang J., Zhao M., Duan Y., Wang S., An R., Liang X., Yeast efficiently utilizes ribosomal RNA-derived oligonucleotides as bioavailable nutrient sources, Foods, 15: 318, 2026
6. Jun, Y. W., Kant, M., Coskun, E., Kato, T. A., Jaruga, P., Palafox, E., Dizdaroglu, M., Kool, E. T., Possible genetic risks from heat-damaged DNA in food, ACS Central Science, 9: 1170–1179, 2023

Keywords

Dietary nucleic acids, digestion, absorption, dietary risks

Topic: Oral Processing and Digestion

PLANT-MARINE BLENDS: ASSESSING THE IMPACT OF FOOD PROCESSING ON IN VITRO PROTEIN DIGESTIBILITY

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Abstract

Introduction: The increasing demand for sustainable, nutrient-dense plant-based foods has accelerated the development of novel protein ingredients derived from legumes and microalgae. However, these matrices often contain anti-nutritional factors (ANF's) such as phytic acid, phenolics, and complex cell structures which can limit proportion of bioavailable amino acids. This study investigated how processing methods, specifically, blending and high-moisture extrusion (HME) modify the nutritional quality of fava bean protein isolate (Fpi) formulated with three microalgae species (Spirulina, Chlorella, Tetraselmis).

Method overview: For analysis, in vitro digestion followed the INFOGEST standardised static digestion protocol integrated with a recently established analytical workflow that allows the assessment of in vitro protein digestibility and calculation of DIAAS scores. After in vitro digestion, methanol was used to separate the digestible and indigestible fraction. The digestible fraction was used to determine the degree of protein hydrolysis (DH%) using the o-Phthalaldehyde (OPA) assay. To determine protein digestibility, both the digestible and indigestible fraction were hydrolysed using 6M HCl to release free amino acids which were measured using LC-MS/MS for individual amino acids and a nitrogen analyser used to measure total protein. Crude polyphenol content was measured using the Folin-Ciocalteu (F-C) method, and Phytic acid (IP6) content was measured using the MEGAZYME Phytic Acid Assay Kit following manufacturer's instructions.

Results: Protein hydrolysis varied ($p < 0.05$) between ~46-37% in Fpi and microalgae, with Chlorella having the lowest and Fpi having the highest percentage. Blending moderately improved protein hydrolysis (~46%) by mixing of ingredients, and diluting ANFs. High-moisture extrusion exhibited a neutral effect on protein hydrolysis in the Fpi and Spirulina formulation (~46%) but had a modest non-significant reducing impact on the Chlorella and Tetraselmis formulations (~42-43%, respectively). Overall, the degree of hydrolysis across matrices was like that of Casein, demonstrating potential for high digestibility.

The highest ($p < 0.05$) phenolic concentration was found in Fpi (~19-20 mg GAE/g dry weight). Spirulina exhibited moderate phenolic content (~8 mg GAE/g) whilst Chlorella and Tetraselmis showed much lower ($p < 0.05$) phenolic contents (~3-4 mg GAE/g). Extruded blends had much lower ($p < 0.05$) phenolic content (~11-13 mg GAE/g) compared to unextruded blends (~16-18 mg GAE/g). Lastly, Fpi had significant ($p < 0.05$) phytic acid levels (~0.80 g/100 g dry weight). Spirulina and Tetraselmis had moderate quantities of phytic acid (~0.65-0.75 g/100 g), while Chlorella had the lowest (~0.60 g). All blends had the highest ($p < 0.05$) phytic acid levels (~1.20-1.30 g/100 g), demonstrating an additive effect of blending. After extrusion, phytic acid levels remained similar to raw materials (~0.60-0.78 g/100 g).

Keywords

Digestibility; Indispensable amino acids; Protein; Anti-nutrients; Food processing; INFOGEST

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Topic: Oral Processing and Digestion

BALANCING SOLUBILITY AND ENZYME ACCESSIBILITY: CONCENTRATION-DEPENDENT EFFECTS OF SURFACTANTS ON RICE PROTEIN DIGESTIBILITY

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Abstract

Rice Protein isolates (RPIs) and concentrates (RPCs) are increasingly utilized in sports & clinical nutrition, infant foods among other applications due to their hypoallergenic profile and pro-sustainability properties. However, their relatively low aqueous solubility, particularly near neutral pH, limits their functionality and may restrict proteolytic accessibility during digestion. While enzymatic, chemical and physical methods have been employed for the improvement of solubility, their impact on digestibility remains underexplored. This study investigates the relationship between surfactant-mediated solubility improvements and proteolytic accessibility of rice protein systems. Two surfactants, polyoxyethylene sorbitan 20 (PS20) and lecithin were tested at concentrations between 0.01 - 1% (m/m) in rice protein dispersions. Protein solubility was quantified using both Dumas nitrogen content analysis and dry matter recovery methods. In vitro protein digestibility (IVPD) was assessed using the Infogest protocol 2.0; amino acids release patterns were determined by HPLC analysis; and protein fragmentation, both pre- and post-digestion were examined by SDS-PAGE.

All surfactants significantly increased apparent protein solubility, even at the lowest concentration tested (0.01% m/m). The natural surfactant, lecithin, performed on par with PS20, a synthetic, pharmaceutical industry standard for protein modification. Digestibility exhibited a concentration-dependent response with moderate surfactant levels (0.01–0.05% m/m) enhancing IVPD, consistent with increased protein surface exposure and improved enzyme accessibility, but regressing at higher doses. Despite differences in overall digestibility, amino acid release profiles remained largely surfactant-independent, indicating that surfactant treatment influenced the rate rather than the specificity of proteolysis. Our study validates that solubility gains corelate with proteolytic accessibility at slightly above critical micelle concentration limits, but higher surfactant loads cause micelles to sterically hinder enzyme access, and thus digestion. This provides insight into the need to optimize usage of interfacial modifiers to balance functional performance and nutritional bioaccesibility in rice protein-based formulations.

Keywords

Protein digestibility, Plant protein isolates, In vitro digestion, Protein solubility, Rice Protein

Topic: Oral Processing and Digestion

STRUCTURAL TRANSFORMATION AND IN VITRO STARCH DIGESTIBILITY OF ROASTED AND BOILED WILD AND COMMERCIALLY CULTIVATED YAMS (DIOSCOREA SPP.) IN AUSTRALIA: A CO

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Abstract

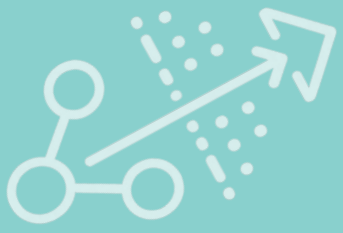
This study employed a multi-scale analytical approach to elucidate structure-function relationships in roasted and boiled Australian yam varieties: commercial D. alata (CDa_C) and three wild species (D. alata, D. bulbifera, D. transversa). X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy, and three-phase in vitro digestion revealed species-specific processing responses driven by distinct cellular architectures. Processing induced specie-dependent structural transformations with Da showing maximum crystallinity reduction (53.6% to 33.2% at B15) corresponding to heterogeneous cellular disruption, while Dt maintained structural integrity (48.5% to 41.0%) due to protective cell wall barriers. Compound granule organisation in Db resulted in threshold-mediated structural collapse at B15 (51.0% to 35.3% crystallinity), whereas CDa_C exhibited minimal processing sensitivity across all treatments. Digestibility patterns revealed hierarchical resistance mechanisms: commercial varieties showed crystallinity-dominated behaviour while wild varieties demonstrated progressively barrier-dominated responses. Roasting treatments preserved crystalline domains while creating surface dehydration barriers, resulting in rapid oro-gastric release but sustained intestinal resistance. These findings establish that digestibility prediction in whole food matrices requires integration across multiple structural scales, with cellular barriers progressively overriding molecular factors in determining enzymatic accessibility. The mechanistic insights provide a framework for optimising processing strategies to achieve targeted digestive behaviours in Australian yams.

Keywords

Yam, thermal processing, SEM, XRD, in vitro starch digestion

SESSION

2 Food Digestion and its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives



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Emmanuelle Reboul is a Director of Research at INRAE (French National Research Institute for Agriculture, Food, and Environment). She heads the "Micronutrients and Metabolic Diseases" research team at the Cardiovascular and Nutrition Research Center in Marseille, France.

Emmanuelle Reboul earned her engineering degree in nutrition and food science from AgroSup Dijon, France, in 2002. She pursued her master's and doctoral research at the INSERM "Human Nutrition and Lipids" laboratory in Marseille, where she investigated the intestinal absorption of carotenoids, vitamin A, and vitamin E.

In 2006, she joined Dr. R.S. Molday's group at the University of British Columbia (Vancouver, Canada), focusing on the molecular mechanisms of ATP transporters. She returned to Marseille in late 2008 as a permanent researcher at INRAE, where her work now centers on the intestinal absorption and membrane transport of fat-soluble micronutrients, such as vitamins A, D, E, K, and carotenoids. Emmanuelle also dedicates part of her research to evaluating how more sustainable diets affect the bioavailability of micronutrients, a topic aligned with the themes of ICFD2026 Session 2: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives.

ABSTRACT

New Insights on Factors Influencing Fat-Soluble Micronutrient Bioavailability

The factors influencing the digestion and absorption of fat-soluble micronutrients—such as fat-soluble vitamins and carotenoids—remain only partially understood.
On one hand, the food matrix and overall diet composition play a critical role in determining the bioaccessibility of these micronutrients.
In today's context, a major challenge is to ensure sustainable diets for all, and transitioning from animal-based to plant-based protein sources may offer a viable solution. However, plant-based diets often contain high levels of antinutrients, which can disrupt the digestion and absorption of lipids and fat-soluble micronutrients. Indeed, in vitro digestion models have been used to explore micronutrient bioaccessibility in test meals containing various legumes or specific antinutrients. Our findings first revealed that the inclusion of legumes in a test meal significantly reduced micronutrient bioaccessibility, primarily due to their high content of fiber, phytates, saponins, and tannins - compounds shown to inhibit lipolysis. Among these, tannins were the most potent inhibitors of lipase activity, followed by phytates and saponins.
Recent studies have shown that using specific bean varieties with low antinutrient content - particularly low phytate levels - could significantly enhance carotenoid bioaccessibility. Furthermore, experiments demonstrated that chickpeas with reduced antinutrient content led to a marked improvement in carotenoid bioaccessibility compared to antinutrient -rich chickpeas.
Antinutrients also interfered with the absorption process of fat-soluble micronutrients, as shown in both Caco-2 cell and mouse models, likely by disrupting the function of membrane transporters at the brush border level.
On the other hand, recent findings emphasize that enterocytes are not merely passive gateways for fat-soluble micronutrients into the body. Clinical evidence has shown that enterocytes can store these micronutrients and later release them into chylomicrons during subsequent postprandial phases, depending on the fat composition of meals. Furthermore, efflux pathways - mediated by proteins such as ABCB1 (P-glycoprotein) and ABCG5/G8 - have been identified in both cell and mouse models, facilitating the transport of fat-soluble micronutrients from the enterocyte cytosol or blood compartment back into the lumen. These discoveries underscore the intestine's active regulatory role in delivering fat-soluble micronutrients to the body.
Overall, these findings highlight that food and diet composition can influence both the bioaccessibility and bioavailability of fat-soluble micronutrients, and that micronutrient absorption involves specific bidirectional pathways. These factors should be taken into account to develop more precise nutritional recommendations for different population subgroups.

ORAL PRESENTATIONS

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IMPACT OF SEAWEED-DERIVED POLYSACCHARIDES ON PROTEIN DIGESTION IN IN VIVO MODELS

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Abstract

Seaweed-derived polysaccharides such as agar, alginate, carrageenan, and cellulose are increasingly used as functional food ingredients; however, their impact on gastrointestinal digestion and on the nanostructural organisation of digestion products remains poorly understood. This study investigated how selected dietary fibres modulate protein digestion and nanoassembly processes in vivo using a pig model. Two model food proteins, casein and whey protein isolate, were combined with different polysaccharides, introduced in nutritionally balanced diets, and fed to pigs. Ileal digesta were collected from the distal ileum via a surgically installed T-cannula. Samples were characterised using complementary compositional, rheological, microstructural, and nanostructural analyses to assess protein digestibility and digesta multi-scale structure.

Results showed that proteins were extensively hydrolysed and absorbed before reaching the distal ileum across all formulations. However, specific peptide fragments were more resistant to digestion when agar was added to whey protein isolate. In contrast, seaweed polysaccharides were not digested and exhibited distinct structural behaviours within ileal digesta. Alginate formed dense, network-like structures, whereas agar led to more homogeneous digesta organisation. Notably, cellulose induced the formation of ordered nanomicellar structures through interactions with bile salts, suggesting enhanced bile salt secretion and/or altered bile salt diffusion and reabsorption.

These findings demonstrate that seaweed-derived polysaccharides do not impair protein digestibility but significantly influence the micro- and nanostructural organisation of ileal digesta by modulating viscosity and colloidal assembly. Understanding how dietary fibres affect intestinal digesta structure and nutrient transport is therefore essential for the rational design of functional foods with improved nutritional performance.

Keywords

Polysaccharides, gastrointestinal digestion, dietary fibres, bile salts, ileal digesta

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

GOAT MILK IMPROVES INTESTINAL BARRIER FUNCTION AND LACTASE EXPRESSION IN A QUADRICELLULAR MODEL OF INTESTINAL EPITHELIUM AFTER DYNAMIC IN VITRO DIGESTION

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Abstract

Introduction

Goat dairy products are often considered easier to digest than cow dairy products. Some studies suggest that their proteolysis and lipolysis may be facilitated by milk composition. Others report benefits on intestinal barrier function and inflammation in mice and humans. This study characterized the dynamic in vitro digestion of goat and cow milks and yogurts and evaluated the effects of their digestates on intestinal health using a quadricellular model of the intestinal epithelium.

Materials and Methods

Iso-protein and iso-lipid goat and cow milks and yogurts were digested using the DIDGI® dynamic in vitro digestion model. Lipolysis kinetics and fatty acid profiles were analyzed by GC-MS (n=3), and proteolysis kinetics and peptide profiles by the OPA method and LC–MS/MS (n=3). Intestinal digestates were applied to a quadricellular model of intestinal epithelium (enterocytes, mucus-secreting, enteroendocrine, and M cells). Barrier integrity was assessed by TEER over 24 h (n=17–24). Gene expression related to intestinal functions was quantified at 4 h and 24 h by RT-qPCR (SmartChip®).

Results

Goat and cow products showed similar lipolysis and proteolysis kinetics, but the released fatty acids and peptides differed markedly. At the end of intestinal digestion, goat products released more medium-chain fatty acids (13% vs 5%, p0.05) and shared only 21% of their peptides with cow products. Goat digestates induced higher TEER values than cow digestates between 4 and 16 h (p0.05), indicating improved barrier integrity. TEER with goat digestates was also higher than control medium between 3 and 8 h, an effect not observed with cow digestates.

At 4 h, goat digestates upregulated tight-junction protein genes (OCLN, CLDN1, TJP1) compared with cow digestates (p0.05), consistent with the TEER improvements. Lactase expression was significantly higher with goat digestates than with cow digestates and controls at 4 h and remained elevated at 24 h, unlike other genes.

Conclusion

Differences in released fatty acids and peptides may help explain the stronger modulation of genes involved in barrier integrity and lactose digestion observed with goat dairy products. These in vitro results support potential intestinal health benefits of goat milk, although the underlying mechanisms require further investigation.

Keywords

Goat milk; Dynamic in vitro digestion; Intestinal barrier; Lactase

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

PH VARIATION IN THE STOMACH AND DUODENUM AFFECTS CALCIUM BIOACCESSIBILITY: AN IN VITRO STUDY USING THE 3-COMPARTMENT DIDGI DIGESTION SIMULATOR

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Abstract

Calcium absorption is controlled by complex homeostatic mechanisms and highly driven by its solubility in the gastrointestinal tract. Calcium solubility is sensitive to the physicochemical conditions, particularly the pH, which varies considerably along the gastrointestinal tract and differs between the duodenum and the jejunum/ileum. Yet, most in vitro digestion studies have focused on calcium solubility at a fixed pH in the jejunum/ileum compartment without considering the pH in the duodenum.

The objective of this study was, firstly, to design a dynamic in vitro digestion model using the 3-compartment DIDGI® system (stomach, duodenum, jejunum/ileum) and find physiological parameters to program it to simulate the evolution of pH in the 3 compartments. The data used to define the pH variations, particularly in the duodenum, came from in vivo studies conducted on humans and pigs using microelectrodes or a pH catheter after ingestion of a liquid meal. Secondly, the digestive system was tested on four commercially available food matrices (milk, yogurt, enriched calcium soy dessert and beverage) to measure the solubility and bioaccessibility of calcium and the effect of pH on these properties. The soluble and total calcium contents were measured at different stages of gastric, duodenal, and intestinal (jejunal/ileal) digestion using inductively coupled optical emission spectrometry. To avoid biases caused by dilution and matrix depletion, calcium bioaccessibility was determined by the ratio of the soluble calcium fraction to the total calcium fraction present during digestion.

Although the milk and soy beverage matrices did not contain the same amount of calcium (1.2 vs. 1.6g/L, respectively), gastric acidification (pH 7 to 3) induced a same progressive increase in calcium solubility in both matrices. This was also the case during the duodenal phase (pH 5.7 to 4.35), when calcium bioaccessibility in both milk and soy beverage reached nearly 80%, after 3h of digestion. However, calcium bioaccessibility was significantly higher in the first part of the duodenal phase for milk compared to soy beverage (38% vs. 21% at 15min; 48% vs. 33% at 21min). Given the rapid transit time through the duodenum (15 min), this could indicate that at this stage, a larger amount of soluble calcium from milk is absorbed compared to that from soy beverage. Conversely, the pH rose to 6.5 in the intestinal phase and induced a decrease in calcium solubility. Overall, calcium bioaccessibility in the intestinal phase was similar between milk and soy beverage (26-28%, respectively, t120min). For gel matrices, yogurt showed a higher calcium bioaccessibility than soy dessert.

The newly developed three-compartment digestive model allowed a fine assessment of calcium bioaccessibility in the duodenum and jejunum/ileum. The higher calcium content in soy dessert and beverage did not increase the amount of soluble calcium being produced during digestion compared to yogurt and milk.

Keywords

Calcium; Bioaccessibility; dynamic in vitro digestion; Dairy product; Vegetable product

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

BIOACCESSIBILITY AND ASSOCIATED CONCEPTS: WHAT TO USE, WHAT TO AVOID, WHAT'S LEFT TO DEFINE?

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Abstract

Food digestion within the oro-gastrointestinal tract involves many biochemical and physical processes, which are described using more or less well-defined concepts applicable to both in vivo and in vitro digestion. Among these concepts, bioaccessibility is an essential one as it refers to the endpoint of all oro-gastrointestinal tract processes, when nutrients reach their absorbable forms. However, due to the high complexity within the oro-gastrointestinal tract and the large differences in nutrient properties, bioaccessibility is rarely defined in vivo and may be ill-defined in vitro.

To clarify the concept of bioaccessibility, as applied to various nutrients and as measured in vitro, we recently published two articles discussing the terminology currently used to describe the oro-gastrointestinal tract processes involved.1,2 In this presentation, we will first define the elementary digestion processes (release, solubilization, hydrolysis) as well as the combined ones (transfer, digestibility), highlighting ambiguous terms and how their accuracy may be improved. Then, we will define bioaccessibility and how it is currently characterized for each class of nutrients.

We will finally highlight situations where further research is required to define bioaccessibility so that it relates more to the in vivo situations. Such cases include biopolymer nutrients (polysaccharides or proteins) which form multiple oligomers upon digestion. Their bioaccessible fraction does not only depend on their molecular mass, as could be separated through membrane filtration or ultracentrifugation, but on their composition, structure, and properties. Another case is that of nutrients which are metabolized by the gut microbiota (such as polyphenols or dietary fibers) yielding absorbable metabolites, this process being rarely reproduced in vitro. Lastly, most vitamins require micellar solubilization or protein binding for luminal transport before absorption can occur, making only these forms bioaccessible.

This work intends to harmonize our understanding of in vitro digestion concepts so they better represent in vivo processes. Notably, reaching a consensus about the bioaccessibility of nutrients should improve the comparability of in vitro digestion studies as well as their predictability of in vivo absorption behaviors.

M.M.L. Grundy, P.J. Moughan, and P.J. Wilde (2024). Bioaccessibility and associated concepts: Need for a consensus. Trends in Food Science & Technology 145, 104373. https://doi.org/10.1016/j.tifs.2024.104373

M.M.L. Grundy, A. Deglaire, S. Le Feunteun, E. Reboul, P.J. Moughan, P.J. Wilde, D.J. McClements, and S. Marze (2025). Bioaccessibility and associated concepts: Terminology in the context of in vitro food digestion studies. Food Chemistry 485, 144424. https://doi.org/10.1016/j.foodchem.2025.144424

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

INTESTINAL SENSING OF DIETARY PROTEINS AND ITS IMPACT ON GLUCOSE ABSORPTION

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Abstract

With the continuous increase in global protein demand and the rising prevalence of metabolic disorders such as type 2 diabetes, understanding the role of dietary proteins in metabolic regulation has become a major research challenge. Beyond serving as a source of amino acids, dietary proteins exert various biological effects, notably through bioactive peptides generated during gastrointestinal digestion. These peptides are sensed by intestinal epithelial cells via specific receptors and transporters, including PepT1, a di- and tripeptide transporter involved in nutrient sensing.

An exploratory study conducted in our laboratory (Dugardin et al., 2022) first suggested that acute intake of certain dietary proteins may improve glucose tolerance by reducing intestinal glucose absorption. The aim of the present study was therefore to investigate the involvement of PepT1 in this regulatory mechanism.

To address this hypothesis, three dietary proteins of different origins (caseins, pea proteins, and fish gelatin) were selected based on previous findings. An integrative experimental approach was employed, combining in vitro, ex vivo, and in vivo models, together with the use of a PepT1 inhibitor, 4-aminomethylbenzoic acid (4-AMBA).

The results showed that the digested protein-induced reduction in intestinal glucose absorption, as well as the associated decrease in GLUT2 gene expression, was abolished upon PepT1 inhibition. Furthermore, the protein-mediated improvement in glucose tolerance observed in rats was also lost when the inhibitor was administered.

Altogether, these findings provide the first experimental evidence of a functional link between intestinal peptide transport and glucose absorption. Beyond its classical role as a peptide transporter, PepT1 emerges as a metabolic sensor capable of modulating glucose transport in response to peptide-derived signals. The involvement of additional intestinal transporters, receptors, and signaling pathways in this regulatory process remains to be elucidated and is currently under investigation within the framework of the SPIGA research project funded by the French National Research Agency (ANR).

Reference : Dugardin Camille, Léa Fleury, Véronique Touche, et al. « An Exploratory Study of the Role of Dietary Proteins in the Regulation of Intestinal Glucose Absorption ». Frontiers in Nutrition 8 (2022). https://doi.org/10.3389/fnut.2021.769773.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IMPACT OF ENZYMATIC HYDROLYSIS ON THE IN VITRO DIGESTIBILITY AND INSULINOTROPIC ACTIVITY OF PLANT PROTEIN SOURCES

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Abstract

The increasing demand for sustainable protein sources has led to a dietary shift toward plant-based ingredients. However, the nutritional quality of plant products is often limited by lower protein digestibility compared to animal sources. In this context, enzymatic hydrolysis represents a key strategy to improve the nutritional quality and metabolic health effects of these products. The aim of this work was to evaluate the impact of enzymatic hydrolysis on the digestibility, peptidomic profile, and insulinotropic activity of two plant-based ingredients derived from the food industry: rapeseed protein isolate (RPI), a protein isolate from rapeseed cake, and corn protein meal (CPM), a co-product starch production. Micellar casein (MC) was used as control. Protein substrates were hydrolyzed with three different enzymes (pepsin, papain, and alcalase) under optimal conditions and further subjected to the INFOGEST standardized gastrointestinal digestion. The peptide profile of the digests was characterized by nanoLC-MS/MS. To evaluate the insulinotropic response, a physiologically relevant 2-tiered bicompartmental model was used. This system consisted of a Caco-2 monolayer (with or without STC-1 cells) in the apical chamber and pancreatic BRIN-BD11 cells in the basolateral chamber to assess the contribution of hormonal secretion and the absorbable fraction of the digests, respectively. The results showed that enzymatic hydrolysis increased the in vitro protein digestibility of all substrates, particularly in the case of CPM. These findings were aligned with the peptidomic analysis, which revealed a lower number of identified peptides in the digests of the hydrolyzed samples, reflecting an extensive protein breakdown into small fragments (5 amino acids) that fall below the mass spectrometry detection limit. Furthermore, in the 2-tiered model, the papain hydrolysate of RPI and the pepsin hydrolysate of CPM significantly increased insulin secretion compared to their non-hydrolyzed counterparts. This insulinotropic effect was primarily driven by the enhanced GLP-1 secretion, although the absorbable fraction of the digests also contributed significantly in both cases. In contrast, for MC, both papain and alcalase hydrolysates elicited a higher insulin release than the non-hydrolyzed sample, consistent with the increased basolateral GLP-1 levels detected, but the contribution of the absorbable fraction was negligible. In conclusion, the development of enzymatic hydrolysates effectively improves the digestibility and insulin-stimulating response of plant protein sources. In addition to the increased GLP-1 response, the gastrointestinal digests of the plant hydrolysates contained protein-digestion products with insulinotropic effect. Consequently, these findings could serve as a seed to enhance the protein quality and metabolic benefits of plant substrates, although further validation in animal models is required to confirm their efficacy in vivo.

Keywords

Plant-based proteins, Enzymatic hydrolysis, In vitro digestibility, Peptidomics, Insulinotropic activity

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

ANTHOCYANIN-RICH PIGMENTED WHEAT: GASTROINTESTINAL DIGESTS REVEAL DISTINCT IMMUNE-PEPTIDOMIC AND PHENOLIC PROFILES WITH REDUCED GLUTEN IMMUNOGENICITY

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Abstract

Celiac Disease (CD) is an autoimmune enteropathy induced by dietary gluten in genetically predisposed individuals, causing intestinal inflammation, mucosal damage, among other symptoms [1]. Currently, a strict gluten-free (GF) diet remains the only effective treatment; however, GF products are often made with refined starches and poor-quality flours, resulting in micronutrient deficiencies, unfavorable glycemic profiles and other nutritional imbalances [2]. Thus, novel strategies aiming to improve the nutritional and functional properties of cereal-based foods are urgently needed.

There is a growing interest in wholegrain and biofortified cereals consumption and pigmented wheat genothypes present a promising potential, combining a balanced macronutrient composition with high content of bioactive phytochemicals, compounds with antioxidant and anti-inflammatory properties that could modulate oxidative stress and intestinal barrier integrity pathways [3].

It is crucial to understand the capacity of pigmented wheat genotypes to modulate gluten immunotoxicity and CD-related pathways. In this study, wholegrain flours obtained from different pigmented wheat genotypes (yellow, purple, blue and black) were digested using standardized INFOGEST in vitro protocol, followed by characterization in terms of peptide profile and phenolic bioaccessibility. The intestinal effects of digested samples were accessed using duodenal organoid-derived monolayers from CD patients and non-celiac controls, evaluating epithelial response, cytokine secretion, and key regulators of authophagic flux (p62 and pE4BP1). In parallel, gluten-reactive CD4+ T-cell lines derived from CD-patients were used to evaluate adaptive immune response and cytokine production in response to gastrointestinal wheat digests.

The present study aims to clarify the mechanistic role of bioactive-rich wheat genotypes in modulating both biochemical and immunological pathways relevant to CD. Althogether, these findings reveal that pigmented wheat varieties combine a reduced immunogenic peptide load with bioactive compounds capable of attenuating metabolic and inflammatory stress on intestinal epithelial cells, while promoting cytokine responses consistent with epithelial protection. By highlighting both immunomodulatory potential and the complexity of antigen presentation whitin wholefood matrices, this work provides foundation to rethink wheat-based dietary strategies in CD.

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Keywords

Celiac disease, wholegrain wheat digests, anthocyanin-rich genotypes, gluten peptides, immunomodulation, organoids

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

ILEAL PROTEIN DIGESTIBILITY AND QUALITY OF FABA BEAN EXTRUDATE AND HONEY CHLORELLA IN HEALTHY HUMANS

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Abstract

Shifting consumption from animal to alternative proteins has been identified as a prominent way to reduce the environmental burden of food production. Yet, the in vivo nutritional value of alternative proteins, such as legumes and microalgae, remains poorly documented. Therefore, the present study aimed at quantifying the true ileal protein digestibility of faba bean extrudate obtained by high-moisture extrusion and honey chlorella (Chlorella vulgaris). Following overnight fasting, 8 subjects (five males and two females, mean age: 38 ± 14 years, mean body mass index: 24 ± 3 kg/m2) received a test meal containing 81 g of 15N-labelled faba bean extrudate (equivalent to 290 mmol of nitrogen), panfried with 15 g sunflower oil to which agave syrup was added. Seven other subjects (five males and two females, mean age: 34 ± 14 years, mean body mass index: 25 ± 3 kg/m2) received a test meal containing 20 g of 15N-labelled honey chlorella mixed with 150 g of pineapple juice, 70 g of coconut milk, and 20 g of agave syrup. After ingestion, ileal, plasma, and urine samples were collected for eight hours, in which nitrogen, amino acids, and urea were quantified. In these fractions, 15N enrichment was quantified in bulk and in individual amino acids using an isotopic ratio mass spectrometer. The ileal nitrogen digestibility was 82.8 ± 5.6 % for faba bean extrudate (n = 8) and 87.9 ± 1.5 % for honey chlorella (n = 7). Assessment of the ileal protein digestibility corrected amino acid score (PDCAAS) and of the digestible indispensable amino acid score (DIAAS) revealed that valine and particularly sulphur amino acids were limiting for faba bean extrudate, restricting its protein quality (PDCAAS = 0.44, n = 8; DIAAS = 0.46, n = 5; N x 5.4). For honey chlorella, histidine and sulphur amino acids were only marginally limiting (PDCAAS = 0.98; N x 4.78). For faba bean extrudate, preliminary results (n = 5) indicate that the individual amino acid digestibility varied from 75.8 ± 5.5 % for valine to 89.8 ± 2.3 % for histidine. Over eight hours following ingestion, 6.6 ± 2.3 % of the nitrogen originating from faba bean extrudate was recovered in urinary urea, while 11.4 ± 3.7 % was still present in body urea eight hours after ingestion. Summing both deamination and digestive losses allowed calculation of the net postprandial protein utilization (NPPU), being 64.7 ± 6.3 % for faba bean extrudate. In conclusion, the ileal protein digestibility from faba bean extruded meat analogue was moderate (85 %), and its protein quality was strongly restricted by the limiting sulphur amino acids. The ileal nitrogen digestibility of honey chlorella was good (>85 %), and histidine was the first limiting amino acids, but only to a marginal extent. This work allows evaluation of the protein quality of faba bean consumed as processed food, and provides the first value of protein quality for honey chlorella.

Keywords

faba bean extrudate, honey chlorella, in vivo digestibility, protein, amino acids

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IMPACT OF A VEGETARIAN DIET ON PLANT-PROTEIN DIGESTIBILITY AND METABOLISM ACROSS LIFESPAN

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Abstract

Introduction: While plant proteins are generally less digestible than animal proteins, it remains unclear whether this could limit protein adequacy in older individuals. This study then aimed to assess the effects of a vegetarian diet on protein digestibility, postprandial nitrogen distribution and protein synthesis in rats across lifespan.

Material & methods: 52 male Wistar rats of 1 (young), 10 (adult) and 18 months (old) at arrival were fed an omnivorous (OMNI, n=8-9) or vegetarian (VEGE, n=8-11) diet for 3 months. The diets had comparable nutritional composition (22% P/E, 70% C/E, 8% L/E) and met the requirements of rats. The vegetarian diet was richer in fiber content and antinutritional factors. Body weight and food intake were measured three times per week. At the beginning, midpoint, and end of the protocol, rats were placed in metabolic cages to measure fecal nitrogen digestibility and bone health measurements were also performed. At the end of the experiment, a test meal with 15N-labeled pea as sole source of protein was administered 6 h before euthanasia in order to specifically trace the digestive and metabolic fate of dietary proteins. In addition, a 13C-valine injection was performed 30 min before euthanasia to evaluate protein synthesis rates in the liver and gastrocnemius muscle.

Results: Aging significantly affected body weight gain, food intake and bone mineral density, with no effect of diet, as both groups exhibited similar changes. Young rats showed lower fecal nitrogen digestibility at baseline compared with adult and old rats (P=0.0002), an age-related difference that diminished over time and disappeared after 3 months of diet. From mid-point onward, a dietary effect emerged, with lower digestibility in VEGE rats than in OMNI rats (91% vs 93%, P=0.008), particularly in older animals. At the end of the protocol, AA digestibility was influenced both by age and diet, with slightly lower values in young rats than in adults and olds (P=0.01) and in VEGE rats compared with OMNI rats (P=0.003). In contrast, AA transporter gene expression in the duodenum, jejunum and ileum did not differ between groups. Ageing was associated with a significant reduction in postprandial nitrogen incorporation (P0.0001) and protein synthesis rates in the gastrocnemius muscle (P=0.01), whereas postprandial dietary nitrogen utilization and whole-body protein metabolism were comparable between both diets.

Conclusion: Overall, despite modest reductions in protein and amino acid digestibility, vegetarian and omnivorous diets led to comparable postprandial protein utilization and similarly supported growth and maintenance in young and adult rats. Likewise, age-related reductions in muscle anabolic responsiveness, lean mass and bone mineral density occurred to a comparable extent in old rats under both dietary conditions. These results suggest that a well-balanced vegetarian diet is just as capable of meeting protein requirements as an omnivorous diet.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

INTEGRATING DIGESTION KINETICS INTO ACRYLAMIDE RISK ASSESSMENT IN COOKIES VIA THE SEMI-DYNAMIC INFOGEST MODEL

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Abstract

Background: Biscuits are widely consumed baked goods globally, but their contribution to dietary acrylamide (ACR) intake raises growing health concerns. ACR is formed during thermal processing (Maillard reaction) between free asparagine and reducing sugars under low humidity conditions. It is classified as a probable human carcinogen (Group 2A) by the IARC. Children are particularly vulnerable, as the consumption of biscuits and cereal products can contribute significantly to their total ACR intake. Food industry explors mitigation strategies, such as using natural or enzymatic ingredients.

Objectives: The study aimed to assess the health risk associated with exposure to ACR through different biscuit formulations. Specific objectives included: (i) comparing the effectiveness of adding apple pomace (a fibre-rich industrial waste product) and the enzyme asparaginase in reducing ACR levels; (ii) to investigate the fate and kinetics of ACR during simulated gastric digestion; and (iii) to estimate the carcinogenic and non-carcinogenic risks for adults (18-65 years) and children (3-10 years).

Methods: Three formulations were analysed: control, biscuit with partial replacement of flour by freeze-dried apple pomace (adjusted to 5% pectin) and biscuit with asparaginase (500 U/kg). ACR was quantified by GC-MS/MS after derivatisation. Digestion was evaluated using the semi-dynamic in vitro model INFOGEST 2.0, which mimics pH changes and physiological gastric emptying (GE1 to GE4). Probabilistic risk assessment used Monte Carlo simulation (10,000 iterations) to calculate the Estimated Daily Intake (EDI), Exposure Margin (MOE), Target Hazard Quotient (THQ) and Incremental Lifetime Cancer Risk (ILCR).

Results: Asparaginase reduced ACR by approximately 70% (65.0 µg/kg) compared to the control (215.3 µg/kg), while apple pomace provided a 39% reduction (132.3 µg/kg). However, during gastric digestion of control and apple pomace cookies, a marked increase in ACR was observed, suggesting the conversion of precursors (such as Schiff bases) in the gastric environment. At the end of digestion, the total levels released were similar for the control and the pomace (~308-321 µg/kg), although the pomace showed a faster initial release. In the risk assessment, the MOE values for children were below 10,000, indicating a carcinogenic risk, which worsened after digestion (up to 59% reduction in MOE). The ILCR for children exceeded the safety limit (1.0×10^{-4}) in the control and post-digestion bagasse biscuits, posing a serious risk. The THQ remained below 1, indicating no risk of chronic toxicity.

Conclusions: Apple pomace has limited effect to reduce ACR. Gastric digestion significantly influenced the kinetics of ACR release. Risk assessment should integrate digestive kinetics to provide accurate estimates of food safety, particularly for children. The semi-dynamic INFOGEST method is excellent for this purpose.

Keywords

acrylamide, bioaccessibility, semi-dinamic method, risk assessment, cookies

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IN SILICO SCREENING METHOD FOR DISCOVERING NOVEL CANDIDATE PEPTIDES FOR CASR ACTIVATION IN PORCINE DUODENAL DIGESTS

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Abstract

The calcium-sensing receptor (CaSR) is a G-protein–coupled nutrient sensor in enteroendocrine cells and has been implicated in cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) secretion in response to peptides and amino acids (Diakogiannaki et al., 2013; Lao et al., 2024; Nakajima et al., 2012). To facilitate the identification of peptides able to bind and activate CaSR, we developed a structure-based in silico pipeline combining AlphaFold3 modeling, docking, Rosetta refinement, and molecular dynamics.

Known CCK- and GLP-1-secretagogue peptides were first used to define structural filters (RMSD, dCOM, MM/GBSA energies) and key Venus Flytrap (VFT)–domain residues involved in ligand recognition (Lao et al., 2024). The optimized workflow was applied to peptides detected in the duodenum of pigs fed micellar casein. Several candidates showed stable VFT-domain binding and favorable interaction energies. Five peptides meeting structural and energetic criteria were selected for testing, together with one predicted poor binder. All the selected peptides bound the inter-lobe region of the VFT domain and stimulated CCK or GLP-1 release in STC-1 cells, whereas the predicted poor binder showed no activity, supporting the predictive value of the pipeline. CaSR involvement was further confirmed using the CaSR allosteric inhibitor NPS-2143. GLP-1 secretion was partially reduced, while CCK release was completely abolished, highlighting a stronger CaSR contribution to CCK regulation.

Overall, this work demonstrates that combining deep-learning-based structure prediction with docking and molecular dynamics provides an efficient and cost-effective strategy to prioritize food peptides found in gastrointestinal digests targeting CaSR, reducing experimental screening efforts.

References

Diakogiannaki, E., Pais, R., Tolhurst, G., Parker, H. E., Horscroft, J., Rauscher, B., Zietek, T., Daniel, H., Gribble, F. M., & Reimann, F. (2013). Oligopeptides stimulate glucagon-like peptide-1 secretion in mice through proton-coupled uptake and the calcium-sensing receptor. Diabetologia, 56(12), 2688–2696. https://doi.org/10.1007/s00125-013-3037-3

Lao, H., Chang, J., Zhuang, H., Song, S., Sun, M., Yao, L., Wang, H., Liu, Q., Xiong, J., Li, P., Yu, C., & Feng, T. (2024). Novel kokumi peptides from yeast extract and their taste mechanism via an in silico study. Food & Function, 15(5), 2459–2473. https://doi.org/10.1039/D3FO04487D

Nakajima, S., Hira, T., & Hara, H. (2012). Calcium-sensing receptor mediates dietary peptide-induced CCK secretion in enteroendocrine STC-1 cells. Molecular Nutrition and Food Research, 56(5), 753–760. https://doi.org/10.1002/mnfr.201100666

Keywords

in silico screening, CaSR agonists, duodenal peptides, hormone secretion

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

INTACT PLANT TISSUES IN A WHOLE MEAL REDUCE NUTRIENT DIGESTIBILITY AND COLONIC FERMENTATION: EVIDENCE FROM AN ILEOSTOMY STUDY

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Abstract

It has been suggested that the structural composition of food may significantly affect nutrient bioavailability. Specifically, when cellular integrity is preserved in plant tissues, nutrients are naturally encapsulated within cells, which may reduce the rate and extent of their digestion. Whereas this effect has been explored at level of single foods, the magnitude of the effect at a meal level has barely been investigated. This study therefore aimed to investigate the effect of plant tissue integrity on total dietary energy excretion, on macronutrients bioavailbility and on colonic fermentation of undigested material after consumption of a whole plant-based meal.

A randomised, single-blinded, crossover-controlled trial (NCT06921811) was conducted in 11 ileostomates (5M, 6F, 43 +/- 11 years, BMI 30 ± 9). On different days, participants consumed meals with similar macronutrient composition and energy content but differing levels of plant tissue integrity: a meal rich in intact plant tissues (INTACT, 538 kcal; pumpernickel bread, an apple, and a salad containing chickpeas, peanuts, and carrots) and a meal low in intact plant tissue (BROKEN, 501 kcal; rye bread, a chickpea burger, peanut butter, and a smoothie made from apple and carrots).

Ileal fluid was collected over the 8 hours following meal consumption and analysed for residual unabsorbed macronutrients, including available carbohydrates, lipids, and proteins. Additionally, ileal fluid samples were subjected to in vitro faecal fermentation to simulate colonic conditions and evaluate the effects of the two meals on short-chain fatty acid (SCFA) production.

In ileal fluid samples collected post-consumption of the INTACT meal, 2.7% (median; Q1–Q3: 2.2–3.1) of carbohydrates remained unabsorbed, significantly higher than after the BROKEN meal (1.2%; 0.9–1.7; $p = 0.03$, one-tailed Wilcoxon test). More notably, after consumption of the INTACT meal, 5.8% (3.4–7.8) of lipids remained unabsorbed, compared with 1.7% (1.4–2.5) after consumption of the BROKEN meal ($p = 0.02$, one-tailed Wilcoxon test). In contrast, no significant difference was observed for protein.

Ex-vivo modelling (ileal fluid + in vitro faecal fermentation) demonstrated a clear effect due to the structural composition of foods with ileal fluids derived from the BROKEN meal, resulting in higher SCFA production compared to those from the INTACT meal.

These findings confirm that plant tissue integrity is preserved during digestion and that meals rich in intact plant structures limit enzymatic accessibility, reduce production of colonic SCFAs, and may consequently contribute to lower metabolizable energy intake and greater energy excretion.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

MODULATION OF MOLLUSK ALLERGENICITY BY FOOD PROCESSING AND GASTROINTESTINAL DIGESTION: A MULTI-SPECIES ASSESSMENT

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Abstract

Mollusk allergy is an IgE-mediated reaction triggered by proteins present in marine/terrestrial mollusks, including bivalves, gastropods, and cephalopods. It is considered less prevalent than crustacean allergy, but often presents comparable clinical severity, ranging from cutaneous symptoms to anaphylaxis. Mollusks are highly appreciated for their nutritional value and organoleptic properties, but their consumption can pose serious health risks for the sensitized/allergic individuals. In this work, it was intended to test how different food processing conditions followed by in vitro digestion would impact protein IgE-reactivity.

In this sense, 13 different species of marine/terrestrial mollusks, namely Octopus vulgaris, Mytilus galloprovincialis, Solen marginatus, Loligo vulgaris, Ruditapes decussatus, Sepia officinalis, Helix aspersa, Cerastoderma edule, Crassostrea angulata, Donax trunculus, Pecten maximus, Patella aspera and Buccinum undatum were freshly acquired at local markets. Each species was submitted to different treatments (boiling, oven-cooking, grilling) under varied temperature/time conditions. Proteins were extracted with Tris-HCl 0.1 M (pH 8.0) buffer and quantified by Pierce™ BCA Protein Assay Kit. Protein profile was analyzed by SDS-PAGE and mass spectrometry (MS), while the IgE-reactivity was assessed by immunoblotting with sera from mollusk-allergic patients. Selected raw and thermally treated mollusk species were digested following the INFOGEST 2.0 protocol using pancreatin for the intestinal phase [1].

SDS-PAGE results generally revealed a more protein bands in processed mollusk species than their raw counterparts, suggesting protein fragmentation induced by thermal treatments. Immunoblotting further showed that, in most cases, processed mollusks (boiled or oven-cooked) exhibited enhanced IgE-reactivity, presenting several intense bands around 37, 75 and 150 kDa. The cooking water from boiled mollusks contained substantial amounts of IgE-reactive proteins, indicating that a fraction of allergenic components is water-soluble and can be partially removed by discarding the boiling liquid. MS analysis confirmed that bands at 37 kDa corresponded to tropomyosin subunits, while bands at 75/150 kDa matched their aggregated forms. Most allergens retained their IgE-binding capacity following gastric digestion (pepsin-resistant), which was markedly diminished after subsequent intestinal digestion using pancreatin. These results indicate that thermal processing may enhance the allergenic potential of mollusks, whereas gastrointestinal (GI) digestion appears to attenuate it. To our knowledge, this study represents the first comprehensive assessment of the IgE-binding properties of allergens from multiple mollusk species as influenced by individual and combined food-processing treatments, following simulated GI digestion.

References

[1] Brodkorb A et al. 2019, 10.1038/s41596-018-0119-1

Keywords

Mollusks, IgE-reactivity, Gastrointestinal digestion; Food processing, Sera from mollusk-allergic patients

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

STARCH BIOACCESSIBILITY, GLYCAEMIA AND GUT HORMONE RESPONSES: INSIGHTS FROM A HUMAN NASO-ENTERIC INTUBATION STUDY OF CHICKPEAS

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Abstract

Food structure plays a critical role in regulating nutrient bioaccessibility and metabolic and gut hormones responses, yet the spatio-temporal dynamics of nutrient release in vivo remain poorly defined. In whole cooked pulses (for example, chickpeas), starch encapsulated in cotyledon cells[1] must be partially digested and released from cells to be detected by nutrient-sensing receptors in the gastrointestinal tract, and/or to be absorbed. Processing-induced plant cell breakage is hypothesised to increase the rate and location of nutrient release during gastrointestinal transit, with consequences for glycaemic and gut hormone responses.

We used naso-enteric intubation techniques[2] for time-resolved intestinal sampling of the stomach, duodenum and ileum, with parallel blood sampling, to investigate the relationship between the digestive behaviour of chickpea porridge with contrasting plant cell intactness and the postprandial blood glucose, insulin and gut hormone responses. In the randomized crossover study[3], ten healthy participants consumed three chickpea meals with contrasting structures while fitted with naso-enteric tubes and a cannula. Blood and gastrointestinal samples were collected postprandially and analysed for glucose, insulin and gut hormones, as well as digesta composition and microstructure. This trial was approved by the Health Research Authority and London-Campden and King's Cross Research Ethics Committee (REC 19/LO/0962) and prospectively registered (ISRCTN18097249). The same chickpea test meals were also studied in vitro, using INFOGEST v2 and starch amylolysis assays.

Microscopy analyses confirmed the persistence of cells through the stomach, duodenum and ileum and provided new insight into meal transit through the upper-gastrointestinal tract. Distinct structure-dependent differences in the spatio-temporal breakdown of test meals were evident from analyses of starch, maltose, and microstructure of samples. Despite having the same nutrient composition, the test meals elicited significantly different glycaemic and gut hormone responses, which were attributed to differences in starch bioaccessibility.

Overall, this study provides rare in vivo evidence linking food structure, starch bioaccessibility, and metabolic responses across human gastrointestinal regions. Together, these findings provide mechanistic in vivo evidence that spatio-temporal starch bioaccessibility, governed by food structure, is a key regulator of postprandial metabolic and gut hormone responses in humans. The findings are valuable for understanding and improving the physiological relevance of in vitro digestion methodologies and have clear applications in structured functional food design for public health.

References:
[1] Edwards CH, et al. Nat Food 2(2), 118-126 (2021).
[2] Ph van Trijp, M. et al. Am. J. Clin. Nutr. 114, 843–861 (2021).
[3] Cai M, et al. Nat Metab 7, 1459–1475 (2025).

Keywords

naso-enteric intubation; food structure; randomised controlled trial; starch digestion

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

FORMULATION, IN VITRO DIGESTION, SENSORY ACCEPTABILITY AND NUTRITIONAL POTENTIAL OF CLIMATE-SMART GLUTEN-FREE PASTA

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Abstract

Ecological and nutritional challenges are accelerating the shift toward climate-smart crops and the enhancement of ‘staple foods’ nutritional quality. The European project Innofood Africa was launched to address these issues by fostering the production, processing, and consumption of African climate-smart crops. Within this context, the present study aimed to develop nutritionally optimized gluten-free pasta made from wholegrain flours of climate-smart legumes, cereals, and leafy vegetables, targeting both African and European adult populations.

Nutritional optimization was achieved through linear programming to meet the adult macro- and micronutrient recommendations established by the Food and Agriculture Organization of the United Nations (FAO) for a single meal, based on a three-meal daily intake, with particular emphasis on women’s nutritional requirements. Four optimized gluten-free pasta formulations were developed, all centered on cowpea, either alone or combined with teff and/or amaranth leaves. The pasta were produced using low-temperature extrusion and drying processes without additives in order to preserve their nutritional quality.

A standard portion (100 g dry weight) of pasta met FAO recommendations for protein, fiber, iron, zinc, vitamin B9, and, when amaranth leaves were included, beta-carotene. In vitro starch and protein digestibility were assessed using the COST method (Brodkorb et al. 2019, Minekus et al. 2014). i-PDCAAS obtained was compared to the i-PDCAAS obtained by the commercial Megazyme Kit (Kit K-PDCAAS). Cowpea-based pasta exhibited a low in vitro glycemic index, protein digestibility comparable to conventional wheat pasta, and markedly improved protein quality, with a PDCAAS approximately twofold higher due to a well-balanced essential amino acid profile. The i-PDCASS obtained via Megazyme kit was clearly overestimated whatever the pasta formulation. Sensory evaluation confirmed good consumer acceptability.

In addition, the study evaluated consumer acceptability and the potential contribution of these products to meeting nutritional recommendations in four African countries. The potential dietary impact was modeled by replacing 25%, 50%, or 100% of cereal-based foods in baseline diets derived from consumption surveys conducted in Kenya, Ethiopia, Uganda, and South Africa. Depending on the country, consuming between 12.5 g and 100 g of cooked pasta contributed substantially to meet zinc and iron recommendations in Kenya and Uganda and enabled achievement of vitamin A recommendations in South Africa.

Overall, these findings highlight the potential of linear programming–based formulation to enhance the nutritional value of staple foods. Complete substitution of durum wheat semolina with gluten-free climate-smart crops such as cowpea, teff, and amaranth leaf flours, maintained consumer acceptability while improving nutrient density and preserving a low glycemic index.

Keywords

Pasta, linear programming, protein and starch Digestibility, Sensory acceptability, Nutritional need coverage

Acknowledgements

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DIGESTION-RESISTANT IMMUNITY: NEW EVIDENCE FROM HUMAN MILK DIGESTOMICS.

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Abstract

Over the past decade, our research has investigated the intrinsic antiviral properties of human milk, demonstrating its activity against pediatric viruses such as human cytomegalovirus (HCMV), human rotavirus (HRoV), and respiratory syncytial virus (RSV), as well as emerging viruses, including Zika and Usutu. While our studies, and others in the field, have examined undigested human milk, little is known about how gastrointestinal digestion influences its antiviral activity. This gap in knowledge is particularly relevant, since digestion may alter, preserve, or even generate novel bioactive molecules capable of exerting systemic antiviral effects.

The current study aims to analyze the antiviral activity of breast milk using a static in vitro digestion protocol simulating the preterm infant digestive environment [1]. Colostrum, transitional, and mature milk samples were collected from 16 very preterm mothers (33 weeks GA), pooled according to lactation stage and donor serological status against HCMV, and subjected to digestion. The antiviral activity against HCMV, HRoV and RSV was evaluated using standard antiviral assays at the end of gastric phase, and at end of digestion.

Results confirmed the previously reported anti-HCMV activity of raw milk samples, and indicated that this antiviral effect is significantly enhanced following digestion, regardless of the mother's serological profile. Additionally, our results showed that the anti-HRoV activity of all milk samples was preserved throughout the entire digestive process. Assays on the evolution of anti-RSV activity are underway. Control experiments ruled out digestive enzymes as the source of this antiviral activity, suggesting either the resilience of immunological and bioactive components or the generation of new antiviral agents during digestion. Mass spectrometry has been employed to follow the protein digestion and peptide release in each sample, and bioinformatic tools are being employed to predict a structure-function relationships for milk-derived antiviral molecules.

To evaluate the retention of antiviral activity following intestinal absorption of the digested samples, a two-dimensional in vitro co-culture model of differentiated human epithelial and mucus-secreting cells is being used to mimic intestinal transport. Cell viability will be assessed using an MTT assay to determine non-cytotoxic concentrations for permeability studies. Bioavailable milk-derived components transported across the intestinal barrier will then be subsequently identified by UHPLC-HRMS.

By elucidating the mechanisms underlying the antiviral activity of digested human milk, our study provides a crucial link between epidemiological evidence of reduced viral infections in breastfed infants and in vitro data on the antiviral potential, offering a deeper understanding of how human milk contributes to neonatal protection.

[1] Vincent M, et al. 2020. doi: 10.1016/j.foodchem.2020.126927

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STRUCTURAL DIFFERENTIATION OF TUNA AND ALGAE OIL AND FUNCTIONAL IMPACT OF DHA CARRIERS ON BIOACCESSIBILITY, INTESTINAL UPTAKE, AND CELL INCORPORATION

Daniëlle W.R. Wessels [1 2 3] , Carlo Roberto de Bruin [1] , Henk-Jan Keijzer [1] , Elisa Mons [2] , Lonneke Janssen Duijghuijsen [4] , Shanna Bastiaan-Net [4] , Nicoletta Pelegrini [5 2] , Harry J. Wichers [4 1] , Vincenzo Fogliano [2] , Paula Perez Pardo [3] , Tamara Hoppenbrouwers [2 4] , Marie Hennebelle [1]

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Abstract

Docosahexaenoic acid (DHA) supports brain development, cognitive performance, cardiovascular health, and regulation of inflammation. Yet, population intake remains below recommended levels. Commercial supplements deliver DHA in different molecular forms: triacylglycerols (TAGs), phospholipids (PLs), free fatty acids (FFAs), or ethyl esters (EEs), which may influence digestion and absorption. Within TAGs, positional distribution on the glycerol backbone (sn-1/3 vs. sn-2) determines lipase processing, but its biological relevance remains unclear due to analytical limitations in resolving TAG positional isomers.

In this study, cyclic ion mobility mass spectrometry (cIMS-MS) was used to characterize DHA-containing TAG species in tuna and microalgal oil. Subsequently, seven DHA sources (two TAG oils, two phospholipid oils, free DHA lysine salt, free DHA, and EE-DHA) were evaluated on bioaccessibility, intestinal uptake using Caco-2 cells, and cell membrane incorporation of THP-1 macrophages.

The distribution of DHA on TAGs differed strongly between tuna and microalgal oils. Within the DHA-containing TAGs, Tuna oil was dominated by TAGs containing one DHA molecule (79.5 ± 0.1%), whereas microalgal oil contained substantially fewer (34.2 ± 0.4%), but markedly more TAGs containing either 2 (48.9 ± 0.4%) or 3 DHA molecules (16.9 ± 0.4%). In microalgal oil TAGs, 22.8±0.04% of sn-2 fatty acids were DHA, versus 12.5±0.04% in tuna oil. Despite structural differences in TAG composition, tuna and microalgal oils did not differ significantly in DHA bioaccessibility, epithelial uptake, or macrophage membrane incorporation. In contrast, krill oil , containing DHA in phospholipids , consistently outperformed other carriers, showing the highest DHA bioaccessibility post-digestion, superior Caco-2 transport, and increased DHA incorporation into macrophage membranes.

These findings indicate that lipid class, specifically phospholipid-bound DHA, exerts a greater influence on functional delivery than TAG sn-position alone.

Keywords

INFOGEST, Caco-2, DHA, Macrophages

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

PERFECT MATCHMAKING: FOOD PAIRING TO TARGET GASTRIC STARCH DIGESTION AND MODULATE POSTPRANDIAL GLYCAEMIA IN HEALTHY ADULTS UNDER FREE-LIVING CONDITIONS

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Abstract

Background

Mechanistic evidence from our previous in vitro and in vivo studies has confirmed that hydrolysis by salivary α-amylase during oral and gastric digestion phases is a critical step of starch digestion which can play a key role in the glycaemic response to starch-rich foods and meals. Building on this foundation, we have also investigated the digestive and metabolic impact of inhibiting this enzyme by lowering meal pH. This effectively delayed starch hydrolysis before the duodenal digestion step in vitro, and significantly decreased the glycaemic response in vivo under controlled conditions. Whether this strategy remains effective within the complexity of real-world dietary patterns and free-living conditions is not yet established.

Objective

To translate these in vitro and in vivo mechanistic insights into a real-world setting and evaluate whether targeting the gastric phase of starch digestion, through the combination of low-pH foods with starch-rich meals, effectively modulates postprandial glycaemia in humans across three levels of increasing dietary freedom.

Methods:

Twenty-three (n=23) healthy adults completed a three-phase intervention trial at The Azores (Portugal) (registered as NCT05456672). Phase 1: A randomized crossover design was used to compare entirely controlled low-pH versus neutral-pH daily meal days. Phase 2: The second phase introduced more flexibility by replacing only one meal a day with a standardized low- or neutral-pH option over four days to assess the effects at different times of the day. Phase 3: In the final phase, participants were provided with a neutral-pH food basket for 3 days, followed by an equivalent 3-day food basket with added low-pH items and were asked to follow specific guidance to self-select and match starch-rich foods with low-pH items. Throughout the study, participants' glucose levels were monitored using the FreeStyle Libre continuous glucose monitoring (CGM) system. Dietary adherence and intake were documented using detailed food diaries and photographic records.

Main Findings

Combining starch-rich foods with low-pH components consistently attenuated postprandial glycaemia across different levels of dietary freedom. In Phase 1, the controlled low-pH day resulted in lower average postprandial glycaemia compared to the neutral-pH day. Notably, in Phase 3, despite the level of freedom given to the participants and the free-living setting, following the food pairing guidance provided to include low-pH items in starch-rich meals successfully reduced average postprandial glycaemia levels compared to the neutral-pH basket period.

Conclusions

This study corroborates our previous findings that when it comes to starch digestion, the stomach matters as a strategic location for "perfect food matching" for glycaemic control. Moreover, the effectiveness of the proposed approach, validated by dietary tracking, highlights that precision food pairing can be highly effective for glycaemic control.

Keywords

Starch digestion , salivary α-amylase, continuous glucose monitoring, food pairing, meal pH.

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POSTER PRESENTATIONS

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EVALUATION OF THE CONTENT AND BIOACCESSIBILITY OF MINERALS FROM ENRICHED WITH IRON AND MAGNESIUM RICE PRODUCTS

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Abstract

The total contents of nutrients in food are not the only criterion determining its nutritional quality, as it also depends on their bioavailability from the product.

The objective of this study was to determine the content and the bioaccessibility of iron, magnesium, and calcium in commonly consumed enriched and unenriched crispy rice cakes purchased from the local market.

The analyses were carried out on three types of crispy rice cakes from one manufacturer: magnesium-enriched, iron-enriched, and unenriched. Mineral content in foods was determined using flame atomic spectrometry (ZA 3000 Hitachi) after mineralization in a Microwave Digestion system (Mars 2TM System).

The bioaccessibility of minerals was determined after enzymatic in vitro digestion. Samples of a finely ground food product were weighed and treated with deionised water and shaken. In order to create suitable conditions for pepsin action, pH was brought to 2 using 0.1 M HCl aqueous solution, then pepsin solution was added to the homogenate. Subsequently, samples were placed in a thermostat shaker for 2 h. During the incubation process, pH was assured or corrected by the addition of 6 M HCl aqueous solution, when necessary. After 2 h digested samples were treated with 6% $NaHCO_3$ aqueous solution to bring the pH to 6.8–7.0, subjected to pancreatin solution, and placed in a thermostatic shaker (37°C) for 4 h. Afterwards, the digested samples were centrifuged, and the clear solution was mineralised. The content of minerals in food products was expressed in mg/100 g dry mass, while the degree of a mineral released (bioaccessibility) was expressed as the amount of mineral (mg) liberated during the enzymatic digestion in vitro from 100 g of product and a percentage of a mineral released vs. its total content. Each product was analysed in triplicate.

It was found that significantly higher content of iron was found in the enriched product than in the unenriched crispy rice cakes. However, the bioaccessibility of iron was lower in the enriched product than unenriched product. The concentration of magnesium and its bioaccessibility in the enriched product were comparable to unenriched one. The content of calcium was significantly higher in unenriched than in enriched products, and bioaccessibility was comparable in the analysed crispy rice cakes.

In conclusion, enriched crispy rice cakes are not a better source of potentially bioavailable iron, magnesium, and calcium than unenriched ones.

Keywords

bioacessibility, minerals, enriched food

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EFFECT OF STRUCTURAL CONFORMATION ON THE DIGESTIBILITY OF PLANT PROTEINS

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Abstract

With the increasing pressure for transition from carbon-intensive animal protein production to more sustainable protein solutions for the increasing world population, various alternative protein (AP) sources including plant proteins have recently been under research and industrial investigation. However, their post-consumption challenges associated particularly with adverse immune reactions, low digestibility and bioavailability require attention and are crucial to ensure consumer safety, adequate nutrient provision and to build trust in APs.

In this study, we discussed the digestibility of different plant-based protein (PP) extracts and how the structural conformations can influence the protein digestibility and bioactive peptide release by a semi-dynamic in-vitro digestion model. Physiochemical characterisation was performed by dynamic light scattering (DLS). The secondary structural conformation was evaluated by CD and FTIR, microstructure was observed by confocal laser scanning microscopy, protein hydrolysis was tested by electrophoresis and amino acid release was done by OPA assay. All tested PP samples demonstrated large particle size with heterogeneous size distribution during gastric and intestinal phase. Proteins with higher % of α-helix or random coiled conformation showed a higher degree of enzymatic hydrolysis and amino acid release than the one with higher β-sheet confirmation i.e., hemp protein. Hemp also showed a slower gastric emptying rate with lowest digestibility/more stability during digestion among all proteins. This study highlights the effect of the protein conformation on DH/ digestibility of any proteins which will impact future food processing and guide future design of AP-based foods.

Reference: 1 Mulet-Cabero, A. I., Egger, L., Portmann, R., Ménard, O., Marze, S., Minekus, M. & Mackie, A. (2020). A standardised semi-dynamic in vitro digestion method suitable for food–an international consensus. Food & Function, 11(2), 1702-1720.

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Keywords

Plant-proteins, Semi dynamic In-vitro digestion, bioactive peptides, structural confirmations

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FOODOMIC STUDY OF BIOACTIVE PEPTIDES WITH ANTICANCER POTENTIAL GENERATED DURING GASTROINTESTINAL DIGESTION OF BUCKWHEAT PROTEIN (FAGOPYRUM ESCULENTUM)

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Abstract

Colorectal cancer is the third most common malignant neoplasm worldwide, and its incidence is projected to increase by approximately 60% by 2030. Nutritional epidemiology has increasingly highlighted the strong relationship between diet and cancer morbidity and mortality. Several dietary patterns with anticancer potential are associated with protein sources of plant, animal, or microbial origin, which can release bioactive peptides during gastrointestinal digestion. These peptides may exert biological activity by modulating molecular pathways involved in carcinogenesis, making the evaluation of their bioaccessibility and biological effects essential. Plant-derived peptides, in particular, have shown promising in vivo bioactivity and in situ anticancer effects against colon cancer. Buckwheat (Fagopyrum esculentum) is a pseudocereal characterized by high fiber content and a substantial protein fraction (8–18%), along with essential vitamins and bioactive compounds. In this study, buckwheat proteins were extracted and subjected to simulated gastrointestinal digestion using the standardized INFOGEST protocol in a dynamic digestion model that mimics oral, gastric, and intestinal phases. Peptide fractions obtained from each digestion phase were evaluated through cell-based bioassays to determine cellular viability, cytotoxicity, and in situ effects. Peptide identification was performed using liquid chromatography–high-resolution mass spectrometry (LC-HRMS). The results demonstrated the generation of bioactive peptides with antioxidant and anti-inflammatory activities during gastrointestinal digestion, with a higher abundance observed in the intestinal phase. These findings suggest that bioactive peptides derived from buckwheat proteins may represent a promising nutritional strategy and therapeutic complement for colorectal cancer prevention and management.

Keywords

Keywords: Buckwheat, bioactive peptides, anticancer, anti-inflammatory

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IMPROVING THE GLYCAEMIC PROPERTIES OF GLUTEN-FREE BAKED GOODS USING REPURPOSED FOOD INDUSTRY BY-PRODUCTS

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[1] Hungarian University of Agriculture and Life Sciences, Institute of Food Science and Technology, Department of Food Chemistry and Analysis, [2] University of Cambridge, Christ's College

Abstract

Demand for gluten-free foods has expanded beyond celiac patients to health-conscious consumers, leading to increased use of gluten-free grains and pseudocereals in baked goods. These ingredients typically have lower protein and higher carbohydrate content than wheat, and the absence of gluten often results in unfavourable glycaemic properties. High glycaemic index (GI) foods can trigger rapid blood glucose rise, increasing long-term risk of type 2 diabetes and obesity. Strategies to reduce GI include protein enrichment, which may slows carbohydrate digestion, and the addition of bioactive compounds such as polyphenols that may inhibit digestive enzymes.

In this study, two milk protein concentrates (MPC and iMPC with improved DIAAS) and a polyphenol-rich apple pomace extract (POE) were incorporated into corn-based gluten-free breads at varying concentrations to lower the GI of the gluten free bread. Products underwent in vitro digestion using a hybrid Infogest protocol, with glucose release measured at multiple timepoints via UV-Vis spectrophotometry after isolation and hydrolysis of the bioaccessible fraction. The in vitro GI-like value was calculated and compared to i) white bread and ii) plain corn bread controls.

Results showed that the corn bread had indeed higher glucose response than the wheat based white bread therefore the effect of the absence of the gluten network was measurable using our chosen method. The addition of the MPC and iMPC into the dough of the corn bread lowered small intestinal starch digestion. Although the changes have not been statistically significant in either cases the release shifted closer to the release registered from white bread (with gluten network slowing the digestion). The addition of the polyphenol-rich apple pomace extract (POE) was able to lower the gastric glucose release when combined with the iMPC but not with MPC. In this instance, i.e., the combination of iMPC and POE, not only the gastric digestion was influenced but in addition the small intestinal glucose release was lowered significantly (at time points 120 min ($p=0.017$), 180 min ($p=0.020$), and 210 min ($p=0.039$).

Corn-based gluten-free bread showed higher glycaemic response than wheat bread, confirming the impact of gluten absence. Adding milk protein concentrates (MPC, iMPC) moderately reduced starch digestion, though not significantly. The combination of improved milk protein (iMPC) and polyphenol-rich apple pomace extract (POE) significantly lowered gastric and intestinal glucose release, demonstrating a synergistic effect on glycaemic reduction.

Keywords

Gluten free, Bakery products, Hybrid Infogest, Glycaemic index, By-product valorisation

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

ENHANCING PROTEIN RECOVERY AND DIGESTIBILITY FROM OAT OKARA: A PROMISING PLANT-BASED INGREDIENT

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Abstract

The increasing popularity of oat-based beverages has resulted in higher production of oat okara, a nutrient-rich by-product that is typically used as animal feed. With a high protein content of 26%, fat content of 12%, and a significant amount of fibre, oat okara shows great potential as a sustainable plant protein source. This project aimed to enhance its functional properties and digestibility for human consumption, as well as to investigate its applications in oat-based beverages.

Oat okara was analysed for its composition and then processed using pH-shift extraction combined with mechanical methods like Ultrasonication and Ultra-Turrax. Protein solubility showed considerable variation with pH, highest at pH 12 (35.12 ± 1.93%) and reaching its lowest at pH 4 (2.76 ± 0.96%). Alkaline extraction achieved the highest protein recovery at 43.56 ± 3.26%. This increased to 55.69 ± 2.29% with ultrasonication (280 W for 3 minutes) and to 55.18 ± 2.16% with Ultra-Turrax (22,899 RPM for 3.25 minutes). Freeze-drying preserved protein integrity better than spray-drying, resulting in higher recovery (93.01 ± 2.52 vs. 67.67 ± 1.03%). Digestibility was evaluated using the in vitro model, and the degree of hydrolysis showed significant improvement with mechanical treatments. Ultrasonication increased gastric and intestinal digestibility by 18.44% and 47.63%, respectively, while the Ultra-Turrax method achieved similar enhancements, reaching up to 47.93%. Additionally, combining these treatments followed by freeze-drying further improved protein accessibility.

These findings demonstrate that processing strategies significantly enhance the recovery, solubility, and digestibility of oat okara protein. As a result, this improved form of protein can be successfully integrated into high-protein, stable oat-based beverages. This research not only underscores the potential of oat okara as a valuable ingredient but also emphasises its role in promoting sustainable plant-based diets and advancing circular food systems, ultimately contributing to a more environmentally friendly food landscape.

Keywords

Oat okara, Digestibility, Solubility, Ultrasonication, Ultra-turrax, Valorisation.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

CELLULAR HETEROGENEITY IN PULSES: THE MISSING LINK BETWEEN COTYLEDON CELL PROPERTIES AND DIGESTION KINETICS

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Abstract

Understanding which structural and compositional features govern macronutrient digestion in complex biological materials is essential for advancing the design of healthy plant-based foods. In pulses, starch and protein are bioencapsulated within cotyledon cells, attenuating digestion kinetics. However, the specific cell properties that steer these kinetics remain unclear. This study investigated how morphological and compositional characteristics of individual cotyledon cells (ICCs) from seven pulse batches (both the same and different pulse types) relate to in vitro digestion behavior (INFOGEST 2.0). ICCs were isolated from seeds cooked to batch-specific equivalent cooking times and characterized for composition and cell morphology.

Substantial variability in ICC properties was observed both within and across pulse types. Chickpea ICCs consistently exhibited the slowest amylolysis, although no direct associations with measured ICC descriptors could be established. Proteolysis kinetics correlated negatively with applied cooking time and with cell wall–related barrier features. Other hypothesized drivers of macronutrient digestion kinetics, such as cell size, showed no discernible relations at the ICC batch level.

Importantly, pronounced morphological heterogeneity within batches was observed and hypothesized to hamper the identification of causal links between averaged ICC properties and digestion outcomes. To clarify whether this batch heterogeneity masked underlying mechanisms, the impact of cell size on black bean ICC digestion was investigated upon fractionating ICCs into four size classes. Smaller ICCs showed markedly accelerated starch and protein digestion, explained by an increased surface-to-volume ratio facilitating enzyme penetration and reduced intracellular diffusion distances. Microscopy confirmed cell size-dependent patterns of cellular emptying.

These results demonstrate that batch heterogeneity can hide mechanistic determinants of macronutrient digestion in pulses. Accounting for intra-batch heterogeneity is therefore critical for future work aiming to elucidate mechanisms steering digestion in heterogeneous biological matrices such as pulses.

Keywords

in vitro digestion, starch, protein, heterogeneity, pulses, cell size

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

MICROSTRUCTURAL CHANGES IN HUMAN MILK DURING INFANT IN VITRO DIGESTION

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Abstract

Human milk (HM) is a biocolloid whose composition and structural organization of lipids, proteins and carbohydrates evolve to meet the changing nutritional and immunological needs of the infant. Breastfeeding is widely recognized to provide both short and long-term health benefits; however, the regulatory mechanisms governing the interactions between maternal milk and the neonate remain incompletely understood. These interactions primarily take place within the gastrointestinal tract and involve digestion-driven structural transformations of milk components, which in turn influence the breakdown, bioaccessibility, and utilization of macronutrients.

Our extensive study focused on variations that reflected digestion-driven differences between colostrum and mature HM and highlighted key contrasts relative to cow's milk, which constitutes the primary basis of most commercial infant formulas used for infant feeding. We applied a miniaturised semi-dynamic in vitro digestion model designed to reproduce the physicochemical and enzymatic conditions of the infant gastric environment. This model constitutes a key innovation, as it enables digestion experiments to be carried out with small volumes (ca. 5 mL) of biological material, addressing a major constraint in HM research, particularly in studies involving early lactation samples. Structural evolution of HM during gastric digestion was monitored using light-scattering–based analytical approaches, providing insight into digestion-induced changes in particle size distribution, aggregation behaviour, and phase separation over time. Progressive gastric emptying was reproduced by collecting digesta at defined time intervals.

The behaviour of HM was strongly influenced by the gastric enzymatic hydrolysis, which contributed to microstructural changes. Due to continuous acidification of the gastric digestion mixture, we observed that the milk digesta underwent substantial reorganisation, including fat droplet flocculation and coalescence, protein aggregation and formation of the gel that was gradual broken down by the enzymes. These structural changes of HM resulted in phase separation, with lipid-rich fractions rising to the top and protein aggregates settling at the bottom of the simulated stomach. The cow's milk behaved differently, with sedimentation of aggregated proteins being a dominant phase separation process.

Our results highlight a close relationship between the digestive fate of HM and its microstructural transformations under gastric conditions. Understanding these processes is crucial not only for developing physiologically relevant models of digestion for term and preterm infants, but also for guiding the design of infant formulas (IFs) that replicate the structural and functional behaviour of HM during digestion. IFs should go beyond matching the concentrations of selected HM components, such as macronutrients, and aim to achieve comparable structural dynamics and functional performance.

Keywords

human milk, microstructural changes, in vitro digestion, infant model

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

PROTEIN FORTIFICATION OF HOME-DELIVERED SOUPS: IMPROVING NUTRITIONAL QUALITY AND DIGESTIBILITY FOR SENIORS

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Abstract

Aging is associated with various health problems, including a higher risk of chronic diseases, mobility limitations, and cognitive decline. The growing need for healthcare and support for older adults represents a major economic challenge. Personalized nutrition, such as meal delivery services tailored to individual health needs, offers a promising avenue to foster healthy aging and reduce healthcare costs. However, current home-delivered meals often fail to meet the specific nutritional requirements of older adults. This work aims to develop protein-enriched recipes that are nutritionally balanced, easy to digest, and palatable.

Five soups—pumpkin, lentil, mushroom, chickpea, and split pea—were selected from menus used in home-delivered meals and a nursing home in Ambert. Each soup was enriched using one of four methods: ham and La Vache qui rit, La Vache qui rit+, egg yolk, and spirulina. A sensory panel first evaluated the 25 soup variants through hedonic testing, followed by in vitro digestion studies using the Minekus protocol. Released peptides and amino acids were quantified by Kjeldahl and HPLC analyses, and oxidation levels were assessed via TBARS.

Enrichments using La Vache qui rit+ and the combination of ham with La Vache qui rit were the most appreciated, particularly in pumpkin and mushroom soups. Spirulina was poorly accepted due to its strong, unpleasant flavor. Pumpkin soups received the highest ratings overall, partly due to an unintended addition of cream that enhanced flavor and texture. Chickpea soups enriched with egg yolk were less appreciated, as the neutral base failed to mask sulfurous and metallic notes from lipid oxidation.

Protein enrichment significantly increased protein content, particularly in soups initially low in protein. For example, protein levels rose 3.2-fold in pumpkin soup but only 1.2-fold in the protein-rich lentil soup. The “Ham & La Vache qui rit” combination produced the highest overall protein content. Statistically significant differences (P 0.05) were observed in mushroom-, pumpkin-, and split pea–based soups, which also showed higher bioaccessible protein, amino acid levels, and improved digestibility compared with controls.

Spirulina and egg yolk enrichments enhanced bioaccessible essential amino acids, particularly branched-chain amino acids (BCAAs) such as isoleucine and valine. In contrast, La Vache qui rit+ and ham-based enrichments led to higher release of low–molecular-weight peptides, indicating superior protein digestibility.

This approach enhances the nutritional quality of soups by increasing protein content, improving digestibility and amino acid bioaccessibility, and stabilizing lipid oxidation. Effects depend on the soup composition: pumpkin- and mushroom-based soups show the best performance, while lentil soups benefit less. A larger-scale hedonic evaluation is ongoing with residents of the Ambert nursing home and recipients of local home-delivered meals.

Keywords

Protein enrichment, Older adults, Digestibility, Amino acid bioaccessibility, Functional soups

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IMPACT OF ULTRASOUND PRE-TREATMENT ON CELL WALL STRUCTURE AND PROTEIN DIGESTIBILITY OF EDIBLE SEAWEEDS

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Abstract

The growing global demand for food, coupled with the scarcity of arable land, has intensified the search for sustainable, non-animal protein sources. Seaweeds have emerged as promising candidates due to their high protein and fiber content, as well as their low environmental footprint. However, the bioaccessibility and digestibility of seaweed proteins are strongly limited by the complexity and mechanical resistance of their cell wall structures. Moreover, since seaweed species differ markedly in cell wall composition and organization, a single processing strategy may not be equally effective for all of them. Understanding how different species respond to specific treatments is therefore essential to identify the most suitable form in which each seaweed should be consumed in order to maximize protein digestibility.

This study focused on evaluating the effect of an ultrasound (US) pre-treatment applied to the biomass of three edible seaweeds (Ulva spp., Porphyra spp., and Gracilaria spp.), assessing the structural modifications induced by the treatment and the potential effect on the accessibility of digestive enzymes, thereby improving protein digestibility. To this end, three US exposure times were applied to examine the extent of cell wall disruption and its relationship with protein accessibility. Structural changes were characterized using confocal laser scanning microscopy (CLSM), X-ray diffraction (XRD), and small-angle X-ray scattering (SAXS), while protein digestibility was determined using the harmonized INFOGEST in vitro digestion protocol.

The results showed that the effect of ultrasound was strongly species-dependent. In Ulva, short treatment times were sufficient to disrupt the cell wall, and longer exposures led to a clear improvement in protein digestibility. In Gracilaria, ultrasound induced structural rearrangements associated with agar gelation, resulting in only minor increases in digestibility. In contrast, Porphyra exhibited high protein digestibility in the untreated state, and although ultrasound altered cell wall structure, it did not significantly enhance digestibility.

Overall, these results demonstrate that the effectiveness of mechanical pre-treatments in improving protein digestibility in seaweeds depends on the intrinsic cell wall structure of each species, highlighting the need for customized processing strategies to enhance the nutritional quality of proteins in seaweed-based food formulations.

Keywords

Seaweeds, alternative proteins, polysaccharides, ultrasounds, in vitro gastrointestinal digestion

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

FRACTIONATION ROUTE AND HEAT TREATMENT SHAPE IN VITRO PROTEIN AND STARCH DIGESTIBILITY IN OAT PROTEIN INGREDIENTS

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Abstract

As demand for sustainable plant-based proteins grows, oats are gaining attention because of their relatively high protein quality (DIAAS) and their dietary fiber, which can modulate postprandial metabolic responses. However, the processing routes used to concentrate oat protein markedly alter both composition and matrix organization, which can in turn influence nutrient digestion. This study examined how fractionation route and heat treatment affect the in vitro digestibility of protein and starch in oat protein ingredients.

Three oat protein concentrates were produced by dry fractionation (fine milling and air classification), alkaline extraction followed by isoelectric precipitation (AE-IEP), and water-only extraction. These routes generated materials with markedly different starch–protein ratios, from starch-rich dry-fractionated concentrates to highly protein-enriched wet-extracted isolates. Each ingredient was subjected to thermal treatment at 70 °C and 110 °C, and characterized for macronutrient composition, protein profile (SDS-PAGE), thermal behavior (DSC), and microstructure (SEM). Protein and starch digestibility were determined after the INFOGEST static digestion protocol using OPA and DNS assays.

Protein digestibility depended strongly on fractionation route. Oat flour (66%) and the dry-fractionated concentrate (61%) showed the highest values, followed by the AE-IEP isolate (44%), while the water-only concentrate showed the lowest digestibility (26%). These differences were not explained by protein profiles, which were similar by SDS-PAGE. Instead, SEM and DSC indicated that wet-extracted proteins, particularly in the water-only concentrate, remained largely native and formed compact structures, whereas dry fractionation produced a heterogeneous protein–starch matrix; these structural differences coincided with differences in proteolysis. Heating to 110 °C reduced protein digestibility most strongly in oat flour and the dry-fractionated concentrate, which showed complete protein denaturation by DSC, while the wet-extracted ingredients showed incomplete denaturation. Proteins in all fractions were largely native before heating, indicating that fractionation itself did not cause denaturation and that the observed heat effects reflect matrix-dependent thermal responses. Starch digestibility differed between extraction routes mainly because the water-only concentrate showed lower starch hydrolysis, a difference that disappeared after thermal treatment.

Overall, fractionation route and thermal treatment jointly shape protein and starch digestibility in oat ingredients. The differences between dry-fractionated and wet-extracted materials reflect matrix organization and macronutrient distribution rather than protein composition alone, showing that processing history can be used to tune digestive behavior.

Keywords

in vitro digestion, INFOGEST, oat protein, oat starch, wet extraction, dry fractionation

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

HOW DRYING TECHNOLOGIES SHAPE THE BIOACCESSIBILITY AND RELEASE OF POLYPHENOLS IN DRIED FRUITS AND VEGETABLES DURING DIGESTION: A CRITICAL EVALUATION

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Abstract

Drying is widely used to extend the shelf life of fruits and vegetables, yet its implications for the nutritional functionality of polyphenols are often evaluated solely in terms of compound retention. Emerging evidence suggests that preservation alone is insufficient to predict nutritional impact, as drying-induced microstructural and chemical changes strongly influence the release and bioaccessibility of polyphenols during gastrointestinal digestion. This work presents a critical synthesis of 16 in vitro digestion studies investigating the effects of diverse drying techniques, including convective hot-air, freeze-drying, vacuum drying, microwave-vacuum drying, infrared drying, and ultrasound-assisted drying, on the digestive behaviour of total phenolics, flavonoids, and anthocyanins in dried fruits and vegetables.

Across matrices, the oral phase contributed minimally to polyphenol release, whereas the gastric phase represented the dominant liberation step due to acid-induced swelling, matrix softening, and enhanced diffusivity. The intestinal phase emerged as a decisive bottleneck, where alkaline instability, enzymatic interactions, and complexation often reduced the bioaccessible fraction, particularly for anthocyanins. Drying methods that induced moderate structural disruption under reduced oxygen (e.g., vacuum-based, microwave-vacuum, and infrared-assisted drying) consistently enhanced polyphenol bioaccessibility compared with freeze-drying, which preserved compounds but restricted release, and hot-air drying, which promoted release but often accelerated oxidative losses.

This critical review further highlights the critical role of bound polyphenols, whose liberation during digestion is governed by both microstructural accessibility and chemical stability. Collectively, these findings demonstrate that drying technology determines not only how much of a polyphenol remains in a food product, but also how much becomes nutritionally available. This work advocates for a paradigm shift from retention-based evaluation to bioaccessibility-centered assessment in dried foods, with implications for process optimization, functional food design, and nutritional labeling.

Keywords

drying technologies, polyphenols, bioaccessibility, in vitro digestion, microstructure

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

FOOD STRUCTURE MODULATION OF ANTIOXIDANT BIOACCESSIBILITY AND GUT EPITHELIAL PROTECTION: THE CASE OF BREAD AND PASTA MADE WITH PIGMENTED WHEAT

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Abstract

Food structure plays a key role in modulating nutrient bioaccessibility and antioxidant activity during gastrointestinal digestion of cereal-based foods. This study examined how semolina type [pigmented grains (Grano Mischio, GM) vs. traditional wheat (Senatore Cappelli, SC)], and the food matrix (bread vs. pasta) influence product quality, in vitro starch digestibility, antioxidant capacity, and intestinal barrier integrity of Caco-2 cells. GM-bread had lower specific volume and was harder than SC-bread, while GM-pasta showed greater cooking loss and significantly higher starch digestibility compared to SC-pasta, probably due to polyphenol-induced structure weakening. Antioxidant activity, measured by ORAC, DPPH, and ABTS assays, varied by matrix and method, with GM-pasta exhibiting higher radical scavenging capacity than GM-bread in the DPPH and ABTS assays. Caco-2 cells treated with digested GM-pasta showed increased viability, enhanced transepithelial electrical resistance, and reduced inflammatory markers (IL-1β, IL-11, NF-κB) under pro-inflammatory conditions. Overall, pigmented wheat products, particularly pasta, retain antioxidant properties upon digestion. These findings provide evidence that food processing can modulate the biological properties of raw cereal materials, laying a promising foundation for the rational design of functional foods that leverage matrix architecture to optimize the release and efficacy of bioactive compounds during digestion.

Keywords

Pigmented wheat, Food matrix, In vitro digestion, Starch digestibility, Antioxidant activity, Caco-2 cells.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

POST-DIGESTIVE CYTOTOXIC EFFECTS INDUCED BY OVERHEATED INFANT FORMULAE WITH INTACT COW'S MILK PROTEINS: IN VITRO AND IN SILICO EVIDENCE

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Abstract

Adequate nutrition in the early months of life is essential for the proper development of infant's immune, gastrointestinal and neurological systems. When breastfeeding is not an option, or cannot fully meet nutritional needs, infant standard formulae (SF) are a vital alternative. These products are carefully formulated to fulfil infants' nutritional requirements during this sensitive period, providing complete nourishment until complementary foods are introduced. Formulae undergo industrial and domestic heat treatments to ensure microbiological safety. However, excessive heating has been shown to modify protein structure, reducing digestibility and altering peptide release. This study used in vitro and in silico models to analyse the impact of overheating on the digestion of cow's milk proteins.

A cell model using the Caco-2 cell line was employed to asses cytotoxicity. This line was subjected to various extracts obtained from the INFOGEST's in vitro infant digestion of SF (with intact proteins), with and without prior heat treatment (tyndalisation). Additionally, digested extracts from extensively hydrolysed formulae (with proteins broken down into small peptides) were used as a control. Cell viability was monitored using the non-invasive real-time cell analyze (RTCA-DP) technology. The digested extracts of the tyndalised and non-tyndalised standard formulae were analysed using nano-UHPLC–MS/MS to determine their peptide profile. In silico predictors were employed for toxicity and bioactivity analysis.

The Caco-2 cell line in vitro model showed that the standard tyndalised formulae, with intact milk proteins, had an immediate cytotoxic effect, that was not detected in the non-tyndalised formulae. Besides, the hydrolysed formulae did not exhibit this effect. Similarly, in silico model, indicated that the effect of tyndalisation reduces the number of bioactive peptides, though the profile of biological activities remains unchanged.

In conclusion, these in vitro and in silico approaches demonstrate that tyndalisation significantly alters the peptide profile of infant formulae, quantitatively reducing their expected bioactivity and inducing cytotoxic effects in an intestinal cell model.

Keywords

Infant formulae, tyndalisation, cytotoxicity, bioactivity, cow´s milk-proteins

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

LACTIC ACID BACTERIA FERMENTATION OF BREWERS SPENT YEAST: ENHANCING DIGESTIBILITY, AMINO ACID BIOACCESSIBILITY, AND GUT MICROBIOME MODULATION POTENTIAL

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Abstract

With an ever-increasing global population and dwindling natural resources, a shift towards more sustainable food systems is required. Approaches to aid this transition include the valorisation of sidestreams to minimise food waste, and movement towards non-animal proteins. Brewers spent yeast (BSY) is a brewing by-product which is generally regarded as waste, despite its high nutritional value. This study explored the impact of of lactic acid bacteria (LAB) fermentation on the in vitro protein quality of BSY using the static INFOGEST digestion protocol coupled with a protein quality assessment workflow based on amino acid analysis. The findings showed that fermented BSY (FBSY) had a superior protein quality compared to the control (CBSY), with overall protein digestibility increasing almost two-fold from 40% to 73%. FBSY also demonstrated significantly higher bioaccessibility values for almost all amino acids, and a markedly increased in vitro digestible indispensable amino acid score (DIAAS) (17 % for CBSY vs. 98 % for FBSY). To assess potential effects on the gut microbiome, FBSY was further evaluated using an in vitro colon fermentation model, where it outperformed CBSY by promoting higher α-diversity indices and a greater enrichment of beneficial Mediterranean diet-responsive taxa after 24 h. Together, these findings highlight the capacity of LAB fermentation to transform BSY into a high-quality, digestible protein ingredient with microbiome-modulating potential. Such valorisation contributes to reducing food waste while supporting the development of more resilient and sustainable global food systems.

Keywords

Valorisation, brewing by-product, INFOGEST, protein digestibilty, DIAAS, microbiome

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

INVESTIGATION OF THE IN VITRO PROTEIN DIGESTIBILITY AND QUALITY OF NOVEL BREWERS' SPENT GRAIN PROTEIN ISOLATES USING THE INFOGEST MODEL

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Abstract

Mounting pressures on global food chains call for an urgent shift towards more sustainable food systems. Two approaches to aid this transformation include valorising side streams to minimise food waste and reducing reliance on animal-derived proteins in favour of plant-based alternatives. As plant-based proteins are gaining popularity as alternatives to dairy and meat due to the rising demand for healthy, natural foods and their ethical appeal, it is essential that the protein quality of novel, alternative protein sources is evaluated. Brewers' spent grain (BSG), a nutrient-dense by-product of the brewing process, has been upcycled to generate two barley-rice protein isolates: everpro™ Dark Fraction (EDF) and a decolourised counterpart, everpro™ Light Fraction (ELF). This study assesses the in vitro protein and amino acid digestibility, as well as the in vitro digestible indispensable amino acid score (DIAAS) of EDF and ELF in comparison to legume (pea and soy) and dairy (whey) protein benchmarks using the static INFOGEST digestion protocol. All protein sources exhibited high overall digestibility (>80%). EDF and ELF achieved in vitro DIAAS values of 51% and 37%, respectively, lower than those of soy or whey, yet comparable to or higher than the values obtained for the pea proteins examined. Differences in the digestibility of individual amino acids in EDF and ELF were observed, indicating that the decolourisation process may be impacting the nutritional profile. Overall, these findings demonstrate the potential of everpro™ ingredients as sustainable, plant-based protein alternatives capable of supporting the ongoing transformation of the food industry.

Keywords

Brewers' spent grain; valorisation; digestibility; in vitro DIAAS; sustainability, plant-based proteins

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

PROTEINS AND PHYCOCYANIN FROM PHORMIDIUM VERSICOLOR: FUNCTIONAL, NUTRITIONAL, AND APPLICATION POTENTIAL

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Abstract

Abstract

Proteins and C-phycocyanin extracted from Phormidium versicolor were evaluated for their nutritional, structural, and technofunctional properties. The protein isolate exhibited a high water-holding capacity (3.8 g water/g), good oil-holding capacity, excellent emulsifying activity at low concentrations (1–2%), and a remarkable foaming capacity reaching 221%, with high foam stability, supporting its application in emulsified and aerated food systems. Nutritional analysis revealed a balanced essential amino acid profile, with amino acid scores exceeding reference values for both adults and children, along with good in vitro digestibility and Hazard and Risk Index values well below safety thresholds.

Purified C-phycocyanin retained its structural integrity, as confirmed by SDS-PAGE (α ~17 kDa, β ~19 kDa) and FTIR analysis, and showed an amorphous structure (XRD) favorable for solubility. Thermal analyses (TGA/DSC) indicated typical protein–pigment stability. Functionally, C-phycocyanin displayed moderate water-holding capacity, good oil-holding capacity, and excellent emulsifying activity at 1–2%, with increased stability at higher concentrations. Overall, these results highlight P. versicolor as a safe, nutritionally valuable, and multifunctional microalgal resource for sustainable food and nutraceutical applications.

Keywords

Keywords : Phormidium versicolor, proteins, phycocyanin, essential amino acids, digestibility, technofunctional pro

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

DYNAMIC GASTRIC PROCESSING AND ITS IMPACT ON NUTRIENT AND BIOACTIVE AVAILABILITY

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Abstract

In vitro digestion models are widely used to investigate food breakdown and nutrient release during gastrointestinal transit; however, most rely on static or semi-dynamic gastric phases that cannot reproduce the mechanical forces, secretion dynamics, or spatial heterogeneity of the human stomach.

This study examined how dynamic gastric processing influences food disintegration and nutrient and bioactive availability using a physiologically validated Dynamic Gastric Model (DGM) that simulates the fundus, main body, and antrum under fed-state conditions. Model food systems representing structured protein matrices and lipid-containing composite foods were digested using both a conventional static gastric model and the DGM. Structural breakdown, particle size evolution, and nutrient and bioactive release were assessed using time-resolved sampling.

Compared with static digestion, dynamic gastric processing resulted in distinct disintegration kinetics, heterogeneous and size-selective emptying behaviour, and time-dependent structural transformations only observable under dynamic conditions. Dynamic simulation also enables investigation of broader food science questions, including nutrient and phytochemical bioaccessibility from complex meals, effects of mastication and food structure on digestion, probiotic survival, and predictors of glycaemic response.

These results demonstrate that while static gastric models remain valuable for reproducible studies, they cannot resolve key mechanistic processes governing food structure transformation, emptying, and nutrient and bioactive availability. Dynamic gastric simulation provides a physiologically relevant complement to existing in vitro approaches, improving mechanistic interpretation and the predictive value of digestion studies relevant to food formulation, processing, structure-function relationships, and functional food design.

Keywords

Dynamic gastric model, in vitro digestion, nutrient release, food matrix, gastric-emptying

Acknowledgements

The Dynamic Gastric Model (DGM) was originally created at the Quadram Institute Bioscience (formerly the Institute of Food Research).

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECT OF FAT SCAFFOLD TYPE AND FREEZING ON LIPID AND PROTEIN DIGESTIBILITY PLANT-BASED BURGER PATTIES

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Abstract

The incorporation of structured fats is a key strategy to improve the functionality of plant-based meat analogues; however, their effects on digestive behavior remain poorly understood. In this study, two plant-based burger patty formulations were developed using texturised vegetal protein and other plant ingredients, combined with different proportions (0, 10, 15, and 20%) of fat scaffolds based on methylcellulose–coconut oil (MCO) or methylcellulose–sunflower oleosomes (MSO). Patty replicas were frozen for 30 days to assess the effect of storage and cooking on protein and lipid digestibility using the standardized INFOGEST 2.0 protocol.Protein solubility during digestion in MCO patties was significantly reduced by freezing (p0.05), with higher soluble protein values observed in non-frozen samples (>1,200 µg BSA/mL). In contrast, MSO patties showed no significant differences (p>0.05) between frozen and non-frozen treatments; instead, soluble protein levels were primarily driven by fat scaffold proportion. Overall, the range of soluble protein values observed in the developed formulations after digestion (700−1,200 µg BSA/mL) was comparable to that of commercial plant-based burgers (650−1,100 µg BSA/mL), while oleosome-based patties exhibited protein solubility patterns more similar to beef patties than MCO formulations. Peptide release during digestion was negatively affected by increasing fat scaffold proportion in all formulations, regardless of fat type. Nevertheless, during the oral and gastric phases, all experimental formulations exhibited higher peptide release than commercial plant-based burgers (p0.05). During the intestinal phase, peptide release remained higher (904−1380 µg/mL) than that of commercial plant-based products (760−1,609 µg/mL), with the exception of beef patties (1,804 µg/mL), which showed the highest values (p0.05). After digestion, patties containing 15 and 20% MSO exhibited significantly higher lipolysis (p0.05), reaching values up to 6-fold higher than those observed in formulations with 0 and 10% fat scaffold. MCO patties also showed increased lipolysis, up to 4-fold, but these values were significantly lower (p0.05) than those obtained with MSO-based systems. These findings demonstrate that fat scaffold composition and proportion strongly influence the digestive behavior of plant-based burgers. MSO enhanced lipid digestibility and promoted protein solubility patterns closer to beef patties, while maintaining protein solubility ranges comparable to market alternatives. Despite reduced peptide release at higher fat levels, the developed formulations showed superior early-stage proteolysis compared to commercial plant-based burgers, highlighting their potential for improved nutritional performance rather than texture mimicry.

Keywords

Alternative proteins, lipolysis, in vitro digestion, oleosomes, meat analogues, proteolysis

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IN VITRO GASTROINTESTINAL DIGESTION OF THE FUNCTIONAL CEREAL BARS ADDED WITH BIXIN ANNATTO

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Abstract

Bixin extract, encapsulated by ionic gelation using three distinct polymer systems, was characterized to identify the optimal delivery vehicle for integration into a cereal bar formulation. Free bixin and encapsulated bixin microparticles, as well as the fortified cereal bars, were evaluated using the INFOGEST in vitro gastrointestinal digestion protocol. During the oral phase, samples were homogenized with simulated salivary fluid (SSF) and human salivary α-amylase solution (75 µg.mL-1). The resulting mixture was adjusted (pH 7). The gastric phase involved mixing the oral bolus with simulated gastric fluid (SGF) and porcine pepsin solution (2000 µg mL-1) at pH 3.0 for 2 hours. Finally, the intestinal phase was conducted using simulated intestinal fluid (SIF) containing pancreatin (100 µg mL-1) based on trypsin activity) and bile salts (10 mM) at pH 7.0 for 2 hours. Post-digestion, supernatants were analyzed spectrophotometrically to determine bixin release and bioaccessibility. Among the encapsulated systems, the release rates followed the trend: alginate+gelatin (AG) > alginate+carrageenan > pure alginate. Specifically, AG microparticles showed the highest release, with wet microparticles (AGU) reaching 59.79% and freeze-dried microparticles (AGL) reaching 61.68%. Correlation with microscopy suggests that highly porous microparticles with low release, such as pure alginate (ALL), likely allowed enzyme and acid penetration, leading to bixin degradation. The cereal bar formulation demonstrated effective bixin delivery, with the highest concentration occurring during the intestinal phase, (>60%) followed by the gastric and oral phases. These results demonstrate a successful strategy for stabilizing annatto extract, providing a functional food alternative with enhanced bioactive delivery.

Keywords

Encapsulated bexin, digestibility, cereal bars

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

PROCESSING-INDUCED CHANGES IN IRON UPTAKE FROM LEGUME-BASED FOODS

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Abstract

Legumes are key contributors to iron intake in plant-based diets; however, iron bioavailability is often limited by phytate, which strongly chelates iron and other minerals, reducing their intestinal absorption (López et al., 2025; Reddy et al., 1982). Food processing can be used to substantially reduce phytate content, thereby increasing iron bioaccessibility and uptake (Erkan et al., 2020). This study investigated how different processing strategies affect phytate–iron interactions and iron uptake from soy, faba bean, grey pea, and yellow pea.

Legumes were processed by soaking and cooking, protein coagulation (tofu-like products), or fungal fermentation (tempeh-like products). Iron and phytate concentrations were quantified, and phytate-to-iron molar ratios were calculated as indicators of iron bioavailability. To assess functional iron uptake, samples were subjected to in vitro digestion followed by exposure to a Caco-2/HT29-MTX intestinal co-culture model. Cellular ferritin formation was used as a marker of intracellular iron uptake.

Protein coagulation resulted in the highest iron concentrations across all legumes but also retained or concentrated phytate, leading to elevated phytate-to-iron molar ratios associated with low predicted iron bioavailability. In contrast, fermentation caused a pronounced reduction in phytate levels in all legumes, with phytate falling below detection limits in faba bean and grey pea tempeh. This resulted in phytate-to-iron molar ratios 1, indicating minimal chelation of iron with phytate. Consistent with these findings, fermented products induced significantly higher ferritin formation in intestinal cells compared to tofu-like products, consistent with improved iron uptake at the cellular level

Overall, the results demonstrated that fermentation is a highly effective processing strategy for improving iron uptake from legumes by reducing phytate in legumes, whereas protein coagulation may increase total iron content without proportionally enhancing bioavailability. These findings underlined the importance of considering phytate–iron interactions, rather than iron concentration alone, when designing legume-based foods aimed at improving iron nutrition in plant-based diets.

Erkan, S. B., Gürler, H. N., Bilgin, D. G., Germec, M., & Turhan, I. (2020). Production and characterization of tempehs from different sources of legume by Rhizopus oligosporus. Lwt, 119, 108880. https://doi.org/10.1016/j.lwt.2019.108880

López, M., Adrián, M., García, C., Roldán, A., Viña, I., & Bertotti, G. (2025). Plant - Based Diet and Risk of Iron - deficiency Anemia . A Review of the Current Evidence and Implications for Preventive Strategies. Current Nutrition Reports. https://doi.org/10.1007/s13668-025-00671-y

Reddy, N. R., Sathe, S. K., & Salunkhe, D. K. (1982). Phytates in legumes and cereals. Advances in Food Research, 28, 1–92. https://doi.org/10.1016/S0065-2628(08)60110-X

Keywords

Phytate reduction; Fermentation; Iron uptake

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

METHOD DEVELOPMENT FOR HIGH-THROUGHPUT PROTEOMIC CHARACTERIZATION OF HUMAN MILK USING HIGH-RESOLUTION MASS SPECTROMETRY

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Abstract

Human milk is a dynamic biofluid with a highly complex composition that supports the early development of immunological, neurological, and metabolic regulatory systems (Lugonja et al., 2024). These processes are particularly significant for preterm infants, who represent more than 10% of global births (Blencowe et al., 2013). Because inadequate nutrition can have long-term consequences, feeding preterm infants requires an individualized approach. Despite its clinical importance, the human milk proteome remains analytically challenging due to its high complexity, with approximately 2500 protein species (Beck et al., 2015) and the current lack of a standardized MS-based workflow. The human milk fractionation method was optimized for low sample volumes and evaluated by SDS-PAGE. A comparative experiment of the PreOmics iST and the Filter-aided Sample Preparation (FASP) protocol was performed, after which method development was focused on FASP optimization. LC-MS/MS analysis was performed using a SCIEX M3 microLC coupled to a SCIEX TripleTOF 6600 mass spectrometer in data-dependent acquisition (DDA) mode. MS1 scans were acquired over 400-1250 m/z, followed by MS/MS scans over 100-1500 m/z. Raw files were processed in PEAKS Studio X Plus, and data filtering was performed using Python-based scripts within an Anaconda environment to maximize throughput. Fractionation optimization revealed that increasing the volume of Phosphate-buffered saline (PBS) and a 20-minute centrifugation step improved phase separation. SDS-PAGE showed significant protein carryover in the first two washes, with minimal protein remaining after the third, confirming that three washing steps are necessary. The comparison of the PreOmics iST kit and the FASP revealed that iST yielded a slightly higher number of identifications at the raw analysis level, while FASP showed similar results after filtering (e.g., 174 shared proteins for iST in the whole milk/skim milk/milk fat globule membrane fractions vs 180 for FASP), showing that fraction-specific proteomic profiles were consistent across different digestion methods. Since both methods revealed similar results, FASP was selected for further optimization due to lower cost and high-throughput compatibility. Filter comparison also revealed valuable insights: Cytiva filters showed high variability, whereas Merck filters provided more consistent raw data. Although Cytiva filters yielded a broader proteomic profile after filtering, Merck filters were chosen for further optimization experiments because they demonstrated lower technical variability and higher reproducibility across replicates. These findings establish a solid basis for a high-throughput human milk proteomics workflow. The optimized sample preparation method will serve as a foundation for upcoming large-scale analyses and advanced quantitative data-independent acquisition (DIA), providing the analytical basis for subsequent in vitro digestion studies of human milk.

Keywords

High-throughput proteomics, Human milk composition, FASP, LC-MS/MS

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

FROM BEEF TO PLANT-BASED ALTERNATIVES: COMPARING NUTRITIONAL QUALITY AND DIGESTIVE BEHAVIOUR IN PATTY ANALOGUES

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Abstract

A broad range of meat analogues has recently been commercialised, reflecting the growing potential of alternative proteins to partially or fully replace animal derived proteins in the human diet. In this study, six commercial patty analogues (PAs) were selected, grilled under standardised conditions and compared with a conventional beef patty (BP) with respect to nutritional composition, protein quality and in vitro digestive behaviour. Initially, the nutritional quality of the PAs and BP was assessed by comparing their amino acid profiles with the FAO (2013) reference pattern for infants aged 6 months to 3 years, and by determining in vitro protein digestibility (IVPD) using the pH-drop method to calculate IVPD-corrected amino acid score (IVPDCAAS). Based on this parameter, BP (46.7) ranked fifth of the seven samples (12.4 – 82.6) analysed. In parallel, the static INFOGEST digestion model (Brodkorb et al., 2019) was applied, which is considered a more physiologically relevant protocol, to digest both PAs and BP for comparative analysis. The results indicate that PAs released higher amounts of free amino acids (5.98 – 16.27 g/100 g protein) at the end of the intestinal digestion phase, whereas BP exhibited superior overall protein digestibility, as evidenced by a higher degree of hydrolysis (37.49%), soluble protein content (78.80 g/100 g protein), and peptide level (72.12 g/100 g protein). Although a moderate correlation was observed between nutritional values estimated by the pH-drop method and those derived from INFOGEST ($R2 = 0.6595$), the pH-drop method underestimated BP's nutritional value. This underestimation can be attributed to a near complete digestibility of the limiting amino acids (Cys + Met) in BP (~100%), despite an overall IVPD of 85.5%. When nutritional values were adjusted using individual amino-acid digestibility obtained from INFOGEST, BP's (54.7) rank improved to second among the seven samples (14.2 - 57.9). Surprisingly, neither the PAs nor BP fully met the FAO (2013) infant reference requirements. Given that beef protein is conventionally regarded as a high-quality protein source, these findings suggest that grilling adversely affected the amino acid profile and/or digestibility. Therefore, further optimisation of PAs formulation and cooking processes is needed to preserve nutritional quality. Overall, the current development of PAs has demonstrated meaningful progress towards improved nutritional quality, but further refinement is needed. In addition, the use of the INFOGEST digestion protocol is strongly recommended for evaluating the nutritional value of multi-ingredient products, as it provides a more physiologically relevant estimate of amino-acid bioaccessibility than single-parameter methods such as the pH-drop assay.

Keywords

Alternative proteins; meat analogues; digestion; protein quality; nutritional value

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IN VITRO DIGESTIBILITY OF THE STABILIZED ACTIVE COMPOUNDS AND FOOD PRODUCTS

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Abstract

In this work, encapsulated active compounds, and functional foods have been studied to simulate gastrointestinal digestion. In the first step, stabilized anthocyanins from jabolan fruits were studied, and in the next step, betacyanin (BTNs) from pitaya residue will be evaluated. Anthocyanins and betacyaninswere stabilized onto edible nanoclay and applied in food formulaion. The in vitro digestibility tests were performed according protocol defined by INFOGEST with modifications. The simulation was performed sequentially for the oral phase, gastric (stomach) phase and intestinal (small intestine) phase. The fluids of each stage had the following composition: Salivary simulation fluid (SSF): 15.1 mM KCl, 3.7 mM KH2PO4, 13.6 mM NaHCO3, 0.15 mM MgCl2(H2O), 0.06 mM (NH4)2CO3, 1.1 mM HCl and 1.5 mM CaCl2(H2O)2; Gastric simulation fluid (SGF): 6.9 mM KCl, 0.9 mM KH2PO4, 25 mM NaHCO3, 47.2 mM NaCl, 0.12 mM MgCl2(H2O), 0.5 mM (NH4)2CO3, 15.6 mM HCl and 0.15 mM CaCl2(H2O)2; Simulated intestinal fluid (SIF): 6.8 mM KCl, 0.8 mM KH2PO4, 85 mM NaHCO 3, 38.4 mM NaCl, 0.33 mM MgCl2(H2O), 8.4 mM HCl and 0.6 mM CaCl2(H2O)2. Oral phase simulation was initiated by the addition of SSF 1:1 (m/v) and human salivary α-amylase solution (75 U/mL, Sigma-Aldrich A1031). The mixture was then adjusted to pH 7 and incubated at 37 °C at 100 rpm for 2 min. The oral bolus was then mixed with SGF 1:1 (v/v) and its pH adjusted to 3 with 1 M HCl and added with porcine pepsin solution (2000 U/mL, Sigma-Aldrich P7012). The mixture was then incubated at 37 °C under shaking at 100 rpm for 2 h. For the small intestine phase, the gastric sample was mixed with SIF 1:1 (v/v), containing pancreatin (100 U/mL based on trypsin activity,) and bile salts (10 mM) at pH 7 adjusted using 1 M NaOH and incubated at 37 °C for 2h at 100 rpm. Samples were collected for all digestion phases (initial, oral, gastric, and intestinal). The bioaccessibility of the samples to ACNs, BTNs and FT was obtained by DPPH. In vitro digestibility showed changes in anthocyanin or betacyanin content, total phenolics, and antioxidant activity during in vitro gastrointestinal digestion. When the oral phase digestibility of the gelatin candies was evaluated, free ACNs in the candies showed a release of 95% due to the melting of gelatin at 37 °C. In addition, oral digestion is a rapid step and there was no degradation of ACNs, even in a neutral medium (pH 7). On the other hand, candies containing stabilized anthocyanins presented a release of 16% due to the adsorption of ACNs in the nanoclay. Candies with free ACNs (EXT) showed a higher concentration after gastric digestion (96.72%). For betacyanins from pitaya residues stabilized the samples are being analyzed. However, both compounds show high potential for application in food products.

Keywords

Jambolan, anthocyanin, residue, pitaya, betacyanin

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

BIOACTIVE PEPTIDES FROM ROLLED OAT DIGESTS FOR DIABETES AND OBESITY PREVENTION

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Abstract

Oats are one of the oldest cultivated plants known to mankind and have been grown for over 2000 years in many parts of the world. They are cereals with unique characteristics because they contain many nutrients suitable for human consumption, animal feed, and ingredients in cosmetics and dietary supplements. The nutrients contained in oats have cardioprotective effect by exhibiting e.g., antidiabetic, antioxidant, and other properties. Therefore, the food industry has shown interest in developing new food products using oats and incorporating them into e.g. bread, bars, baked goods or beverages. In our earlier study we demonstrated that ACE and DPP IV inhibitors can be released by gastrointestinal digestion after oat product intake [1]. α-Amylase, α-glucosidase, and pancreatic lipase have been shown to be inhibited by certain plant proteins and the peptides released from them. The objective of this study was to assess the anti-diabetic and anti-obesity activities of digests of proteins separated from rolled oats. The in silico part of the study was carried out using computational tools available in the UniProt (http://www.uniprot.org/), BIOPEP-UWM (http://www.biochemia.uwm.edu.pl/biopep-uwm/ and METLIN (http://metlin.scripps.edu/) databases, as well as the Fragment Ion Calculator (http://proteomicsresource.washington.edu/protocols06/). According to the INFOGEST method, the in vitro digestion procedure included the following steps: oral – 2 min, gastric – 2 hours, pH = 3.0, intestinal – 2 hours, pH = 7.0 [2]. Then, the digests were analysed for their α-amylase, α-glucosidase, and pancreatic lipase inhibition. Based on systematic in silico screening for α-amylase, α-glucosidase, and pancreatic lipase inhibitory peptides, the amino acid sequences of bioactive peptides were identified in the digests using the LC-Q-TOF-MS/MS method [1]. It was found that rolled oat digests are a potential source of sequences of α-amylase, α-glucosidase, and pancreatic lipase inhibiting peptides. The duodenal digest demonstrated the highest degree of α-glucosidase inhibition (82 %) and lipase inhibition (74 %), whereas that for α-amylase was the lowest (60 %). α-Amylase (e.g. dipeptides GL, HF) and α-glucosidase (e.g. dipeptide VW), as well as lipase inhibitory fragments (e.g., SW), were identified. According to our results, globulins from rolled oats are a source of peptides with α-amylase, α-glucosidase, and pancreatic lipase inhibitory activities. The hydrolysates obtained after the intestinal phase of digestion demonstrated the inhibitory capacity against all tested enzymes.

Keywords

BIOPEP-UWM database; oat, peptides; proteolysis simulation

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

INFLUENCE OF K-CARRAGEENAN- AND KONJAC GLUCOMANNAN-BASED EMULSION GELS ON PHYSICOCHEMICAL PROPERTIES AND IN VITRO DIGESTIBILITY OF TVP-BASED MEAT ANALOGS

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Abstract

This research examined how emulsion gels (EGs) formulated with κ-carrageenan (κCAR) or konjac glucomannan (KGM) affect the physicochemical characteristics and in vitro digestion behavior of textured vegetable protein (TVP)–based meat analogs. The EGs were produced using κCAR or KGM to incorporate grapeseed oil (GO) at a final concentration of 20%. Compared with KGM-EG, κCAR-EG showed greater hardness but lower cohesiveness and viscosity. TVP systems containing GO, κCAR-EG, or KGM-EG were prepared and referred to as TVP-GO, TVP-κCAR, and TVP-KGM, respectively. Confocal laser scanning microscopy revealed that lipid droplets in TVP-κCAR and TVP-KGM were smaller than those in TVP-GO. Textural analysis indicated that hardness decreased in the order TVP-κCAR > TVP-GO > TVP-KGM, while cohesiveness was highest for TVP-KGM. TVP-based emulsions were subjected to a static in vitro digestion model following the INFOGEST protocol. During in vitro gastric digestion, TVP-GO showed the highest release of α-amino groups, whereas TVP-κCAR and TVP-KGM exhibited reduced proteolysis, with more intact protein bands remaining, particularly in TVP-κCAR. These differences were associated with variations in particle disintegration, electrostatic interactions, and digesta viscosity, which followed the order TVP-κCAR > TVP-GO > TVP-KGM. In the intestinal phase, TVP-GO showed the highest α-amino group content and the greatest content of proteins 3 kDa, indicating higher protein digestibility. In contrast, TVP-κCAR retained partially aggregated protein particles in the microscopic image and higher viscosity, while TVP-KGM exhibited well-dispersed particles but lower protein digestibility, likely due to restricted enzyme accessibility caused by KGM–protein interactions. Overall, although κCAR- and KGM-EGs altered the digestive behavior of TVP through different mechanisms, the incorporation of polysaccharide-based EGs consistently reduced protein digestibility. These results highlight the need to balance textural enhancement and nutritional quality when using gel-type fat replacers in TVP-based meat analogs.

Keywords

textured vegetable proteins, emulsion gel, meat analog, protein digestibility, in vitro digestion

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

GASTROINTESTINAL DIGESTION OF MODIFIED MICELLAR CASEIN ISOLATES: INSIGHTS FROM IN VITRO AND IN VIVO MODELS

Mengjia Li [1] , Kristine Holgersen [2] , Søren Drud-Heydary Nielsen [3] , Pingping Jiang [2 4] , Luiza Patrycja Morawska [3] , Ann Bjørnshave [3] , Nina Aagaard Poulsen [1] , Per Torp Sangild [2] , Lotte Bach Larsen [1]

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Abstract

Aim: The study aimed to evaluate the effects of incorporating different micellar casein isolates (MCI, plasmin pre-hydrolyzed MCI (PMCI), or calcium-reduced MCI (CaMCI)) into infant formula on gastrointestinal (GI) digestion characteristics, and to assess the consistency between in vitro and in vivo digestion models.

Method: For in vitro study, a protein mixture of MCIs and whey protein concentrate (WPC, 40:60, w:w) with protein concentration 1.2% was subjected to simulated infant gastric and intestinal digestion using the INFOGEST protocol (1 h per phase), with samples collected at different digested timepoints. For in vivo study, piglets were fed formula containing different MCIs–WPC blends with identical casein-to-whey ratios (40:60) and total protein content of 6%. The collection of GI contents was conducted for 1 h postprandial. The release of free amino terminals, peptides and bioactive peptides was measured.

Result: Plasmin treatment and calcium removal from MCI affected digestibility and peptide generation during GI digestion. By the in vitro digestion model, the level of free amino terminals released increased modestly during gastric digestion and rapidly during early intestinal digestion from all protein mixtures. PMC I blends showed a higher digestion level than other MCI types during gastric phase. PMCI and CaMCI blends maintained higher peptide counts during intestinal digestion than standard MCI blends. Gastric digestion favored generation of opioid and anticancer peptides, while intestinal digestion enhanced levels of antioxidant, ACE-inhibitory, and DPP-IV inhibitory peptides. For in vivo digestion, CaMCI-based formula showed the highest free amino terminals in the gastric phase. Regarding the intestinal phase, CaMCI group showed higher peptide counts compared with PMCI and WPC groups. A total of 197 different bovine milk–derived bioactive peptides spanning 19 functional categories were identified in vivo, markedly fewer than those detected in vitro, with 625 peptides across 25 categories. Peptides with opioid, wound healing, antihypertensive, immunomodulatory, and antithrombotic activities predominated in the in vivo stomach, whereas DPP-IV inhibitory, prolyl endopeptidase inhibitory, and cholesterol-lowering peptides were enriched in proximal intestine. Notably, CaMCI-fed piglets exhibited a higher abundance of bioactive peptides in distal intestine.

Conclusion: The in vitro model based on blends revealed the intrinsic digestive properties of MCI protein blends, whereas in vivo model captured digestion within a complete formula matrix. Both models revealed the potential for improved digestibility of modified MCI. PMCI improved gastric digestion in vitro but showed no clear advantage in vivo. CaMCI demonstrated enhanced in vivo digestion and bioactive peptide generation in both models. The difference between in vitro and in vivo results likely reflects variations in enzyme dynamics, matrix effects, and nutrient absorpti

Keywords

in vitro digestion, in vivo piglet model, micellar casein isolate, calcium removal, plasmin hydrolysis, peptides

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECTS OF PROCESSING ON STRUCTURAL MODIFICATIONS AND DIGESTIBILITY OF PROTEINS IN YELLOW PEA READY-TO-EAT FOOD PRODUCTS

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Abstract

The impact of processing techniques on the in vitro protein digestibility (IVPD) of dry yellow pea based real-life, ready-to-eat food products was evaluated by the static INFOGEST protocol. To investigate increasing levels of processing, whole yellow peas were processed into seven different ready-to-eat products, hummus, tofu, soup, tempeh, bread, pasta, and a meat analogue, which was prepared to simulate ingestion of the products. The protein content of the yellow pea products varied considerably ranging from 5.0% to 20.2% dependent on the recipe. Products prepared from whole yellow peas, thus hummus, tofu, soup, and tempeh, generally exhibited the lowest protein contents (5.0-11.2%), primarily due to the addition of water required to soften the dried peas. In contrast, bread, pasta, and meat analogue, formulated with pea flour, concentrate, or isolate had higher protein contents (12.6-20.2%). Overall, the ready-to-eat pea products exhibited high IVPD between 85.2%-100.0%, which was significantly higher than the 68.4% of the original dried yellow pea. The IVPD was to some extent dependent of the degree of processing. Hummus, with the mildest processing, demonstrated the lowest IVPD of 85.2%. Soup, tempeh, and bread, though varying considerably in recipe and processing, showed very similar IVPD results of 92.0%, 92.1% and 91.3% respectively. Pasta and the meat analogue, which are based on pea concentrate and isolate, exhibited very high IVPD of 100% and 98.5%. Tofu had a IVPD of 100% due to being formed from the soluble pea proteins. To explain the IVPD results, Coherent Raman Scattering (CRS) microscopy, a label-free and non-destructive imaging technique, was used to visualize structural changes in the pea storage cells. The dried peas showed, as expected, a compact cellular structure of intact storage cells that adhere tightly to each other. The peas in the hummus had similar structure of adhering cells, however, some cell walls appeared disrupted resulting in leakage of starch and protein. In the soup, the storage cells did not adhere to each other and a considerably amount of starch and protein leakage was observed. Only a few cells remain intact in the flour-based bread, hence the cellular structure is extensively disrupted, with numerous fragmented cells as well as large irregular shaped aggregates. Though, the storage cells retain their original structure and no observed leakage in Tempeh, a clear interactions between the Rhizopus mycelium and the cells occurred. The original structure was completely broken down in tofu, pasta, and the meat analogue, all appearing as one assembled mass with individual structures. These CRS imaging together with complementary Fourier Transform Infrared (FTIR) spectroscopy is currently under in-depth analysis to provide a comprehensive understanding of how increasing processing level influences the nutritional value of yellow pea proteins.

Keywords

Yellow pea, In vitro protein digestibility, processing, protein integrity, Coherent Raman Scattering (CRS), Fourier

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

THE EFFECT OF AGING ON AMINO ACID DIGESTIBILITY OF SKIMMED MILK, SORGHUM AND BLACK BEANS IN VITRO AND COMPARISON WITH IN VIVO DATA.

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Abstract

Background

The aging gastrointestinal tract undergoes functional and structural changes that may reduce digestive capacity and protein digestion efficiency. This study evaluated the impact of aging on indispensable amino acid (IAA) digestibility of skimmed milk, sorghum and black beans in vitro and compared these results with previously obtained in vivo data from the same protein sources.

Methods

IAA digestibility was assessed using the standardized INFOGEST protocol for adults and older adults. In each condition, 80 mg of protein from skimmed milk, sorghum or black beans, was subjected to digestion, with a protein-free cookie as blank. The older adult protocol included a 40% reduction of pepsin and trypsin activities, increased gastric pH (3 to 3.7), extended gastric incubation time by one hour, and 30% reduction of bile salts (i.e. 3.3mM). IAAs in the absorbable fraction were quantified to calculate IAA digestibility. In vitro results were compared with in vivo IAA digestibility values for the same black beans and sorghum, previously measured with the dual isotope method in 10 young and 10 older adults (1). Differences between age in vitro conditions were assessed using Student's t-test. The aging effect on IAA digestibility across in vitro and in vivo approaches was examined using multiple linear regression and linear mixed-effect models.

Results

In vitro digestibility of black beans and skimmed milk proteins was similar between age conditions for all IAAs, except for histidine in black beans (99.1±1.2% versus 92.7±0.7%). In contrast, sorghum showed reduced digestibility in older adult conditions for histidine (98.0±1.4% versus 72.7±6.5%), threonine (91.7±5.9% versus 69.1±7.0%) and methionine (62.4±1.0% versus 48.4±3.7%) with a similar trend for valine, leucine, isoleucine, and phenylalanine being observed. Lysine and tryptophan were unaffected.

The relative aging effect on protein digestibility was consistent between in vitro and in vivo methods, with a regression slope of 0.99. However, absolute digestibility values differed between approaches. In vitro estimates were on average 30 units higher than in vivo values, and the in vitro–in vivo regression slopes were 0.62 and 0.68 for young and older adults, respectively.

Conclusions

These findings demonstrate that aging predominantly affects the digestibility of less digestible protein sources such as sorghum, whereas skimmed milk and black beans remain largely preserved. Although both in vitro and in vivo methods show comparable age-related patterns, discrepancies in absolute digestibility values highlight the need for further optimization and validation of in vitro models. Future work should include additional protein sources and refine digestion protocols to enhance the accuracy of IAA digestibility assessment in older populations.

1) Differences in amino acid digestibility between young and older adults determined using the dual tracer method. Hinssen et al. Submitted

Keywords

Aging, older adults, amino acid digestibility, protein digestibility

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

ANALYSIS OF BIOACTIVE PEPTIDES RELEASED FROM YOGURTS ENRICHED WITH HIGH-PROTEIN MILK PREPARATIONS USING IN SILICO AND IN VITRO PROTOCOLS

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Abstract

Fermented dairy products, including yogurt, are not only a rich source of macronutrients or minerals, but also contain bioactive peptides that affect various physiological functions. During yogurt fermentation, bioactive peptides are released primarily from casein and whey proteins. Such bioactive peptides may possess significant prophylactic and even clinical properties, including, for example, regulation of carbohydrate or lipid metabolism. The aim of this study was to determine the impact of the addition of high- protein milk preparations on some bioactivities of yogurts, i.e., of dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5), α-glucosidase (EC 3.2.1.20), and pancreatic lipase (EC 3.1.1.3) inhibition. First, yogurts were enriched with two preparations (2%, w/v): micellar casein concentrate (MCC) and milk serum proteins concentrate mixed with buttermilk (SPCB). Control yogurts were produced without MCC or SPCB. All variants of yogurts were prepared in pilot plant scale using the thermostatic method [1]. Next, predictions of the release of biopeptides from bovine milk protein sequences using the UniProt and BIOPEP-UWM databases were computed. Then, the digestion of enriched yogurts according to the INFOGEST 2.0 protocol, followed by analysis of respective hydrolysates in terms of inhibition of the above-mentioned enzymes was carried out. Finally, RP-HPLC-ESI-MS/MS method was used to identify released peptides [2]. In silico results showed that 59 biopeptides exhibiting the above-mentioned activities could be produced upon the action of pepsin, trypsin, and chymotrypsin, whereas 36 of them were released from yogurts after the simulated gastrointestinal digestion. They were found in at least one of the three yogurts studied. All yogurt hydrolysates showed DPP-IV, α-glucosidase, and lipase inhibitory activities. Differences between control yogurt and enriched yogurts were observed. Among all yogurt samples subjected to simulated digestion, yogurt fortified with SPCB demonstrated the highest DPP IV inhibitory activity. The highest α-glucosidase and pancreatic lipase inhibitory activities were also shown by this yogurt. Yogurts enriched with SPCB represent promising sources of biologically active peptides, including enzyme inhibitors that are released after digestion .
[1] Żulewska J., Baranowska M., Bielecka M.M., Dąbrowska A.Z., Tarapata J., Kiełczewska K., Łobacz A. Foods 2025, 14, 80.
[2] Darewicz M., Pliszka M., Borawska-Dziadkiewicz J., Minkiewicz P., Iwaniak, A. Molecules 2022, 27, 7907.

Keywords

BIOPEP-UWM database, bioactive peptides, high-protein milk preparations, yogurts

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IN-VITRO DIGESTIBILITY OF THERMO-REVERSIBLE PROTEIN GELS FOR PATIENTS WITH CANCER CACHEXIA ASSOCIATED MALNUTRITION.

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Abstract

Cancer cachexia (CC) is a form of malnutrition that results in sarcopenia or muscle wastage. Cancer cachexia affects 50-80% of all cancer patients and has a significant impact on overall quality of life and tolerance to treatment. In extreme cases CC is responsible for 20% of cancer related deaths. Compliance with current dietary interventions such as calorie-dense diets and protein-rich beverages is poor due to premature satiety and reduced appetite. The TRANSFORM project aims to address this challenge through the development of casein based gels with novel functional and thermo-reversible properties that can be incorporated into patients existing diets, with the goal of increasing overall dietary protein intakes. The gels will melt upon body temperature and thus can act as protein and micronutrient delivery systems that are easily ingested to help reduce loss of lean body mass. The objectives of this study are to 1) develop and optimise casein-based formulations, 2) analyse their composition and macro- and microstructure, 3) perform in-vitro digestion using the INFOGEST protocol, and 4) find correlations between gel structure and digestibility. The texture, microstructure and thermal properties of the gels will also be analysed. After in-vitro digestion trials, digesta collected will undergo analysis of free amino acid and free fatty acid profile, and structural and rheological analysis to determine the physiochemical and microstructure properties. The data generated will provide insights into how ingredient type, processing, and formulation approaches influence digestibility of the thermo-reversible hydrogels aiding product development prior to incorporation in human clinical trials.

https://sites.google.com/view/thetransfromproject/home

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

RELEASE OF ACE-INHIBITORY AND ANTIOXIDANT PEPTIDES FROM YOGURTS ENRICHED WITH AN RUF FRACTION DURING GASTROINTESTINAL DIGESTION

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Abstract

Yogurt represents an important dietary source of milk proteins that, upon gastrointestinal digestion, may release peptides with angiotensin-converting enzyme (ACE) inhibitory and antioxidant properties relevant to cardiovascular health. In this context, the impact of enriching yogurt with an RUF fraction composed of micellar casein concentrate (MCC) and ultrafiltration-derived buttermilk permeate on bioactive potential was evaluated using an in vitro digestion model. A control MCC-based yogurt and RUF-supplemented yogurts, produced with lactose or in a lactose-free variant, were subjected to simulated gastric and intestinal digestion.

Protein hydrolysis during digestion was monitored by electrophoretic separation. Progressive degradation of caseins was observed, leading to the disappearance of protein bands during the intestinal phase, whereas whey proteins exhibited greater resistance to enzymatic digestion. The biological activity of yogurts and their digested products was assessed by determining ACE-inhibitory activity and antioxidant capacity using ABTS and DPPH assays.

All yogurts and their hydrolysates demonstrated the ability to inhibit ACE. Notably, RUF-supplemented yogurts exhibited enhanced ACE-inhibitory activity after digestion compared with the control MCC-based yogurt, indicating an increased potential to generate peptides associated with blood-pressure-modulating effects. Antioxidant activity varied with digestion stage and analytical method, suggesting the release of compounds with distinct radical-scavenging mechanisms.

Targeted and untargeted tandem mass spectrometry enabled the identification of digestion-derived peptides, including sequences previously reported to exert ACE-inhibitory and antioxidant effects. Overall, the results suggest that yogurts enriched with the RUF fraction may serve as an improved dietary source of health-relevant bioactive peptides released during gastrointestinal digestion, while lactose presence did not significantly influence peptide release or bioactivity.

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The task entitled 'Research network of life science universities for the development of the Polish dairy sector – research project' is financed by a targeted grant from the Minister of Science and Higher Education

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

SELECTIVE DETOXIFICATION OF DIGESTA REVEALED HOW APPLE POMACE MODULATE TRANSEPITHELIAL GLUCOSE TRANSPORT AND STIMULATE GLP-1 SECRETION

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Abstract

Achieving glucose-lowering and appetite-suppressing effects through dietary strategies depends not only on the presence of active compounds but also on the timing and rate of nutrient release from the food matrix. This highlights the need for mechanistic research to employ physiologically relevant models that accurately mimic the environment experienced by intestinal epithelial and enteroendocrine cells in the body. This study examined whether whole apple and apple pomace modulate transepithelial glucose transport and GLP-1 secretion under physiologically relevant in vitro conditions, achieved via a laboratory-implementable solubilization and purification method that doubled polyphenol concentrations without inducing cytotoxicity. Samples collected at the end of the gastric and intestinal phases represented pre- and post-intestinal chyme. Purified digesta were incubated with Caco-2 monolayers, and UHPLC-ESI-QTOF-MS/MS analysis of 48 polyphenols across apical, intracellular, and basolateral compartments showed predominant apical retention. Apple and pomace inhibited glucose transport, primarily driven by dihydrochalcones and hydroxybenzoic acids (Spearman $\rho>0.70$). Notably, although phloridzin alone inhibited GLP-1 secretion in mouse STC-1 L-cells, gastric digesta of both apple and pomace enhanced secretion by 50%. However, pomace digesta retained this effect after intestinal digestion, increasing GLP-1 by 179% and 62% compared to the glucose and intestinal blank controls, respectively.
Results showed that coupling the INFOGEST 2.0 digestion protocol with a purification and depuration procedure yielded a robust in vitro platform for cell-based bioassays. By concentrating bioaccessible polyphenols and removing cytotoxic constituents from digesta, the resulting digesta were suitable for both mass spectrometry analysis and direct application to Caco-2 and STC-1 cells. This integrated approach enabled mechanistic investigation of physiologically relevant bolus and chyme. Notably, the distinct mechanisms by which whole apple and apple pomace exert glucose-lowering and appetite-suppressing effects, respectively, underscore the need for greater precision in evaluating how specific food components influence physiological responses.

Keywords

Polyphenols; digestion; bioaccesibility; STC-1 cells; glucose homeostasis; enteroendocrine cells; GLP-1;

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

ENCAPSULATION AND RELEASE OF INULIN FROM CALCIUM ALGINATE BEADS FOR COLONIC DELIVERY

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Abstract

To mediate the harmful side effects of protein fermentation in the distal colon, prebiotics such as inulin are delivered distally to sustain fermentable substrate availability. Yet, without protection, most prebiotics are fermented in the proximal colon and insufficient quantities reach the distal colon. Therefore, hydrogel encapsulation systems are developed to protect prebiotics from earlier fermentation. Calcium-alginate beads represent a promising encapsulation matrix for this purpose; however, the relationship between alginate intrinsic properties and prebiotic encapsulation efficiency remains unclear. This study investigated how the molecular weight and concentration of alginate influence encapsulation efficiency and in vitro release of fructo-oligosaccharide (FOS) and inulin from pH-responsive beads and designed for targeted colonic delivery.

Prebiotic-loaded calcium alginate beads were prepared via co-extrusion using low (20–60 kDa), medium (95–100 kDa), and high (250–300 kDa) molecular weight alginate with FOS or inulin at various concentrations. Rheological properties of polymer solutions and hydrogels were characterized to elucidate the mechanism of encapsulation. Encapsulation efficiency was quantified, and cumulative release of the prebiotics was determined following INFOGEST 2.0 static in vitro digestion.

Medium molecular weight alginate achieved significantly higher encapsulation efficiency of inulin (57.5 ± 3.7%) compared to low (40%) and high (16%) molecular weight types. Rheological analysis revealed that encapsulation performance is governed by both the mechanical strength of the final gel and the intrinsic properties of the polymer solution. We showed that inulin released 18% in the gastric phase and a further 27% in the intestinal phase (~45% cumulative release). On the other hand, FOS showed poor retention (20%) across all formulations, which is most probably due to its small size relative to the mesh size of the gel, and lack of interaction with alginate.

The pH-responsive release behavior and bead integrity throughout the upper GI tract signify the potential of alginate-inulin systems for targeted prebiotic delivery. As a next step, simulated batch fermentations will confirm distal colonic delivery.

Keywords

Calcium alginate beads, encapsulation efficiency, inulin, pH-responsive release, colonic delivery

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

INFLUENCE OF SPROUTING ON IMMUNOGENIC GLUTEN PEPTIDE RELEASE AND NUTRITIONAL QUALITY OF WHEAT

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Abstract

Sprouting is a bioprocess that activates endogenous enzymes inducing significant nutritional and structural changes in grains. Sprouting involves the hydrolysis of proteins into peptides and amino acids, the breakdown of starch into simple sugars such as glucose and maltose, thereby improving the digestibility and the nutritional quality of the grains. Gluten, the primary protein found in wheat, exhibits resistance to digestion, resulting in the formation of long and immunogenic gluten peptides. The accumulation of these peptides can lead to the development of celiac disease in genetically susceptible individuals. Modification of wheat gluten by a biochemical process such as sprouting may be a potential strategy for eliminating these immunogenic gluten peptides. Accordingly, protein and amino acid bioaccessibility can be improved by sprouting. Additionally, starch hydrolysis during sprouting may also impact the nutirional quality of wheat. This study aimed to investigate the effect of sprouting wheat on immunogenic gluten peptide release as well as its nutritional quality.

Wheat sprouting was achieved by incubating steeped wheat at 20°C and 95% relative humidity for a period of 72 hours in darkness. Each 12-hour interval, the wheat grains were washed under tap water. Sprouted wheat were subjected to in vitro digestion procedure (0, 20, 30, 60, 90, 120, and 180 minutes for the intestinal phase), and the analysis of immunogenic gluten peptides, amino acids as well as sugars was conducted afterward.

The results demonstrated a substantial reduction in the concentration of 33-mer, the most immunodominant gluten peptide, following sprouting. At the end of a 24-hour sprouting period, the concentration of 33-mer decreased by 68%, whereas this reduction was further increased to 85% at the end of a 60-hour sprouting period. The changes in nutritional protein quality of wheat during sprouting was assessed by calculating the area under the curve of amino acid concentrations during in vitro digestion, expressed as a percentage of its counterpart of egg, which is considered as a reference food for its exceptional amino acid bioavailability. During sprouting, the nutritional protein quality of wheat significantly improved. The protein quality of wheat increased from 57% to 64% with varying durations of sprouting. Additionally, the sprouting process alters the rapidly and slowly digestible starch contents. These findings demonstrate that sprouting wheat for varying durations can effectively reduce immunogenic gluten peptides while simultaneously enhancing protein digestibility and digestible starch fractions. The overall results suggest that sprouting plant-based materials is an effective bioprocess to obtain ingredients with enhanced nutritional value.

Keywords

sprouting, wheat, immunogenic gluten peptides, protein digestibility, digestible starch

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

FROM DIGESTION TO ABSORPTION: COMPARATIVE EVALUATION OF INFANT FORMULA DIGESTATES USING DYNAMIC DIGESTION AND 3D HUMAN INTESTINAL MODELS

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Abstract

Background: Human breast milk is the gold standard for infant nutrition, combining optimal nutrient composition with bioactive functions that support digestive maturation and epithelial development. Infant formulas increasingly aim to narrow this gap, yet a similar nutrient composition alone is insufficient without functional validation at the digestive and intestinal levels.

Objective: This study aimed to compare the digestion behavior and intestinal absorption profiles of a whey- and cream-enriched infant formula versus competitor formulas, using physiologically relevant in vitro digestion and advanced human intestinal models.

Methods: Infant formulas were subjected to dynamic in vitro digestion using the DIDGI® system, generating standardized gastric and intestinal digestates. Digestates were applied to 3D human intestinal epithelium models to assess cytotoxicity (LDH, MTT assays), epithelial barrier integrity (FITC-dextran permeability), and absorption of bioactive compounds. Basolateral compartments were analyzed using LC–MS/MS-based peptidomics to identify whey-derived peptide signatures and targeted lipidomics (FIA–MS/MS) to quantify digestion-derived lipid species relevant to intestinal uptake and remodeling.

Results: Digestates from the whey- and cream-enriched formula showed distinct absorption profiles compared with competitor products, characterized by enhanced transfer of specific whey-derived peptides and lipid classes across the intestinal epithelium. Barrier integrity was preserved across tested conditions, supporting the physiological relevance of the observed absorption differences.

Conclusion: Combining dynamic digestion with advanced intestinal models provides a robust translational framework to functionally discriminate infant formulas beyond composition alone. This approach highlights how protein and lipid sources modulate digestion outcomes and intestinal absorption, supporting evidence-based differentiation of infant formulas with closer functional alignment to human milk.

Keywords

Infant formula; dynamic digestion; intestinal absorption; whey proteins; lipidomics; peptidomics

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

UNLOCKING THE ANTIOXIDANT POTENTIAL OF MAYA NUT (BROSIMUM ALICASTRUM) THROUGH SIMULATED GASTROINTESTINAL DIGESTION

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Abstract

Underutilized species such as Brosimum alicastrum (Maya nut) hold considerable potential for enhancing food security and supporting the livelihoods of Mesoamerican indigenous communities. Despite its historical importance as a dietary staple, a significant knowledge gap remains regarding the bioaccessibility of its nutrients. The aim of this study was to understand the release of antioxidant peptides of the Maya nut (Brosimum alicastrum) by applying the INFOGEST static in vitro digestion protocol. Three samples were subjected to gastrointestinal digestion: Maya nut free-polyphenol flour (FMNF), protein concentrate (MNPC), and an extensive protein hydrolysate (MNPH). The digestion kinetics were measured by soluble protein (BCA), degree of hydrolysis (DH, measured by TNBS), and SDS-PAGE. Furthermore, fractions below 10 kDa were isolated from both gastric and intestinal stages to assess their antioxidant capacity using ORAC and ABTS radical scavenging assays, alongside iron (Fe2+) and copper (Cu2+) chelating activities.

The soluble protein levels increased across all samples after intestinal digestion, increasing seven-fold for FMNF, 2.5-fold for MNPC, and 3.7-fold for MNPH. Notably, the lack of a statistically significant difference ($P>0.05$) in DH between FMNF and MNPC suggests that the natural matrix of the Maya nut does not necessarily hinder the action of intestinal digestive enzymes compared to concentrated forms. The SDS-PAGE showed remaining bands at 14, 10 , and 24 kDa, indicating that some proteins present in the Maya nut are resistant to digestion.

The antioxidant potential of the intestinal digestates consistently surpassed that of the gastric samples across all assays. In ABTS assay, intestinal fractions reached IC50 values of 0.42 mg/mL (FMNF), 0.37 mg/mL (MNPC), and 26 mg/mL (MNPH); in contrast, gastric samples failed to reach 50% inhibition, thus, IC50 determination was not performed. This enhancement was further evidenced in the ORAC assay, where the intestinal phase yielded values of 123.06 and 191.22 mM TE/mg protein for FMNF and MNPC, respectively, a nine-fold increase over gastric levels. While metal chelation was observed in both phases, intestinal copper chelation IC50 values improved significantly (0.20 mg/mL for FMNF and 0.14 mg/mL for MNPC) compared to gastric results. For iron chelation, gastric FMNF (14.49%) and intestinal MNPH (15.45%) showed the highest capacities at maximum protein concentrations (1 mg/mL). Distinctly, FMNF and MNPC generally performed comparably ($P>0.05$), the whole flour (FMNF) demonstrated a significantly higher efficacy in iron chelation than the protein concentrate.

In summary, these findings indicate that Brosimum alicastrum products possess antioxidant properties that are released during gastrointestinal digestion. Notably, the high performance of FMNF suggests that industrial processing, such as protein concentration or pre-hydrolysis, may not be necessary for enhanced health benefits.

Keywords

Maya nut proteins, protein hydrolysates, antioxidant capacity, in vitro digestion, protein concentrate

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

DESIGNING FOODS THAT DELIVER FUNCTIONALITY AND PLEASURE: THE ROLE OF INGREDIENTS, PROCESSING, AND DIGESTION

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Abstract

Mucositis is a common and debilitating adverse effect of chemotherapy and radiotherapy in cancer patients, characterized by inflammation and ulceration of the oral and gastrointestinal mucosa. This condition causes severe pain and dysphagia, leading to impaired nutrition, reduced treatment tolerance, frequent dose reductions or interruptions, and ultimately worse treatment efficacy and survival.

That is why this study proposes the design of two functional desserts that promote health and pleasure in chemotherapy patients with this adverse effect. We analyzed the conservation of flavonoids, polyphenols, and antioxidant activity in each phase of the digestion process of golden berries (Physalis peruviana) eaten without transformation compared with two different food matrixes which used fruit as a basis. To achieve that, in the first place, two pastry techniques were selected – mousse and semifreddo – considering their similarities in processing, based on the low quantity, availability, and cost of ingredients – cream milk, egg whites, sugar, gelatin, and fruit pulp. For each technique, the best distribution of each ingredient was found increasing golden berries content to enhance the functionality; each sample -mousse, semifreddo and golden berries- were digested according to the INFOGEST model.

Samples of each digestion phase were taken, and antioxidant activity (ABTS & DPPH), total polyphenols and flavonoids content were measured and compared, showing significant differences between them in flavonoids (p0,05) and polyphenols (p0,05) between oral and intestinal phases. In antioxidant activity, the results obtained with ABTS assays, shown a potentialized antioxidant activity through the digestion process (p0,05), increasing 28% in golden berries, 74% in mousse, and 50% in semifreddo.

These results are consistent with the bioaccessibility percentages obtained in each assay. Antioxidant activity (ABTS) was 5.8- and 2.04-fold higher in the mousse and semifreddo, respectively, compared with golden berries. Similarly, flavonoid bioaccessibility increased by 1.26 and 2.07 times in the mousse and semifreddo relative to the fresh fruit, while polyphenol bioaccessibility was 2.24 and 1.27 times higher, respectively. These findings highlight the impact of food matrices on the preservation and bioaccessibility of bioactive compounds, such as polyphenols, through their interactions with the gastrointestinal environment and the components involved in the digestive process.

These findings underscore a major opportunity for designing foods with improved functionality and sensory attributes. Strategic ingredient selection and process optimization promote beneficial interactions among food components and with the gastrointestinal environment, ultimately better fulfilling consumer demands and expectations.

Keywords

Food design, physalis peruviana, bioaccesibility, mousse, semifreddo.

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECT OF KOREAN-STYLE MEAL CONTAINING RESISTANT STARCH TYPE 4 (RS4)-INDUCED RICE ON POSTPRANDIAL BLOOD GLUCOSE AND SATIETY

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Abstract

Rice is a major staple food in Asia and is mostly consumed as cooked intact kernels. Because rice is generally considered a high-glycemic index food, modulating its starch digestibility by increasing resistant starch (RS) content is demanding, particularly in cooked intact grain. This study aimed to establish processing conditions for RS type 4 (RS4)-enriched rice with quality attributes suitable for use as a ready-to-eat cooked rice (RTE-rice) product and to evaluate its effects on postprandial glycemic response and satiety in humans when consumed as a complex meal. For RS4 formation in rice kernel, the effects of cultivar of rice with different amylose contents, the type and concentration of organic acid, and thermal reaction time were examined. The optimized process was as follows: high-amylose brown rice was immersed in 1.5% (w/v) malic acid solution for 4 h, dried at 40 °C to a moisture content below 10%, and then heat-treated at 130 °C for 7 h. The resulting RS4-enriched brown rice showed an approximately 5-fold increase in RS content compared with raw brown rice. The processed rice was incorporated into RS4-containing RTE-rice product (RS-BR), and compared with products containing white rice (WR) and brown rice (BR). RS content of WR, BR, and RS-BR products was 18.50%, 22.54%, and 26.78% RS, respectively, as determined by the Englyst's assay. A typical Korean-style complete meal consisting of an RTE-rice, stew, a main dish (beef Bulgogi), and four small side dishes (total 942 kcal) was provided to 20 healthy subjects, and blood glucose levels and perceived fullness (9-point scale) were recorded before and after consumption. Blood glucose levels following the BR and RS-BR meals reached a peak at 15 min after consumption and then gradually declined, whereas the WR meal induced a continuous increase in blood glucose up to 30 min postprandially, reaching a higher level. The RS-BR meal showed a significantly (p0.05) lower incremental area under the curve (iAUC) compared with the other meals. The degree of fullness gradually decreased from 8.4 immediately after the meal to 5.9 at 120 min; however, no significant differences were observed among the test meals, indicating that postprandial blood glucose responses did not directly influence satiety. These results suggest that RS4 induction into cooked rice may exert a blood glucose-lowering effect, even when consumed as part of a complex meal with diverse accompanying dishes.

Keywords

Starch digestion, Resistant starch, Complex meal, Postprandial blood glucose, Satiety

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECTS OF HIGH-MOISTURE EXTRUSION ON THE NUTRITIONAL QUALITY OF PROTEIN ISOLATES AND CONCENTRATES FROM DIFFERENT SOURCES

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Abstract

Plant-based meat analogues (PBMAs) are a sustainable alternatives to animal protein and are increasingly found in vegetarian or flexitarian diets. However, concerns have been raised over their nutritional quality, driven by their nutrient profile and overall processed nature. To provide insights on the nutritional quality and impact of processing, this study evaluated anti-nutritional factors (ANFs), in vitro protein digestibility, amino acid scores, and mineral bioaccessibility before and after high-moisture extrusion (HME) in four commercial plant protein ingredients: faba bean isolate (FBI), pea protein isolate (PPI), soy protein concentrate (SPC), and vital wheat gluten (VWG). HME decreased two of the three evaluated ANFs; the total phenolic content by 8-35%, and the trypsin inhibitor units by 44-67%, while phytic acid levels remained stable. HME increased the in vitro protein digestibility in SPC by 15-16%, whereas FBI, PPI, and VWG were unaffected despite the ANF reductions. The amino acid scores declined slightly, with PPI showing the lowest reduction (0.9%) and mineral bioaccessibility remained stable across most ingredients and minerals upon HME. This study provides a detailed, ingredient-specific overview of the nutritional effects of HME. Overall, the findings show that HME does not compromise the nutritional value of plant-protein ingredients, suggesting that ingredient choice has a far greater impact on nutritional quality than the extrusion process itself.

Keywords

Plant-based meat analogues; protein transition; extrusion; in vitro protein digestion; mineral bioaccessibility

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

THE BEAN LOTTERY? HOW SEED-LEVEL DIFFERENCES MATTER FOR IN VITRO STARCH DIGESTION

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Abstract

Pulses are nutrient-dense staple foods whose cotyledon cell structure affects in vitro starch digestibility and glycemic response. Even within a single bean batch, variability in seed physical properties (e.g., seed size) and composition leads to non-uniform post-cooking hardness, with yet unknown effects on digestion. Previous studies have linked post-cooking hardness to in vitro starch digestion kinetics, but this relationship may be partially due to seed size, since seed size affects the cooking time for seed softening and the resulting hardness. The described seed-to-seed heterogeneity of pulses creates complexity in the processing and quality steering of whole seeds. Therefore, this work aims to unravel the respective impact of seed to seed heterogeneity (seed size and seed hardness) on in vitro starch digestion kinetics and included two approaches: (i) a population-based approach, where seeds of more uniform characteristics where pooled to evaluate the impact of seed sorting on in vitro digestion kinetics; (ii) a seed-specific approach in which we managed to evaluate seed size, hardness and in vitro digestion properties on a single seed level. For the latter, specific methodological downscaling challenges were taken.

When the impact of seed size was investigated at the population level, small-sized seeds (0.6 g dry seed weight) proved higher maximum starch digestion rate constants and shorter lag phases than medium/large-sized seeds. Next, the medium-sized bean seeds (0.6-0.8g dry seed weight) were sorted into different hardness categories after cooking. Within this medium-sized bean seed class, the hardness categories showed no significant effect on in vitro starch digestion kinetics, indicating that the detected hardness differences did not impact in vitro starch digestion after the seeds were size-sorted.

When the seed-to-seed heterogeneity was studied for individual seeds, one cotyledon of an individual bean was digested and linked to the hardness of the other half cotyledon. For individual beans with hardness levels 70-80N and 90-100N, a ~20% spread in the level of starch digestion at 30 min of small intestinal simulation was observed. However, across individual seeds, seed hardness did not clearly correlate with the level of starch digestion. Importantly, the individual-seed approach revealed even more pronounced seed-to-seed variability masked by population-based digestion analyses, even after sorting.

In summary, this work showed seed heterogeneity issues within a single common bean batch. They were documented at the level of seed size, post-cooking hardness, and in vitro starch digestion properties. This work shows the benefit of sorting approaches combined with downscaling to grasp seed-to-seed differences and potentially elucidate the mechanisms behind these differences. Insights into the relation between seed properties and digestion can lead to easier tailoring of the digestion properties of pulse-derived foods.

Keywords

Heterogeneity; seed size; hardness; starch digestibility; infogest method;

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

DIGESTION DYNAMICS OF QUINOA (C. QUINOA WILLD) PROTEINS: UNCOVERING THE ANTIOXIDANT, ANTIDIABETIC AND HYPOTENSIVE POTENTIAL OF RELEASED BIOACTIVE PEPTIDES

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Abstract

In recent years, bioactive peptides have attracted considerable interest as promising agents for the development of functional ingredients to mitigate oxidative stress. Quinoa is a pseudocereal characterized by high protein content and a complete essential amino acid profile, represents a promising source for the identification and characterization of bioactive peptides. The aim of this study was to assess and compare the protein digestion patterns and the in vitro antioxidant, antidiabetic & hypotensive properties of 3 quinoa varieties: black quinoa protein isolate(PIBQ), yellow quinoa protein concentrate(PCYQ), and red quinoa protein concentrate(PCRQ) using the INFOGEST2.0 digestion protocol. Each sample was digested individually, aliquots were collected at oral phase, gastric(G) (1h/G1 and 2h/G2) and intestinal(I) (1h/I1 and 2h/I2), respectively. Finally, the G2 and I2 from all quinoa digestates was assessed for peptide identification by DDA-PASEF/LC-MS/MS. After digestion PCYQ(I2) showed the highest degree of hydrolysis (59.23%) among all samples. Notably, this behaviour was also observed in the SDS-PAGE, where the PCYQ exhibited bands of low molecular weight (MW) for the I1 and I2 digestates (10.2–15.2kDa). In general, all quinoa digestates showed a progression on proteolysis along digestion stages and smaller MW bands at the intestinal phase. Regarding peptide content, PIBQ(I1) presented the highest peptide concentration (225mg/mL). The antioxidant capacity showed that PCYQ and PCRQ maintained high copper-chelating capacity (61.20% and 60.98% respectively) at I2. TEAC inhibition assay showed the highest radical scavenging activity at oral phase: 38.69%(PIBQ), 42.58%(PCYQ), and 28.39%(PCRQ) at 1000µg/mL, respectively. About α-glucosidase inhibition, this was observed for all quinoa digestates across the tested concentration range (100–1000µg/mL). PCYQ(G2) showed the highest α-glucosidase inhibition (61.23% at 1000µg/mL). DPP-IV inhibition increased in a dose-dependent manner for all quinoa digestates; PCRQ(OP) and PCYQ(I2) yielded the lowest concentration EC50 values (421.25µg/mL and 425.86µg/mL respectively), indicating the strongest DPP-IV inhibition. Finally, the ACE-I inhibitory activity was the highest in the intestinal phases (I1 and I2). Across samples, the highest inhibition was observed for PCRQ(I1) with 79.5% at 1000µg/mL. The peptidomic analysis revealed that G2 showed 1218 peptides with the same characteristics among all quinoa digestates, while I2 presented 2972 peptides (7–20 aa). PCYQ showed the highest number of peptides (1219 peptides) at G2, while PIBQ presented 560 peptides at I2. In conclusion, the in vitro gastrointestinal digestion showed effectively released peptides with metal chelating and radical scavenging activities. PCYQ exhibited the highest antioxidant, α-glucosidase and DPP-IV inhibitory capacity at I2. While, PCRQ had the highest at ACE-I. Further research is needed to validate these results.

Keywords

Quinoa, digestion, bioactive peptides, peptidomic, antidiabetic, antioxidant, hypotensive

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECT OF THERMAL PROCESSING AND IN VITRO DIGESTION ON THE BIOACTIVITY OF TROUT PROTEIN HYDROLYSATES

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Abstract

Fish proteins are increasingly recognized as valuable dietary sources of bioactive peptides with potential health-promoting properties. Among freshwater species, rainbow trout (Oncorhynchus mykiss) is characterized by high protein digestibility and wide consumption, which makes it a promising carrier of food-derived bioactives. The aim of this study was to evaluate trout muscle proteins as precursors of bioactive peptides and to assess the effect of thermal processing on the bioactivity of protein hydrolysates obtained during simulated gastrointestinal digestion.

In silico analysis of trout myofibrillar and sarcoplasmic proteins was conducted using UniProt and BIOPEP-UWM databases to assess the profile of encrypted bioactive sequences. The analyses indicated that trout proteins contain a wide range of peptide fragments with predicted biological activities, among which angiotensin I-converting enzyme (ACE) inhibitory, dipeptidyl peptidase IV (DPP-IV) inhibitory, and antioxidant peptides were the most prevalent. Based on these predictions, raw and thermally treated trout meat protein extracts were subjected to simulated gastrointestinal digestion in vitro according to the INFOGEST protocol.

The obtained protein digests exhibited biological activity, confirming that trout proteins can act as precursors of bioactive peptides released during digestion. Thermal processing influenced the digestion process and affected the bioactivity of the resulting hydrolysates, indicating that heat-induced modifications of protein structure may alter the pattern of peptide formation during gastrointestinal digestion.

Overall, the results support the potential of trout proteins as a source of digestion-derived bioactive peptides and highlight the importance of integrating food processing conditions with digestion models when evaluating the bioactivity of protein hydrolysates. These findings contribute to a better understanding of how thermal treatment and digestion jointly influence the biological properties of food protein digests.

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Keywords

trout proteins, bioactive peptides, in silico analysis, in vitro digestion, thermal processing

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

HOW B-CASEIN ENRICHMENT EFFECTS IN VITRO INFANT DIGESTION OF REASSEMBLED CASEIN MICELLES

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Abstract

Abstract:

Casein micelle structure strongly influences protein digestion in infant formulas. Most infant formulas are produced from bovine milk, in which casein micelles contain a lower proportion of β-casein compared with human milk, the gold standard for infant nutrition. As a result, enrichment of bovine milk–based formulas with β-casein have been explored as a strategy to improve its digestive behavior. However, enriching β-casein directly in milk inevitably alters multiple components and does not control if and how β-casein is incorporated into the casein micelle. This study therefore investigates how β-casein enrichment, and its mode of incorporation, modify micellar structure and digestion behavior. Native casein micelles (NCM), reassembled casein micelles (RCM), and two β-casein–enriched RCMs were studied. In the enriched systems, β-casein was added either during micelle reassembly (R60-D) or after reassembly (R60-A). Digestion was evaluated at two casein concentrations representing bovine milk (2.8%) and human milk (0.48%), using an in vitro infant digestion model. Structural and digestive changes were assessed using soluble protein content, degree of hydrolysis, SDS–PAGE, clot mass, and confocal laser scanning microscopy. Digestion behavior differed markedly depending on the mode of β-casein incorporation. β-casein incorporation during micelle reassembly (R60-D) resulted in more gastric coagula and higher digestibility compared to after-reassembly (R60-A). Native casein micelles formed denser coagula with the lowest digestibility, whereas RCM exhibited faster hydrolysis. These digestion trends were consistent across both casein concentrations. These findings demonstrate that micellar structure, as determined by the mode of β-casein incorporation, governs enzyme accessibility and digestion, providing insights for the structural redesign of caseins in infant formula.

Keywords

infant digestion, casein micelles, β-casein enrichment, reassembled casein micelles, protein concentrati

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

COMPREHENSIVE ANALYSIS OF PROTEIN DIGESTIBILITY IN 20 ALTERNATIVE PROTEINS PRODUCTS: INFLUENCE OF COMPOSITION AND PROCESSING FROM FLOURS TO ISOLATES.

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Abstract

Alternative proteins are central to achieving a more sustainable and nutritionally resilient food system. However, introducing new protein sources requires a detailed understanding of their digestibility, amino acid composition, antinutrient content, and how processing affects protein quality. This study assessed 20 commercial protein-rich ingredients derived from diverse sources (e.g., microalgae, bacteria, insects, rapeseed, oats, fava beans, chickpeas, and lentils) to explore how composition, solubility, and processing level (flour, concentrate, or isolate and deflavouring) influence protein digestibility and quality. Analyses included nitrogen content (Dumas), amino acid composition (ion-exchange chromatography), in vitro digestibility and DIAAS (INFOGEST protocol), trypsin inhibition activity, solubility, and peptide profiling via SDS-PAGE and Size Exclusion Chromatography (SEC).

Results showed wide protein content variability (16–90%) related to source and degree of processing. In vitro digestibility ranged from 60 to 100%, with Chlorella vulgaris exhibiting the lowest digestibility. Generally, protein isolates showed higher digestibility and degree of hydrolysis than concentrates or flours. Deflavouring processing clearly increased digestibility. Legumes typically displayed sulphur-containing amino acids (SAA) as limiting, whereas microalgae and microbial and insect proteins were limited in histidine and isoleucine, respectively. Lysine is limiting in rapeseed and oats. Trypsin inhibition was markedly higher in air-classified plant concentrates where native structures were preserved, while isolates and deflavoured ingredients exhibited lower inhibition, suggesting denaturation and aggregation mitigate enzyme inhibition. Under physiological conditions, no residual inhibition was detected, confirming that structural and compositional factors primarily drive digestibility outcomes.

SEC and electrophoretic analyses tracked protein hydrolysis throughout digestion, highlighting the dominant effect of intestinal conditions over gastric hydrolysis and the strong correlation between solubility and final digestibility. Overall, this research provides a comprehensive overview of nutritional functionality across alternative protein classes and identifies processing pathways that maximise protein digestibility and quality, an essential step to accelerate the adoption of sustainable, nutritionally balanced protein ingredients in future food systems.

Keywords

Alternative proteins, Protein digestibility, In vitro digestion, DIAAS, Solubility, Processing, Nutritional quality

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

STRUCTURAL REFORMATION STRATEGIES TOWARD LOW-GLYCEMIC RICE FLOUR

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Abstract

This study proposes a multi-step structural modification strategy to enhance the low-glycemic potential of rice flour, which has attracted increasing attention as a gluten-free alternative ingredient. Rice flour typically contains a high starch content (70–90%) and exhibits a high glycemic index (GI), while its resistant starch (RS) content is inherently low. Furthermore, thermal processing commonly used in cereal product manufacturing—such as steaming, baking, and extrusion—further reduces RS content and intensifies high-GI characteristics.

To retain or increase RS after heating, esterification (i.e., type 4 RS formation) has been widely applied to irreversibly modify starch structures. However, effective GI reduction requires not only enzyme-resistant structures through the modified functional groups, but also the reduction of rapidly digestible starch (RDS). Accordingly, three structural modification strategies were compared:

(1) malic acid–induced esterification (MA-esterification),

(2) MA-esterification followed by α-amylase hydrolysis, and

(3) annealing in malic acid solution followed by esterification.

Rice flour (FRF, cultivar Baromi2) was esterified via two-step heating at 45 °C and 90 °C (MA-FRF). MA-FRF was subsequently treated with α-amylase to partially hydrolyze RDS (MA-Ez-FRF). In the third approach, FRF was annealed in malic acid solution at 60 °C prior to esterification (MA-ANN-FRF).

In vitro digestion showed that RDS contents decreased from 56.20% (FRF) to 46.43% (MA-FRF) and 16.03% (MA-Ez-FRF), while RS contents increased from 10.85% (FRF) to 50.35% (MA-FRF) and 81.67% (MA-Ez-FRF). Estimated GI (eGI) values decreased proportionally: 95.63 (FRF), 63.21 (MA-FRF), and 45.59 (MA-Ez-FRF), indicating that RDS reduction is an effective strategy for producing low-GI foods (GI ≤ 55).

Notably, MA-ANN-FRF exhibited a lower eGI of 41.74 and a markedly higher dietary fiber content (64.6 g/100 g) compared with MA-Ez-FRF (10.5 g/100 g). Clinical GI testing further confirmed an exceptionally low GI of 26.7 for MA-ANN-FRF, corresponding to only 27% of that of FRF (97.9).

These findings suggest that annealing in malic acid solution promotes molecular rearrangement between amorphous and crystalline starch domains, enabling preferential esterification within amorphous regions and effectively restricting primary enzymatic access. This approach allows efficient ester group placement under relatively mild acidic conditions, offering a promising alternative to conventional high-acid esterification for producing low-GI rice flour ingredients.

Keywords

glycemic index, resistant starch, rapidly digestible starch, esterification, α-amylase, annealing

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECT OF BRASSICA INGREDIENTS ON ACRYLAMIDE FORMATION AND BIOACCESSIBILITY IN CRACKERS

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Abstract

Acrylamide is a processing contaminant with toxicological relevance formed in cereal-based foods during thermal treatment. Beyond its concentration in foods, acrylamide bioaccessibility after digestion plays a key role in determining consumer exposure. Previous studies showed that food matrix and interactions between food components can influence acrylamide bioaccessibility.1 Michael-type addition reactions acrylamide-nucleophilic compounds have been reported, leading to a reduction in acrylamide bioaccessibility.1,2 Our study evaluated acrylamide bioaccessibility in crackers added with broccoli and cabbage, Brassica vegetables rich in sulfur-containing amino acids and other nucleophilic compounds. Crackers were produced by partially replacing refined wheat flour (control) with 15% Brassica powders and baked under standardized conditions (200 °C; 14 min). In broccoli, inflorescences and stems were assessed. Samples were subjected to in vitro gastrointestinal digestion (INFOGEST) and acrylamide was quantified by LC–ESI–MS/MS in the undigested samples as well as in the bioaccessible and non-bioaccessible fractions after digestion. Acrylamide levels in the control crackers were 155 µg/kg, whereas higher concentrations were observed in crackers enriched with broccoli inflorescences (1084 µg/kg) and cabbage (961 µg/kg), and to a lesser extent in those with broccoli stems (546 µg/kg). Acrylamide bioaccessibility reached 90.9% in the control crackers, while lower values (81.6–84.0%) were shown in Brassica-enriched formulations, with recoveries between 89.8-101.1%. Although acrylamide formation increased during baking in Brassica-added crackers, its bioaccessible fraction (%) was reduced compared with the control. This effect is likely associated with interactions between acrylamide and sulfur-containing amino acids or thiol-containing compounds released during digestion, potentially via Michael addition reactions,3 limiting acrylamide availability in the gastrointestinal environment. Data suggest that incorporating Brassica ingredients into cereal-based products may represent a promising strategy to mitigate acrylamide exposure by reducing its relative bioaccessibility during digestion. Nevertheless, the increased formation of acrylamide after Brassica inclusion must also be considered, as new ingredients may provide higher levels of precursors that promote acrylamide formation during thermal processing. Therefore, despite the reduction in the percentage of bioaccessible acrylamide, high formation rates also led to raised amounts of total bioaccessible acrylamide, which could still compromise food safety and health. Findings highlight the need to evaluate both formation and bioaccessibility together when assessing acrylamide risk in food products.

1.González-Mulero et al. (2022). Food Res. Int., 161, 111820.
2.González-Mulero et al. (2023). Food Res. Int., 164, 112409.
3.Hamzalıoğlu & Gökmen (2015). Food Func., 6, 109-114.

Keywords

acrylamide bioaccessibility; brassicas; broccoli; cabbage; food safety

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECT OF PECTIN ON CALCIUM RELEASE FROM ACID-INDUCED PEA PROTEIN GELS DURING DYNAMIC IN VITRO DIGESTION

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Abstract

Plant-based dairy alternatives often require calcium fortification and texture modifiers or stabilisers to meet both the nutritional and functional standards of their dairy counterparts. Dietary calcium intake is determined not only by the amount consumed but also by the efficiency of its delivery, which depends on its release from the food matrix during digestion. Structural changes arising from texture modifiers, as well as interactions between calcium and these modifiers, may influence calcium release rates from the food matrix into the GIT; however, research on this topic remains limited. In this study, a plant-based yoghurt alternative model was developed using 5% pea protein and 1% GDL to induce acid gelation. The acid-induced gels were fortified with insoluble $CaCO_3$ (30 mM; 1200 ppm Ca) and modified using different doses (0, 0.2, and 1.0% w/w) of a gelling agent, low-methoxyl (LM) pectin. Gel textural and structural properties were characterised using a texture analyser and confocal laser scanning microscopy. The calcium-fortified gels were subjected to dynamic in vitro gastric digestion using a Human Gastric Simulator, combined with static oral and intestinal phases. Digesta were collected at 30-min intervals to determine pH, total solids, and calcium profiles. Total, soluble, and ionic calcium were quantified using microwave plasma atomic emission spectrometry (MP-AES) and a calcium-selective electrode. Results showed that the addition of pectin altered the microstructure of pea protein gels. A high dose of pectin (1.0%, w/w) resulted in thicker protein strands and a more compact network, leading to significantly higher gel firmness. In contrast, a low dose of pectin (0.2%, w/w) contributed to the formation of a homogeneous, intertwined pectin–protein network; however, this did not alter the mechanical properties compared with the control sample without pectin. During in vitro gastric digestion, the gels with higher hardness and more compact microstructure slowed gastric emptying, which in turn delayed total calcium release rates in both gastric and intestinal phases. However, ionic and soluble calcium release appeared to be unaffected by gel firmness or its breakdown during digestion in this acidic gel system. In addition to enhancing gelation, LM pectin might bind ionic/soluble calcium through electrostatic interactions in the intestinal phase, thereby preventing precipitation with phosphate or carbonate present in simulated intestinal fluid (SIF). This resulted in a more sustained and higher ionic calcium release in the 1.0% pectin sample. Overall, these findings indicate that while gel firmness does not govern soluble/ionic calcium release in acid-induced gels, the incorporation of LM pectin can modify the kinetics of total calcium release during digestion. This work provides insights for designing plant-based dairy alternatives, highlighting how other ingredients in yoghurt formulations can alter calcium release patterns.

Keywords

dynamic in vitro digestion, calcium fortification, acid-induced gels, pea protein

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECTS OF FERMENTATION ON PROTEIN DIGESTION, PEPTIDE RELEASE AND PEPTIDE ABSORPTION IN AN INFOGEST IN VITRO ADULT MODEL

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Abstract

This study aims to investigate the effects of fermentation on protein digestion, peptide formation, and peptide absorption using the INFOGEST in vitro digestion model combined with a Caco-2 monolayer model. Fermented milk samples were prepared from pasteurized whole milk (W) using Lactobacillus delbrueckii subsp. bulgaricus in combination with Bifidobacterium animalis subsp. lactis (Y1), or with L. delbrueckii subsp. bulgaricus alone (Y2). This study investigated the gastric and intestinal digestion of samples and compared their digesta peptide profiles (10 mins intestinal digestion) before and after absorption with the range of 3-25 amni acid. Y1 and Y2 showed higher protein hydrolysis degree and higher intensity of overall peptides than W, both in gastric and intestinal digesta. PCA analysis of peptidomics data showed that fermentation altered the peptide profile of the gastric digesta but not that of the intestinal digesta. Further analysis of gastric digesta peptides showed that subgroup of peptides significantly more abundant and only present in fermented milk was generally longer than those more abundant in whole milk. The cleavage site patterns of these peptide subgroups also differed between fermented and unfermented samples. During absorption, brush border enzymes further cleaved most peptides, and cleavage patterns were similar for fermented and whole milk digesta samples. Cleavage after proline was limited during both digestion and absorption, and peptides containing the “PP” motif were most abundant in the digesta compared to other motifs with “P”. Fermented milk exhibited higher intensity of absorbed bioactive peptides than whole milk, which may be explained by the higher proportion of long peptides in fermented milk digesta that were not further cleaved into smaller fragments during absorption. This study provides insight into how fermentation influences protein digestion and peptide absorption, with implications for the bioavailability and bioactivity of proteins.

Keywords

fermented milk, digestion, absorption, peptidomics

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECTS OF PROCESSING AND IN VITRO PROTEIN DIGESTION ON IMMUNOREGULATING PROTEASE INHIBITORS FROM LEGUME SEEDS

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Abstract

Background: Bowman Birk and Kunitz trypsin inhibitors (BBI and KTI, respectively) are two well-characterized families of serine protease inhibitors (PI) abundant in legume seeds such as soybeans. Active PI have been shown to modulate proteolysis in the gastro-intestinal (GI) tract (T), reducing key pro-inflammatory proteases and thereby acting as potential anti-inflammatory compounds. Due to their rigid structure stabilized by disulfide bonds, PI exhibit notable resistance to heat and pH variations. However, common processing methods such as boiling substantially reduce PI activity in legume seeds, which may limit their potential benefits in the gut. Despite this, the effects of in vitro protein digestion on PI remain poorly understood.

Aims: The aims of this work were to assess the effects of processing on the activity of PI in whole legume seeds and to investigate whether PI are affected by in vitro protein digestion.

Methods: Trypsin and chymotrypsin inhibitor activity (TIA and CIA, respectively) assays were used to measure the activity of semi-pure PI (commercially available) and of raw and boiled peas and faba beans. To simulate adult digestion conditions in vitro, the INFOGEST method was performed (oral, gastric and intestinal phases) using: semi-purified BBI and KTI separately mixed with BSA (~ 1:10) as model to mimic PI content in food on a protein basis, raw peas (PR), cooked peas (PC), raw faba beans (FR), and cooked faba beans (FC). The OPA method was applied to quantify the free amino groups after digestion and to determine the degree of hydrolysis (DH), and SDS-PAGE to visualize protein profile of the digests.

Results: TIA and CIA were significantly reduced after soaking + boiling peas (TIA: -91%, CIA: -62%) and faba beans (TIA: -95%, CIA: -61%). After in vitro digestion, the DH of BSA + 10% BBI/KTI was similar to that of PC, FR and FC. Soaking and boiling improved protein digestibility in peas but not in faba beans, although the TIA and CIA after cooking were similar between the two legume seeds. SDS-PAGE revealed that both BBI and KTI bands were intact in BSA + BBI/KTI digests, suggesting the digestive resistance of PI. However, BBI and KTI bands were not visible in PR, PC, FR, or FC, maybe due to lower content of PI within these samples.

Conclusions: As expected, processing highly reduced both TIA and CIA of peas and faba beans. Processing increased protein digestibility of cooked peas but, unexpectedly, not of cooked faba beans. Here, possible pre-germination during the soaking step, and matrix effects need to be further investigated. Both BBI and KTI bands appeared in BSA+ BBI/KTI digests, indicating their structural resistance through digestion conditions, as expected. Further research will evaluate if PI in these digests are still active to investigate their putative anti-inflammatory activity in the GIT.

Keywords

Plant protease inhibitors, Bowman Birk inhibitor, Kunits trypsin inhibitor, legume seed, in vitro protein digestion

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

MODIFYING MACRONUTRIENT DIGESTION THROUGH ADDITION OF CELLULAR LEGUME FLOURS TO PASTA

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Abstract

Conventional legume milling techniques destroy legume cellular structure and thereby impact nutrient digestion. Previous in vitro research repeatedly showed that intact legume cells lower starch digestion rate compared to broken cells, leading to a reduction of post-prandial glycemic responses (Duijsens et al., 2022; Rovalino-Córdova et al, 2018; Bajka et al., 2021). In contrast, evidence regarding whether the cellular structure influences the total amount of digestible starch and protein remains inconsistent (Staes et al., 2025; Zahir et al., 2021; Rovalino-Córdova et al., 2019).

The aim of this study was to compare the impact of cell wall integrity of various legumes (black bean, chickpea, kidney bean, lentil, yellow pea) on in vitro starch and protein digestion. “Cellular” flours, made up of intact cells were obtained by hydrothermal treatment, whereas the “broken” flours made up of mechanically disrupted cells were produced by cryomilling the cellular flours. Next to this, the penetration of pancreatic α-amylase within intact cells during digestion was studied by measuring the α-amylase activity inside cellular flour after 10 and 20 minutes of intestinal digestion. In vitro protein and starch digestion, assessed using the INFOGEST and the Englyst method respectively showed that for all legumes cellular integrity had no significant impact on total protein and starch digestion with the exception of chickpea flour (7% decrease in total starch digestion in cellular flour). The impact of the cellular integrity on starch digestion rate varied across legumes. Cellular disruption had the largest impact on digestion rate of kidney bean and lentil flour leading to a 30-45% increase in rapidly digestible starch (RDS) compared to intact cells. Interestingly, the differences in starch digestibility between broken and cellular flours did not strongly correlate with the α-amylase activities measured in the intact flours. This demonstrates that the protective effect of intact cell walls on starch digestion probably involves other factors such as differences in cell wall cellulose contents which can bind and inhibit pancreatic amylase (Dhital et al., 2015).

To explore the effect of cellular integrity on starch digestion in a real food system, cellular or broken yellow pea flours were incorporated in wheat spaghetti in different amounts (0, 10, 30, 50%). Interestingly, in pasta with 30% yellow pea flour, legume cellular integrity did not significantly affect starch digestion. However, the replacement of 30% wheat flour with yellow pea flour increased RDS by 50 and 17% in lab and pilot scale produced pastas. Yellow pea flour may have increased nutrient accessibility through weakening the gluten network. This hypothesis was substantiated by the positive correlation of the pasta cooking losses with starch digestion. Our findings provide guidance on ways to tailor food processing to optimize starch and protein digestion using legume flours.

Keywords

Cellular integrity, Legume, Pasta, Extrusion, Starch digestion, Protein digestion

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

FROM OXIDATION TO DIGESTION: THE GASTROINTESTINAL FATE OF LIPID OXIDATION PRODUCTS IN SUNFLOWER OIL

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Abstract

High-lipid shelf-stable foods are prone to oxidative reactions during processing and storage, leading to the formation of lipid oxidation products (LOPs). Lipid oxidation initially generates primary oxidation products, namely lipid hydroperoxides, which are unstable and decompose into secondary oxidation products, mainly volatile compounds including aldehydes, ketones, alcohols, carboxylic acids. Although these compounds can impart unpleasant sensory attributes, even strongly oxidised foods are often still perceived as acceptable by consumers.

LOPs are associated with oxidative stress and the development of non-communicable chronic diseases, including obesity, cardiovascular and neurodegenerative disorders, and cancer. However, the fate of dietary LOPs during gastrointestinal digestion and the role of digestive conditions in modulating the oxidative status of ingested lipids remain poorly understood, with available evidence being fragmented and often contradictory.

This study aimed at investigating the impact of in vitro gastrointestinal digestion on primary and secondary lipid oxidation products, using sunflower oil as a simplified model system representative of high-lipid, shelf-stable foods.

Oil samples with different initial oxidative status were obtained through temperature-accelerated oxidation, producing peroxide values (PV) ranging from 10 to 80 $mEqO_2/kg$. Samples were subjected to the Infogest simulation of gastrointestinal digestion, and PV was determined as an indicator of primary oxidation products before and after digestion, using the official COI method. Volatile secondary oxidation products were determined by solid-phase microextraction coupled with gas chromatography–mass spectrometry (SPME-GC-MS) on the headspace of undigested and digested oil samples.

Digestion significantly affected LOPs. Specifically, PV remained largely unchanged during the gastric phase, while the intestinal phase led to a marked reduction in PV, particularly in samples with advanced oxidative status, where decreases exceeded 50%. Regarding secondary oxidation products, overall, 37 volatile compounds were identified, mainly aldehydes and ketones, followed by alcohols and carboxylic acids. In undigested oils, 25 volatile compounds significantly increased as a function of the initial PV. After digestion, only 12 volatile compounds presented significant variations as a function of the initial PV, generally increasing with few exceptions.

These findings suggest that the intestinal phase of digestion plays a central role in modulating lipid oxidation, coinciding with a reduction of hydroperoxides associated with the formation and persistence of secondary oxidation products. In light of evidence indicating that consumers may still consider highly oxidized shelf-stable foods acceptable, the dietary intake of potentially harmful volatile LOPs may represent an underestimated risk to human health.

Keywords

Dietary lipids; Oxidation products; Peroxide value; Volatile compounds; SPME-GC-MS; Food safety; Shelf-stable foods

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

FROM GRAIN TO COLON: HOW BARLEY GENOTYPE AND PROCESSING SHAPE FIBER FERMENTATION AND PHENOLIC BIOTRANSFORMATION

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Abstract

Context: Barley is a cereal rich in fermentable dietary fibre and phenolic compounds with recognized benefits for gut health. However, the nutritional and functional properties of barley-based foods are strongly influenced by both genotype and processing. Biofortified genotypes and technological treatments such as extrusion may substantially modify fibre structure, polyphenol profile, and their physiological fate along the gastrointestinal tract.
Objective: To evaluate the impact of extrusion and genotype on the bioaccessibility of β-glucans and phenolic compounds, as well as on colonic fermentation and phenolic metabolism, using an integrated in vitro gastrointestinal digestion–colonic fermentation model.

Methods: Four barley genotypes differing in β-glucan content, type of starch, and phenolic profile were processed by extrusion. Samples were subjected to standardized in vitro gastrointestinal digestion followed by 48 h colonic fermentation. The bioaccessibility of β-glucans and phenolic compounds was determined after digestion. Short-chain fatty acids (SCFA) were quantified by GC-FID, and phenolic-derived metabolites were characterized by HPLC-MS/MS. Changes induced by extrusion on starch, fibre and phenolic composition were also evaluated.

Results: Extrusion significantly modified the composition of the barley genotypes, increasing amylose content while slightly reducing β-glucans and total phenolic compounds. After in vitro digestion, 40-55% of β-glucans but only 15–20% of total phenolics were recovered in the bioaccessible fraction, whereas most phenolics, especially bound phenolic acids, remained in the non-bioaccessible fraction, indicating their potential availability for colonic fermentation. During fermentation, fermentable carbohydrates were rapidly utilized, leading to a marked increase in SCFA production, particularly butyrate, with a stronger effect observed in β-glucan-rich genotypes. In parallel, native phenolic compounds were progressively degraded and converted into a wide range of low-molecular-weight phenolic metabolites, reflecting an extensive biotransformation of barley polyphenols.

Conclusions: Although extrusion partially reduced the content of native bioactive compounds, it generated barley-based foods with enhanced fermentability and strong functional potential. The combined stimulation of SCFA production and the conversion of polyphenols into bioactive metabolites provide mechanistic insight into how genotype selection and processing can shape the gut health potential of cereal-based functional foods through synergistic fibre–polyphenol interactions.

Keywords

Barley; Dietary fibre; In vitro digestion; Colonic fermentation; Polyphenol metabolism; Short-chain fatty acids; Fu

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STATIC IN VITRO SIMULATION OF GASTROINTESTINAL FOOD DIGESTION (INFOGEST) TO ASSESS SAFETY AND BIOAVAILABILITY OF ALTERNATIVE PROTEIN SOURCES

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Abstract

The increasing global population and demand for animal protein, alongside extensive use of natural resources, have led to a scarcity of traditional protein sources, driving the search for alternative proteins (APs) such as plant-based, insect-based, microbe-derived and ocean-based [1,2]. To properly integrate these APs into food systems, it is necessary to demonstrate their nutritional and safety adequacy for human consumption. This study evaluated the safety profile and bioavailability of 12 alternative protein ingredients (APIs) from various sources, including insect, pea, fava, rapeseed, krill, fungus, and bacteria, as well as and 16 alternative final products by in vitro assessment that analysed amino acid composition, digestibility using the INFOGEST protocol [3], and intestinal absorption through differentiated Caco-2 cells. Cytotoxicity was evaluated by exposing Caco-2 cells to varying concentrations of digestates for 4 hours, measuring cell viability, and then assessing barrier integrity and permeability after exposure to sub-toxic digestate concentrations. Additionally, the bioavailability of Branched Chain Amino Acids (BCAAs) in the digestates was quantified using a commercial assay kit. Overall, the results indicated that the different APs tested demonstrated high bioavailability of essential nutrients and no detectable toxicity following intestinal absorption. In conclusion, the assessed APs exhibited no detectable toxicity and high bioavailability after intestinal absorption, supporting their potential incorporation into food systems for human consumption.

[1] Quintieri, L., Nitride, C., De Angelis, E., Lamonaca, A., Pilolli, R., Russo, F., & Monaci, L. (2023). Alternative Protein Sources and Novel Foods: Benefits, Food Applications and Safety Issues. Nutrients, 15(6), 1509. https://doi.org/10.3390/nu15061509

[2] Malila, Y., Owolabi, I.O., Chotanaphuti, T. et al. Current challenges of alternative proteins as future foods. npj Sci Food 8, 53 (2024). https://doi.org/10.1038/s41538-024-00291-w

[3] Brodkorb, A., Egger, L., Alminger, M. et al. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat Protoc 14, 991–1014 (2019). https://doi.org/10.1038/s41596-018-0119-1

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

LIPID OXIDATION AND PROTEIN (GLYC)OXIDATION DURING HEATING AND IN VITRO GASTROINTESTINAL DIGESTION OF COMMERCIAL MEAT AND PLANT-BASED BURGERS

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Abstract

I. INTRODUCTION

Meat analogue burgers are increasingly positioned as alternatives to meat-based burgers, due to environmental and societal concerns. However, little is known about how plant-based and meat-based burgers differ in their chemical stability during heating and gastrointestinal digestion. These products differ markedly in lipid- and protein composition, matrix structure, and pro-oxidant and antioxidant constituents, including the presence of heme iron in meat and its absence in plant-based analogues. In this study, we directly compared commercial meat and plant-based burgers to investigate how these compositional differences influence lipid oxidation and protein (glyc)oxidation during heating and subsequent in vitro gastrointestinal digestion.

Ⅱ. MATERIALS AND METHODS

Commercial meat-based (n=7) and plant-based (n=7) burgers were purchased from the local supermarket, subdivided into 100 g portions, and grilled for 7 min at a surface temperature of 180 ℃. Amino acid (AA) and fatty acid (FA) composition were determined in cooked samples by HPLC-FLD and GC-FID, respectively. Cooked samples were subsequently subjected (in quintuplicate) to an in vitro gastrointestinal digestion model. Raw, cooked, and digested samples were analyzed for: (i) glycoxidation, assessed as protein-bound pentosidine (PEN; HPLC-FLD) and Maillard reaction products (MRPs; spectrophotometry); (ii) lipid oxidation, determined by 4-hydroxy-2-nonenal (4-HNE), propanal (PROP), and hexanal (HEX) using HPLC-FLD; (iii) protein oxidation, measured as protein carbonyl content (PCC; spectrophotometry); and (iv) dry matter digestibility, determined gravimetrically.

Ⅲ. MAIN FINDINGS

Plant-based burgers contained significantly higher polyunsaturated fatty acid (PUFA) contents and elevated PUFA/SFA ratios, and their amino acid profiles were especially enriched in glutamic acid, whereas meats contained higher levels of methionine and lysine. Maillard reaction products remained low in raw and cooked samples, and their levels increased substantially following digestion, though no significant differences were observed between meat and plant-based digests. Similarly, no significant inter-group differences were found in pentosidine levels in digest samples. Lipid oxidation products (HNE and hexanal) were significantly higher in raw plant-based burgers (vs. meats), increased during digestion, with no significant differences between groups post-digestion. Protein oxidation (PCC) was significantly higher in plant-based burgers compared with meats both prior to heating and following digestion. No significant digestibility differences were found between cooked plant-based and meat burgers.

Ⅳ. CONCLUSIONS

These results demonstrate that raw plant-based substitutes showed lower oxidative stability, likely due to their high PUFA content and prior processing steps. However, following digestion, only PCC still differed significantly between groups.

Keywords

4-HNE, pentosidine, fatty acid composition, protein carbonyl compounds

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

BIOACTIVITY AND FUNCTIONAL PROPERTIES OF NOVEL FERMENTED DONKEY MILK BEVERAGES ENRICHED WITH COMMERCIAL PROBIOTIC BACTERIA

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Abstract

In recent years, donkey milk is considered as a promising non-bovine milk alternative due to its unique composition and its potential functionality and positive effect on human health. In addition, fermentation and added probiotics could further enhance donkey's milk health benefits to the consumer. In this study, a starter culture (Streptococcus thermophilus) and three probiotic strains (Lactobacillus rhamnosus LGG, Lactobacillus paracasei subsp. paracasei L. CASEI 431 and Lactobacillus helveticus R0052) were used to ferment donkey, enhance the milk's nutritional value and prolong its shelf life. Probiotics were used in pure cultures and in co-cultures with and without prebiotic (inulin) addition. Fermentation temperature was 37oC. The process was subsequently stopped by cooling milk at 4oC; i.e. when milk had reached pH 5. The bioactivity of the fermented beverages was evaluated by assessing antimicrobial activity against selected pathogens, antioxidant activity using ABTS, DPPH and Total Phenolic Content assays, α-glucosidase inhibitory activity and Angiotensin-Converting Enzyme (ACE) inhibitory activity. All bioactivity assays were performed both before and after static in vitro simulation of gastrointestinal digestion following the INFOGEST protocol. The results, with the exception of antimicrobial activity, indicated that fermentation enhanced the bioactivity of donkey milk beverages, while the addition of inulin further increased these effects. Beverages fermented with LGG and R0052 exhibited the highest bioactive responses across all assays, whereas L. CASEI 431 remained statistically significant at lower levels compared with the other probiotic strains. Moreover, the same trends were maintained after in vitro digestion, with digesta from fermented beverages showing significantly improved bioactive properties compared with the control samples (pasteurized donkey milk). Overall, the findings demonstrate that fermented donkey milk, particularly when combined with selected probiotic strains and inulin, represents a promising functional beverage with enhanced bioactive properties that are retained after simulated gastrointestinal digestion. These results support the potential of fermented donkey milk as a non-bovine dairy alternative with added health related functionality and justify further investigation under in vivo conditions.

Keywords

Non-bovine dairy milk, donkey milk, nutritional functionality, probiotic fermentation, in vitro digestion

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IN VITRO DIGESTIBILITY AND PROTEIN QUALITY OF MYCOPROTEIN

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Abstract

Mycoprotein is a sustainable wholefood alternative protein source from fungal biomass. Due to its high nutrient content, containing protein, fiber, micronutrients and bioactives, it is a promising ingredient with potential applications in various food products.

The objective of this study was to evaluate the in vitro digestibility and protein quality (Digestible Indispensable Amino Acid Score, DIAAS) of three batches of mycoprotein dry powder and a more finely milled mycoprotein powder, compared to four plant- and dairy-based reference materials (soy protein isolate, pea protein isolate, chickpea protein concentrate, whey protein isolate). Protein content and amino acid composition of the test materials were determined and all samples were subjected to the standardized static in vitro digestion protocol based on INFOGEST 2.0, followed by LC-MS amino acid analysis. Two experimental approaches were applied for sample processing and analyses following the digestion protocol, differing with respect to blank correction, precipitation of the digesta and hydrolysis of digested samples prior to amino acid analyses. Digestibility coefficients and DIAAS values were calculated for all test materials.

Essential amino acid composition, digestibility and DIAAS were very similar for the two mycoprotein materials, with very limited batch-to-batch variation for both. Protein content of the mycoprotein materials was lower compared to plant- or dairy-based reference protein ingredients. Using the first sample processing method (based on free amino acids in digesta corrected for background from pancreatin), DIAAS values were calculated to be on average 105 and 103%, respectively, for the reference pattern for children aged 6 months to 3 years. However, the second approach following most recent INFOGEST recommendations (protein-free cookie for blank correction, precipitation of digested samples followed by hydrolysis and amino acid analyses) yielded average DIAAS values of 49 and 53%, respectively. In both cases, methionine and cysteine were identified as most limiting amino acid. DIAAR values for all other essential amino acids were determined to be >95%. The impact of the two different methods varied across the reference samples, with DIAAS values either lower or higher, or comparable results from both approaches.

The present results highlight the potential impact of specific protocols used for in vitro determination of digestibility and protein quality of mycoproteins. In contrast to traditionally used relatively pure, globular proteins, complex biomass-derived proteins obtain specific characteristics such as fibrous structures and cell wall compounds potentially interfering with digestive enzymes and/or methanol precipitation in the in vitro digestion protocol. Therefore, further research is needed to clarify how particular aspects of in vitro digestion procedures affect outcomes when applied to structurally complex, single cell protein ingredients.

Keywords

Mycoprotein, single cell protein ingredients, INFOGEST, in vitro digestion, DIAAS

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IN VITRO PROTEIN DIGESTIBILITY AND BIOACCESSIBILITY IN PLANT/ANIMAL RAGÙS: AMINO ACID DYNAMICS AND PEPTIDE MAPPING

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Abstract

Plant-based meat alternatives have advanced in mimicking sensory and structural qualities of animal meat. However, limited literature exists on their protein quality and digestibility, necessitating evaluation to confirm nutritional equivalence. This study assessed in vitro protein digestibility and bioaccessibility of experimental plant-based ragù A (yellow pea protein) vs. 2 commercial plant-based B–C (pea and pea/lentil/corn proteins respectively) and 2 commercial meat-based D–E (beef/pork). The protein fraction was characterized by total content (Kjeldahl), amino acid (AA) profile (total/free AA, EAA sum, AAS via UHPLC-ESI/MS), and integrity (OPA/NAC). Post-characterization, static INFOGEST in vitro gastro-intestinal procedure assessed protein fate, quantifying soluble proteins, degree of hydrolysis (DH), and free AA. Bioaccessibility was evaluated via AA profiles (total AA, EAA, AAS) in gastric/intestinal digesta, enabling DIAAS% computation. Digested peptide profiles were analysed by HR-MS. Results highlighted differences both between samples and in the amino acid profile prior and after in vitro digestion. Samples showed high protein integrity, indicating that the treatments underwent were not too harsh on the protein fraction. All samples exhibited high protein integrity and met FAO/WHO EAA requirements for adults/older children, with lysine (A, B), tryptophan (C, D), and valine (E) as limiting AA (AAS analysis), with higher values recorded for plant-based samples. After in vitro digestion, plant-based samples showed superior protein solubilization and degree of hydrolysis (DH%) vs. animal-based counterparts (ρ 0.05, highest in experimental ragù A). Alongside, values of free AA released coupled with high DH% suggest high release of di-/tri-peptide, suggesting high bioaccessibility. Results suggests a better digestibility of plant-products – probably related to the quality of the raw material used for the animal-ragùs. Differences between samples where anyhow ironed out when comparing EAA, AAS, and DIAAS at the end of the gastro-intestinal digestion, with all samples showing lysine ad limiting amino acid. Indeed, greater differences were noted in the EAA contents before and after digestion, especially for animal-based samples (D, E), which showed higher contents released. Peptide profiling showed, as expected, a good number of peptides, with longer sequences for animal-based samples (D, E). The lower digestibility of meat-based ragùs compared to plant-based ones, despite equivalent EAA content, suggests greater protein release from muscle tissue, whereas insoluble protein likely derives from connective tissue (abundant in low-quality ground meat). Plant-based ragùs emerge as highly digestible, nutritionally equivalent alternatives to meat-based ragùs, driven by raw material quality. Processing optimization could further enhance AA bioavailability in both.

Keywords

Plant-based ragùs, Protein digestibility, Protein bioaccessibility, DIAAS, Peptide mapping

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

SINGLE-CELL PROTEINS FROM XANTHOBACTER SP. SOF1 AS A NOVEL INGREDIENT FOR DAIRY ANALOGUES: A PROTEIN DIGESTIBILITY PERSPECTIVE

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Abstract

The food sector faces increasing pressure to identify sustainable, nutrient-dense protein sources that reduce environmental impact while maintaining sensory and nutritional quality. Single-cell proteins (SCPs) derived from carbon-fixing bacteria represent a promising solution. This study evaluates the digestibility and nutritional quality of foods formulated with Solein®, a microbial protein produced by Xanthobacter sp. SoF1, designed for incorporation into dairy analogues. Two model products (e.g., a beverage and a fermented yogurt) were developed and subjected to in vitro digestion using the INFOGEST static model, complemented by a semi-dynamic gastric digestion approach to capture kinetic aspects of protein hydrolysis. Measurements of total nitrogen, free amine groups (OPA assay), free amino acids, and DIAAS were used as indicators of digestibility and protein quality (DIAAS). Structural changes during digestion were monitored using Confocal Laser Scanning Microscopy (CLSM) and particle size distribution, while peptide profiling was assessed by SDS-PAGE and Size Exclusion Chromatography (SEC).

The static model showed that fermentation slightly reduced protein breakdown. Both matrices exhibited moderate protein digestibility (60% and 64%), with isoleucine identified as the limiting amino acid. The semi-dynamic digestion model revealed distinct digestion kinetics between formulations: yogurt-type systems demonstrated delayed gastric disintegration and slower protein release, attributed to their coagulated microstructure and larger aggregates. In contrast, the beverage matrix displayed faster solubilisation and protein accessibility, leading to higher hydrolysis rates. Microscopy confirmed matrix-dependent differences in structural disintegration and protein network breakdown, highlighting how physical structure influences gastrointestinal behaviour.

These findings establish Solein® as a nutritionally promising SCP ingredient whose moderate digestibility indicates opportunities for optimisation through fermentation or processing adjustments. The digestion kinetics observed provide valuable insight into how structural conformation and matrix interactions affect protein breakdown, emphasizing the importance of the structure–function–digestion relationship. By bridging processing, structure, and nutritional quality, this work supports the utilisation of bacterial single-cell proteins in sustainable dairy analogues and their integration into next-generation food systems.

Keywords

Single-cell proteins, Dairy analogues, Proteins, Structure–function–digestion relationship, Sustainable foods

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

A LOOK BEYOND THE INTERFACE: EFFECTS OF EMULSIFIER BLENDS AND DIGESTIVE CONDITIONS ON IN VITRO LIPOLYSIS KINETICS

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Abstract

Lipids in food often appear as oil-in-water (o/w) emulsions. They deliver energy, essential fatty acids, and lipophilic micronutrients. Research assessing in vitro lipolysis kinetics (i.e., rate and extent) demonstrated that o/w emulsions can be designed to affect nutrient release, which can affect physiological parameters such as satiety. Nevertheless, the majority of studies focus on (i) singular emulsifiers, (ii) the small intestinal phase, and (iii) static in vitro digestion. Contrarily, food emulsions typically contain multiple emulsifiers, and the gastrointestinal tract involves a gastric phase and dynamic processes that are often overlooked.

Our findings revealed that emulsifiers differ in their ability to reduce interfacial tension, indicating varying affinities for the o/w interface. Some emulsifiers adsorb more rapidly and strongly, thereby influencing interfacial composition in mixtures. However, in the small intestinal phase, where bile salts are present, lipolysis kinetics are more affected by emulsion stability and the emulsifiers' capacity to form mixed micelles than by the interfacial composition. Contrarily, in the gastric phase, bile salts are absent. Experiments with o/w emulsions stabilized by Tween 80 and pectin demonstrated that increasing proportions of Tween 80 inhibited gastric lipase adsorption. This inhibitory effect was substantially reduced during subsequent small intestinal digestion, underscoring the importance of including the gastric phase in digestion studies.

Findings above were all demonstrated using static in vitro digestion models, emphasizing their strength in showing the effect of emulsion design on lipolysis kinetics. However, our research showed that certain semi-dynamic parameters can influence lipolysis kinetics. Gradual addition of gastric or pancreatic lipase was negligible during digestion of lecithin-stabilized o/w emulsions. However, lipolysis kinetics were affected by the gradual addition of bile salts, once again demonstrating their importance in facilitating lipase adsorption.

Keywords

Lipid digestion; in vitro; emulsifier competition

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

HOW PEA MIGHT MAKES US ALL HAPPEA? – STEERING PROTEIN DIGESTION IN A RANGE OF PEA PROTEIN RICH SYSTEMS

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Abstract

Pulse proteins, such as pea protein, are increasingly in demand due to the protein transition and the growing need for plant-based functional food ingredients. Although different extraction techniques are known to affect pea protein techno-functional properties, their nutritional consequences remain insufficiently understood. Therefore, the effect of extraction-induced, so-called intrinsic properties, on the protein digestibility of alkaline- and salt-extracted pea protein extracts, pea flour, and pea cells was evaluated using the standardized in vitro INFOGEST protocol. Intact pea cells contained denatured proteins encapsulated within a highly organized cellular matrix, which strongly hindered proteolysis. Alkaline extraction induced protein denaturation, increased hydrophobic protein–protein interactions, and lowered protein solubility, resulting in reduced digestion rate and extent. In contrast, salt extraction preserved a more native and highly soluble protein structure, leading to digestion kinetics comparable to those of pea flour, in which proteins were highly accessible. Overall, extraction- and matrix-induced structural organization governed protein accessibility and in vitro digestibility.

Pea protein ingredients can also be incorporated into plant-based drinks, where they are subjected to extrinsic processing factors to obtain stable and functional dispersions. The effects of high-pressure homogenization (HPH; 0–200 MPa) and temperature–pH treatments (60 °C; pH 7 or 12) on pea protein structure and in vitro digestion kinetics were investigated. Increasing HPH intensity disrupted insoluble protein aggregates, yielding smaller, more homogeneous particles and inducing secondary structure changes, which collectively improved protein solubility and enhanced proteolysis. Similarly, temperature–pH treatments (60°C at pH 7, and 60°C at pH 12) produced dispersions with comparable microstructures to selected HPH conditions (0 MPa, and 100 MPa, respectively), but distinct secondary structures and solubilities, resulting in different digestion kinetics.

Lastly, pea proteins can be structured into heat-induced, solid gel systems. As plant-based gels are typically weaker, enzymatic crosslinking using transglutaminase can be applied to enhance gel strength, although this may affect protein digestibility. Therefore, the effect of increasing transglutaminase activity (0–10 U/g protein) on gel structure and gastric and intestinal in vitro digestion kinetics was evaluated. Transglutaminase markedly reduced both the rate and extent of gastric protein digestion by restricting accessibility of pepsin cleavage sites.

Together, these findings demonstrate how intrinsic ingredient properties and extrinsic processing conditions modulate pea protein structure and digestion kinetics across liquid and solid food systems, providing a basis for rational design of plant-based foods with tailored nutritional functionality for people with specific needs.

Keywords

extraction conditions, pH, temperature, in vitro digestion, pea protein, food structural properties

Acknowledgements

This work has been financially supported by VLAIO and KU Leuven related research funds.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

NATURALLY HIGH FIBRE WHITE BREADS: POTENTIAL FOR INCREASED HEALTH BENEFITS AT REDUCED PROCESSING LEVELS

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Abstract

Consumption of dietary fibre is widely recognised to have positive health effects, but global average intake is about half the recommended daily 30 g. Bread is a staple food for a large segment of the population, and a major potential source of dietary fibre, but increasing preference to low fibre white breads contributes to the observed fibre gap. In this work, newly identified wheat lines with endosperms that are naturally high in dietary fibre, therefore producing naturally high fibre white flours, have been grown, characterised, and further used to test the digestibility of dilute and concentrated solutions, as well as white breads. Flat breads were used as model systems to avoid yeast, which interferes with the bread's dietary fibre content. Five wheat lines (different crosses of Yumai x Valoris) were tested, and the Yumai x Valoris 078 cross showed the highest content of total and water extractable arabinoxylans. Flours were further characterised using enzymatic fingerprinting. INFOGEST static digestion showed that starch digestibility rate and extent was higher in dilute suspensions, compared to the concentrated dispersions and flat breads, likely due to increased level of gelatinisation at high water content. In addition, starch digestibility of the high fibre breads was lower to that of standard breads when normalised to the bread mass (relevant to consumption), but comparable when normalised to starch content. Xylanase was further used as pre-treatment to alter the structure of the fibre. The resulting dough was thinner compared to that without enzyme, as expected, but starch digestibility was marginally affected by the pre-treatment. In large intestinal fermentations, the high fibre bread promoted bifidogenic activity and butyrate production, compared to the standard bread. Xylanase treatment accelerated early fermentation but led to a decline in microbial diversity and increased presence of opportunistic taxa such as Enterobacteriaceae, which was also observed for the dilute solutions. Overall, this work indicates the potential to develop naturally high fibre white breads that can modulate starch digestibility and large intestinal fermentation through controlling fibre content and structure.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IMPACT OF INDUSTRIAL PROCESSING ON PROTEIN DIGESTIBILITY AND NUTRITIONAL QUALITY: A COMPARATIVE STUDY OF LIQUID VS. POWDERED INFANT FORMULAS

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Abstract

Infant formula (IF) is an essential alternative to human breast milk when breastfeeding is not possible. However, the industrial thermal processing required for safety, induces protein modifications that may compromise nutritional quality.1 Since commercially available liquid and powdered IFs are produced using different technological operations,2 it is essential to compare their nutritional profiles and digestive behavior under infant-specific physiological conditions. This study analyzed the effects of manufacturing processes on liquid and powdered commercial IFs of identical composition by evaluating thermal damage, protein hydrolysis profile and digestibility, and total bioaccessible peptides. Two formulations, each available in both formats (IF01/IF02 and IF03/IF04), were selected for direct comparison. Thermal damage was assessed using CIELAB color and the Maillard reaction markers furosine (early-stage, RP-HPLC) and carboxymethyl-lysine (CML, advance-stage, LC-MS/MS). Samples were subjected to INFOGEST in vitro infant digestion protocol3 and digestibility was measured by the o-phthaldialdehyde method4 in the gastric and intestinal bioaccessible fractions (BFs). Protein and peptide profile was analyzed by SDS-PAGE and FPLC in undigested samples and BFs, where total bioaccessible peptides were also determined. Results showed that while furosine values suggested slightly higher early-stage lysine damage in powdered IFs (13.7 vs. 10.0 and 12.2 vs. 10.0 mg/100 mL IF), CML levels revealed higher advanced thermal damage in liquid IFs (22.8 vs. 247.2 and 23.3 vs. 232.4 µg/100 mL IF). Although total protein digestibility (81.2-82.7 %) and bioaccessible peptides (0.72-0.81g/100 mL IF) were similar across all IFs after complete gastrointestinal digestion, gastric digestibility was notably lower in liquid forms (18.9 vs. 13.4 and 17.0 vs. 14.0 %). While SDS-PAGE lacked the resolution to differentiate post-digestion protein profiles, FPLC successfully revealed distinct peptide distributions after gastric phase. Specifically, liquid IFs retained high-molecular-weight proteins in an intact state following the gastric phase, whereas these proteins were partially or completely hydrolyzed in powdered IFs. Our findings indicate that despite a robust nutritional profile post-gastrointestinal digestion, the more intense thermal processing applied in liquid IF results in a nearly ten-fold increase in advanced thermal damage markers like CML. This significantly alters protein breakdown and peptide release kinetics during the gastric phase. Such modifications may influence gastric comfort and amino acid absorption in neonates, warranting further investigation into processing optimizations and their clinical implications for digestive tolerance.

1. Bavaro et al. (2021). Food Chem., 347, 129019.
2. Bakshi et al. (2023). Front. Nutr., 10, 1194679.
3. Menard et al. (2018). Food Chem., 240, 338-345.
4. Sousa et al. (2023). Food Res. Int. 166, 112569.

Keywords

infant formula, thermal damage, protein digestibility, protein profile, bioaccessible peptides

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

DIALYSIS VERSUS ULTRAFILTRATION FOR BIOACCESSIBLE CALCIUM SEPARATION IN INFOGEST DIGESTION USING STABLE ISOTOPIC LABELLING

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Abstract

A critical step in assessing mineral bioaccessibility is separating the absorbable fraction from non-absorbable components after digestion. The INFOGEST protocol recommends the use of dialysis or centrifugation tubes having a molecular weight cut-off (MWCO) ranging from 3 to 10 kDa. The separation mechanism of these techniques is dictated by distinct driving forces. Dialysis is a diffusion-controlled process governed by the concentration gradient between the digesta and the dialysate, whereas ultrafiltration is a pressure-driven technique, in which centrifugation forces the solvent and permeable solutes through a membrane of defined MWCO. Considering these differences, the present study aimed to compare the dialysis and ultrafiltration techniques for separating the bioaccessible Ca fraction during in vitro digestion. Assorted food samples were digested following a previously published modified INFOGEST method with stable isotopic labelling (Muleya et al., 2024), employing the two separation techniques. In the dialysis approach, tubing (12.4 kDa MWCO), containing 17.5 mL of 0.05 M PIPES buffer (pH 6.7), was introduced into the digestion mixture 30 min before the end of the gastric digestion and remained in the mixture throughout the subsequent intestinal digestion. For ultrafiltration, following centrifugation of the digested samples, a 500 µL aliquot of the supernatant was transferred to 3 kDa Amicon Ultra centrifugal filters and centrifuged at 8,000 × g for 30 min. Finally, both the dialysis tubing contents and the filtered fractions were collected for Ca analysis. Regression analysis of ultrafiltration versus dialysis showed a moderate linear relationship ($R^2 = 0.68$). Dialysis generally yielded higher Ca bioaccessibility values than ultrafiltration. A Bland-Altman analysis was also conducted. Although the bias was relatively low at –1.5%, wide limits of agreement were observed, ranging from –22% to +19%. The lack of agreement was mainly driven by oat porridge and cashew nut results, which were 16.4% and 13.0% higher, respectively, when assessed by dialysis, as well as by kale and sweet potato results, which were 19.4% and 10.4% higher when assessed by ultrafiltration. For the remaining samples (long-grain rice, potato, black turtle beans, pinto beans, Brazil nuts, and spinach) the differences were lower than 7.0%. These findings highlight that dialysis and ultrafiltration should not be considered interchangeable methods for assessing Ca bioaccessibility. Differences in driving force, transport kinetics, and susceptibility to matrix effects can lead to discrepancies. This reinforces the need for standardisation of separation procedures to improve comparability of Ca bioaccessibility across studies.

Muleya, M., Bailey, E.F., Bailey, E.H. Food Research International. 2024; 175: 113795.

Keywords

Calcium, bioaccessibility, dialysis, ultrafiltration

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

CHOOSING IN VITRO MODELS FOR THE SIMULATED UPPER-GASTROINTESTINAL DIGESTION OF FOODS

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Abstract

The simulated digestion of food can be achieved using in vitro models that comprise static, semi-dynamic, and fully dynamic systems. Each of these approaches has inherent advantages and disadvantages that must be carefully considered when selecting an appropriate model. Key factors influencing model choice include food composition, structural and matrix effects, the stability and bioaccessibility of nutrients and bioactive compounds, materials and operational costs (e.g. enzymes and reagents), access to specialised equipment, and, ultimately, specific research objectives.

Static digestion models, particularly when combined with the INFOGEST protocol, are widely regarded as the gold standard for assessing nutrient release and bioaccessibility from foods, in a reproducible and standardised manner. However, for mechanistic studies that seek to better replicate physiological conditions such as gastric emptying rates, and dynamic pH and enzyme profiles, semi-dynamic and fully dynamic models may be more appropriate. Furthermore, the potential to integrate upper and lower gut (colon) models provides the capability for simulation and study of food digestion through the entire gastrointestinal tract (GI). For example, the SHIME is widely recognised as one of several comprehensive and well-validated in vitro models (e.g. TIM, SIMGI, MARS and others) for studying the lower GI tract post-upper GI conditioning. Nevertheless, all models have limitations, and it is essential to align the chosen digestion model with the specific research question.

At the Quadram Institute, we have gained experience using a broad range of digestion models for different research applications and in this work, we draw on this experience to propose a comprehensive and practical guide which we believe can support researchers in selecting the most suitable model systems for designing and conducting simulated digestion experiments, thereby enhancing the relevance and translational value of in vitro digestion studies.

Keywords

Digestions, models, foods, static, dynamic, mechanisms

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

BIOACCESSIBILITY OF FE, ZN AND CA FROM NON-CONVENTIONAL FOOD PLANTS WITH RICE AND BEANS ASSESSED USING INFOGEST DIGESTION AND STABLE ISOTOPIC LABELLING

Alexandre Minami Fioroto [1 2] , Molly Muleya [3] , Lolita Wilson [4] , Luísa Moraes Gonçalves [1 2] , Eliana Bistriche Giuntini [2] , Eduardo Purgatto [1 2] , Elizabeth H. Bailey [4]

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Abstract

Non-conventional food plants are not produced on a large scale; thus, they are not widely included in the population diet. Although these plants are an alternative food source, their consumption may be restricted to specific regions and local cultures. Most of them are traditionally regarded as having nutritional potential. Taioba (Xanthosoma sagittifolium (L.) Schott), ora-pro-nobis (Pereskia aculeata Miller), and serralha (Sonchus oleraceus L.) are non-conventional food plants from Brazilian biodiversity and have been increasing in popularity. Their mineral content may be equivalent to or higher than that of conventional leafy vegetables consumed worldwide. Therefore, this study aimed to evaluate the bioaccessibility of Fe, Zn, and Ca in these plants using a modified INFOGEST in vitro digestion method with stable isotopic labelling and to investigate the effect of combining these plants with rice and beans, which compose a typical Brazilian meal. First, non-conventional food plants were analysed on their own, assuming a meal composed exclusively of leafy vegetables. Then, the evaluation of the food combination effect was based on a meal containing 32% cooked rice, 52% cooked beans, and 16% sautéed non-conventional food plants. Rice and beans were analysed on their own and in combination with the leafy vegetables. In vitro digestion of the leafy vegetables alone resulted in Fe bioaccessibility below 2.6% across all plants. Ca bioaccessibility was low across the leafy vegetables, with particularly low values in taioba and ora-pro-nobis (0.35%) and comparatively higher, though still limited bioaccessibility in serralha (11%). Zn showed moderate bioaccessibility values ranging from 7 to 20%. To evaluate the effect of food combinations, the sum of bioaccessible minerals in the final digesta from individually digested foods was compared with that of the mixtures. The bioaccessible Fe fraction in the mixtures did not show significant differences compared with the sum of individual results, except when taioba was digested together with rice and beans, for which a lower Fe bioaccessibility in the meal was observed. Moreover, the combinations of all these plants with rice and beans did not significantly affect bioaccessible Zn fractions. On the other hand, all combinations showed a negative effect on Ca bioaccessibility. Even in the mixture of rice and beans without plants, a decrease of 22% in the bioaccessible Ca fraction was observed. However, in mixtures containing plants, this negative effect was more pronounced, with decreases ranging from 38% to 55%. Although these non-conventional food plants contain significant mineral contents, they showed low Fe and Ca bioaccessibility and may contain antinutritional properties (e.g., fibres, phytate, and oxalates) that reduce mineral bioaccessibility in the meal, mainly affecting Ca.

Keywords

Non-conventional food plants, mineral nutrients, bioaccessibility, rice, beans

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

PEA CREAM AND INTESTINAL HEALTH, AN IN VITRO AND IN VIVO STUDY

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Abstract

The search for sustainable alternative protein sources has intensified, with legumes emerging as promising candidates. In parallel, valorisation of by-products from the food industry has gained interest, through optimisation and reuse for human and animal nutrition. Pea processing for starch extraction generates pea cream (PC), rich in proteins and dietary fibres. Proteins are essential for muscle growth and physiological functions (hormones, enzymes, etc...), whereas dietary fibres can positively modulate digestive processes and intestinal health (Gill et al.,2021). However, the effective valorisation of PC requires comprehensive characterisation of its nutritional and functional properties, particularly protein bioaccessibility and digestibility, which may be influenced by the dietary fibres it contains. The study therefore aimed to evaluate the protein digestibility and impact of dietary fibre of PC on the intestinal barrier function, using a multidisciplinary approach combining in vitro digestion, a cell culture model, and an in vivo study.

Three dietary conditions were studied: pea cream on its own (R1) or as part of meal (R2), with pea flour (R3) used as a control. Protein bioaccessibility and digestibility of these three diets were assessed using the INFOGEST static in vitro digestion model. The diets and digesta were characterised for protein and dietary fibre content, particle size, and microstructure by microscopy. The cell line IPEC-J2 was used to study impact of the digesta obtained during in vitro digestion on the intestinal barrier function. Conjointly an in vivo study was performed on piglets where jejunal tissues were analysed for structural characteristics (crypt depth, villosity height, and mucin quantity).

PC contained 19.6% of protein and 32.7% of dietary fibre on a dry weight basis. PC had a high protein bioaccessibility (81.5% at intestinal phase) which was associated with an important protein hydrolysis (90.4%). R2 and R3 diets also showed very high protein hydrolysis (98.1%). The results from the IPEC-J2 cell model showed that PC did not impair the integrity of the piglet intestinal epithelial barrier. Consistently, in vivo analyses confirmed a positive effect of PC on villus height (p0.05) and mucus secretion (p0.05) compared to the pea flour.

In conclusion, this study showed that PC contained easily digestible proteins despite being rich in dietary fibres. It also contributes to maintaining intestinal barrier integrity probably because of its dietary fibre composition. These results were confirmed in vivo where PC seemed to stimulate intestinal maturation. Further research should investigate the composition of the digesta to identify the specific bioactive compounds (oligosaccharides or peptides) behind the effect observed.

Gill, S. K., Rossi, M., Bajka, B. & Whelan, K. Dietary fibre in gastrointestinal health and disease. Nat Rev Gastroenterol Hepatol 18, 101–116 (2021).

Keywords

Dietary fibres, Proteins digestion, IPEC-J2 Gut health, Pea

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECTS OF CHITOSAN AND CELLULOSE ACETATE AEROGELS ON IN VITRO LIPID DIGESTION

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Abstract

Chronic metabolic diseases represent a growing global health burden. Excessive intake of lipids contributes to postprandial metabolic dysregulation and elevates the risk of obesity, cardiovascular disease, and atherosclerosis. Reducing lipid digestibility may help reduce postprandial burden and improve metabolic outcomes. Aerogels are lightweight, highly porous materials with large surface area and tunable surface chemistry. Chitosan (CS) and cellulose acetate (CA) aerogels provide a potential platform to modulate digestion through interactions with digestive components and substrates. In this study, we investigated the effect of CS- and CA-based aerogels on in vitro lipid digestion and explored the ability of aerogels to bind lipids, lipase, and bile acids. Aerogel microstructure was characterized by scanning electron microscopy (SEM). The interactions of CS and CA aerogels toward linseed oil, pancreatin, and bile acids were evaluated using adsorption assays under the simulated gastrointestinal conditions of the INFOGEST protocol. Lipid digestibility was assessed under in vitro static conditions using constant pH-stat titration during the intestinal phase. After incubation for four hours in simulated gastrointestinal fluids without digestive enzymes or bile salts, CA aerogels showed a slight dry mass loss (−5.4±2.7%). In the presence of oil the dry mass ratio increased markedly to 533.3 ± 123.2%, indicating strong oil binding. In contrast, CS showed limited oil binding (−23.2±10.3% to 27.6±7.6%). Both CS and CA in either aerogel and powder forms (no structured as aerogels) displayed pronounced binding to lipase. After incubation for four hours under simulated gastric and intestinal digestion with CS and CA, the residual lipase activity in intestinal digesta decreased to 1.5% (CS) and ~26.7% (CA) of the original activity. Under the same simulated conditions, soluble bile acid concentration decreased from 3.4 mM to an average of ~2.6 mM following exposure to either CS or CA aerogels. No difference was observed in the bile acid binding capacity between CS and CA. Collectively, these findings suggest that CS and CA have the potential to modulate lipolysis under simulated intestinal conditions.

Keywords

chitosan, cellulose acetate, aerogel, lipid digestion, binding capacity

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

ADJUSTING FOOD STRUCTURE SHAPES NUTRIENT BIOACCESSIBILITY AND POSTPRANDIAL METABOLIC RESPONSES

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Abstract

While nutrient composition is a key element of dietary guidance, the structural integrity of foods, particularly plant-based foods, plays an equally critical role in shaping metabolic outcomes after eating. This study [1] investigated how contrasting cellular structures of chickpea-based meals influence digestion, nutrient bioaccessibility and postprandial physiological responses. In a randomized cross-over design, ten healthy participants consumed iso-nutrient meals differing only in whether their plant cell structures were preserved (“Intact”) or disrupted (“Broken”). Gastric, duodenal, and blood samples were collected to characterise digestive and metabolic trajectories.

The results demonstrated structural effects on nutrient release and metabolic signalling. Meals with Broken cellular structures exhibited high starch digestibility, leading to a rapid rise in gastric maltose within 30 minutes and triggering sharp increases in blood glucose, GIP, and GLP-1 levels. In contrast, Intact structured meals slowed digestive processes and produced sustained hormonal and metabolic responses. These divergent patterns underscore the importance of food microstructure in modulating gut nutrient-sensing mechanisms, glycaemic control, and appetite regulation.

Collectively, the findings provide an insight into how modern food processing, which often disrupts natural plant cell structures, may promote faster nutrient absorption, increased glycaemic load, and diminished satiety. Understanding how food structure affects upper-gastrointestinal digestion and endocrine signalling offers promising avenues for designing foods that support healthier metabolic responses and mitigate risks associated with obesity and type 2 diabetes.

[1] Cai, M., Tejpal, S., Tashkova, M. et al. Upper-gastrointestinal tract metabolite profile regulates glycaemic and satiety responses to meals with contrasting structure: a pilot study. Nat Metab 7, 1459–1475 (2025). https://doi.org/10.1038/s42255-025-01309-7

Keywords

Food structure, Nutrient bioaccessibility, Postprandial metabolism, Gut hormone signalling, Satiety, Glycaemia

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

INFLUENCE OF OVALBUMIN ON PHENOLIC COMPOUNDS STABILITY DURING IN VITRO DIGESTION AND INTESTINAL LACTOBACILLUS FERMENTATION

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Abstract

Phenolic compounds are widely recognized for their bioactive properties; however, their stability can be significantly affected by multiple factors during gastrointestinal digestion. In this study, the effects of ovalbumin on the stability of gallic acid, caffeic acid, cyanidin-3-glucoside, and quercetin during INFOGEST in vitro digestion followed by gut microbiota digestion (L. brevis, L. plantarum, L. casei, L. johnsonii, and L. rhamnosus) was evaluated. To assess the behavior of phenolic compound during gastrointestinal digestion, samples were acidified, purified, and subsequently quantified using HPLC-MS/MS. Quantification was performed using matrix-matched calibration curves over a concentration range of 0.1 to 100 mg/L. As expected, in the absence of ovalbumin, gallic acid, caffeic acid, and cyanidin-3-glucoside exhibited concentrations close to the theoretical value (62.5 mg/L). In contrast, quercetin was highly unstable (10 mg/L) and precipitated due to its limited solubility under acidic conditions. In the presence of ovalbumin, gallic acid concentrations were markedly reduced (15 mg/L), whereas quercetin concentrations increased (33–43 mg/L). These results suggest compound-dependent phenolic–protein interactions, predominantly mediated by non-covalent forces, resulting in a protective effect for quercetin but a negative effect, possibly through precipitation, for gallic acid. Following gastrointestinal digestion, complete degradation of quercetin and cyanidin-3-glucoside was observed in all samples, indicating their high instability under alkaline conditions. Gallic acid was detected only in the sample without ovalbumin (2 mg/L), whereas caffeic acid exhibited greater stability, with similar concentrations in the absence and presence of ovalbumin (29 and 24 mg/L, respectively). The greater instability of gallic acid is associated with its chemical structure, which contains a higher number of hydroxyl groups, making it more susceptible to oxidation and covalent binding with proteins than caffeic acid. However, in the presence of Lactobacillus, only caffeic acid was detected in samples containing ovalbumin (17 mg/L). These findings indicate the preferential utilization of nitrogen sources by bacteria over phenolic compounds; however, under nitrogen-limited conditions (such as in the sample without ovalbumin), phenolic compounds may serve as alternative metabolic substrates. Overall, these results highlight the importance of food matrix composition and gastrointestinal conditions in determining the bioaccessibility and potential biological relevance of phenolic compounds.

Keywords

Egg albumin, simulated gastrointestinal digestion, bioaccessibility, lactic acid bacteria, phenolic acids

Acknowledgements

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EFFECT OF REDUCED PANCREATIN AND BILE ON MACRONUTRIENT DIGESTIBILITY AND FE AND ZN RELEASE USING THE INFOGEST IN VITRO DIGESTION METHOD

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Abstract

In a previous study, Muleya et al. (2021) proposed a stable isotope approach based on a modified INFOGEST static in vitro digestion method to accurately assess Fe and Zn bioaccessibility. The simulated digestion fluids contain high mineral background, which compromises the quantification of the bioaccessible mineral fractions. Thus, in order to distinguish the mineral concentrations derived from samples from those introduced by reagents, the reagents were isotopically labelled by the addition of 57Fe and 70Zn isotopes. Moreover, the amounts of pancreatin and bile were reduced, due to the high contributions of pancreatin and bile to Fe and Zn levels in the digesta, and to the introduction of additional mineral binders/ligands into the system that could affect the bioaccessibility measurements. Therefore, the present study aimed to evaluate the impact of pancreatin/bile reduction on macronutrient digestibility and, consequently, mineral release in food samples with diverse compositions. Furthermore, processing methods to remove minerals from pancreatin/bile were also evaluated. Sonication followed by centrifugation removed 34% and 10% of Fe and Zn from pancreatin, respectively, while minimizing losses in trypsin activity. Thus, treated pancreatin was used for the preparation of digestive fluids under standard INFOGEST conditions. Ten food samples (white long-grain rice, oat porridge, potato, sweet potato, black turtle beans, pinto beans, Brazil nuts, cashew nuts, spinach, and kale) were digested using two digestion conditions: (1) Reduced pancreatin and bile conditions; (2) Standard INFOGEST conditions using treated pancreatin. In both cases, stable isotopes were applied to enable discrimination between reagent and sample Fe and Zn. Macronutrient digestibility was higher under standard INFOGEST conditions, demonstrating that reducing the amounts of pancreatin and bile in the digestive fluids can affect protein and starch hydrolysis. However, Fe and Zn solubility were not consistently modified across food matrices or between the two methods used, although there was a general tendance of increased solubility in the standard INFOGEST conditions. Interestingly, when observing the pattern of Fe and Zn solubility across the samples, those within the same food group tended to show similar responses, suggesting that mineral solubility is strongly influenced by the type of food matrix. Under standard INFOGEST conditions, Fe solubility increased by 64–91% in cereals, beans, and nuts but decreased by 48% in sweet potato. Similarly, Zn solubility increased by 11–75% in oats, nuts, and leafy vegetables, while it decreased by 18–64% in potatoes and beans. Overall, the influence of enzymatic action and background minerals could not be isolated, thus standardisation of the INFOGEST method for mineral bioaccessibility assessment is urgently needed.

References: Muleya, M., Young, S.D., Bailey, E.H. Food Research International. 2021; 139: 109948.

Keywords

iron, zinc, stable isotopes, pancreatin, bile, mineral bioaccessibility, mineral solubility

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DIGESTIVE FATE OF STRUCTURALLY DIVERSE ANTHOCYANINS FROM EDIBLE FLOWERS: A COMPARATIVE IN VITRO STUDY

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Abstract

Anthocyanins are dietary flavonoids whose health potential is strongly influenced by their overall low stability and extensive transformation in the gastrointestinal (GI) tract [1]. Edible flowers (EFs) constitute an emerging source of structurally diverse anthocyanins, ranging from non-acylated glycosides to polyacylated and polyglycosylated derivatives [2]. Acylated anthocyanins have attracted increasing attention due to their enhanced chemical stability compared to non-acylated counterparts, particularly under conditions relevant to GI digestion. Beyond molecular structure, the food matrix plays a key role in modulating anthocyanin bioaccessibility during GI digestion [3]. Nevertheless, comparative information on their behavior in the GI tract remains limited, as most digestion studies have focused on structurally simpler anthocyanins [4]. In this work, anthocyanin-rich EFs with distinct structural profiles were investigated, ranging from butterfly pea flower (BPF), characterized by polyacylated and polyglycosylated anthocyanins, to wild pansy, cosmos, and cornflower, which predominantly contain less complex anthocyanin structures. All EF extracts were characterized by UHPLC-DAD-MS, and were consistent with literature-reported profiles for each EF. Simulated digestions following the INFOGEST guidelines revealed marked differences in digestive behavior among the flowers. BPF exhibited higher resistance to enzymatic and pH conditions, revealing a higher content of free anthocyanins after intestinal digestion. In contrast, less complex anthocyanins from wild pansy were more susceptible to degradation, displaying pronounced losses after the intestinal phase. The effect of food matrices during digestion was evaluated using starch, apple pectin, and soy protein isolate as representative systems. Higher free anthocyanin contents after gastric and intestinal digestion suggested matrix-dependent release and transient stabilization of these compounds. To evaluate the impact of digestion and matrix interactions on anthocyanin transport, transepithelial absorption studies were conducted using gastric (NCI-N87) and intestinal (Caco-2/HT29-MTX, 9:1) epithelial cell models exposed to EF extracts in free and digested forms, both with and without food matrices. Overall, digestion led to a marked reduction in anthocyanin transport, while food matrices differentially modulated absorption in a matrix- and flower-dependent manner. Overall, this comparative approach demonstrates that anthocyanin structure and food matrix interactions strongly influence their stability, digestive transformation, and epithelial transport throughout the GI tract.

References:
[1] Escobar, G. M. V., et al. (2025). Food Nutr Health, 2(1), 28.
[2] Jokioja, J., et al. (2021). Compr. Rev. Food Sci. Food Saf., 20(6), 5570-5615.
[3] Teixeira, M., et al. (2023). Trends Food Sci. Technol., 138, 708-725.
[4] Teixeira, M., et al. (2026). Food Chem., 148137.

Keywords

Anthocyanins, Edible Flowers, Gastrointestinal Digestion, Bioaccessibility, Structural Complexity

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COMPARISON OF THE BIOAVAILABILITY OF KRILL (E. SUPERBA) AND PEA PROTEINS AS ALTERNATIVE PROTEIN FOOD INGREDIENTS BY THE STATIC IN VITRO SIMULATION

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Abstract

Estimation of bioavailability of nutrients in Novel Foods is part of the required knowledge for their authorization for human consumption [1]. The standardized INFOGEST methodology for in vitro estimation of bioavailability presents some challenges, especially for evaluation of alternative protein ingredients and the respective foods with their incorporation. This study evaluated the protein quality and in vitro bioavailability of 2 alternative protein ingredients (APIs) from krill and pea and fish stick products with incorporation of these two ingredients in substitution of fish protein. We analysed the amino acid composition, digestibility using the INFOGEST protocol [2-4], and the intestinal absorption through differentiated Caco-2 cells. Cytotoxicity was evaluated by exposing Caco-2 cells to varying concentrations of digestates for 3 hours, measuring cell viability, and then assessing barrier integrity and permeability after exposure to sub-toxic digestate concentrations. Additionally, the bioavailability of Total (TAA) and Branched Chain Amino Acids (BCAAs) in the digestates and after passage through the Caco-2 monolayer model was quantified using commercial assay kits and HPLC. Overall, the results indicated that the krill and pea APIs tested demonstrate high antioxidant capacity, bioavailability of the contributed amino acids and no detectable cytotoxicity following intestinal absorption, supporting the potential incorporation into novel food formulations.

References:
[1] EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) https://doi.org/10.2903/j.efsa.2024.8946 .
[2] Brodkorb et al., 2019. https://doi.org/10.1038/s41596-018-0119-1
[3] Mulet-Cabero et al., 2020. https://doi.org/10.1039/c9fo01293a
[4] Menard et al., 2023. https://doi.org/10.1039/d3fo00535f.

Keywords

bioavailability, alternative proteins, krill, pea, static digestion, amino acids, antioxidants

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EFFECT ON MYCOPROTEIN DIGESTIBILITY AFTER PROCESSING

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Abstract

Edible filamentous fungi, known as mycoprotein, is a nutrient dense and sustainable alternative to meat. The cell walls are rich in dietary fibres such as β-glucan, chitin and chitosan and encloses large amounts of protein. There is reason to believe that the protein digestibility could be hindered by those rigid cell walls. In this study the aim was to investigate the effect on digestion after different processing affecting or removing the cell walls.

Mycoprotein was treated with several cycles of high-pressure homogenisation to rupture the cell walls and release the protein inside. Micrographs showed that cells were broken into pieces by the homogenisation and some of the protein was released. After a while, cell fragments and protein stick together in large compact clusters. Protein was further extracted using the pH-shift method. The yield from extraction was 32.1 % and the protein concentration was 61.5 % (dry weight). Microstructure analysis reviled that it also contained large aggregates of cell walls.

The protein bioaccessibility was analysed using Infogest 2.0 for fresh, dried, and high-pressure homogenised fungi as well as the protein isolate. Overall, the protein accessibility, measured as protein hydrolysis (DH%), was low and neither the drying nor the homogenisation had influence on it. Although, the isolated protein showed a significantly higher hydrolysation during digestion compared to the other samples.

Keywords

Mycoprotein, digestibility, Infogest

Acknowledgements

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EFFECT OF BACILLUS FERMENTATION AND THERMAL PROCESSING ON ANTINUTRIENT AND PROTEIN DIGESTIBILITY OF OAT AND PEA USING INFOGEST IN VITRO DIGESTION MODEL

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Abstract

Plant-based proteins from oat and pea are increasingly adopted in sustainable diets. However, their nutritional value might be limited due to low protein bioavailability and antinutritional factors. Among these, proteinase inhibitors (PI), such as trypsin and chymotrypsin inhibitors, might reduce digestive protease activity and impair protein hydrolysis. Thermal processing is commonly used to inactivate PI, while microbial fermentation, particularly by protease-producing Bacillus species, may further modify inhibitor stability, protein structure and digestibility. However, the relative contributions of thermal treatment versus microbial proteolysis and their effects across gastrointestinal digestion remain unclear.

In this study, a high extracellular protease-producing Bacillus subtilis strain (PRO64) was selected to investigate PI interactions in oat- and pea-based systems. Cell-free supernatants were first incubated with purified trypsin inhibitors to assess direct effects on inhibitor activity. Subsequently, oat and pea substrates were fermented under controlled conditions, with non-fermented and heat-treated samples included to disentangle fermentation-driven effects from heat-induced inactivation.

Trypsin inhibitor activity (TIA) was quantified prior to digestion using a BAPA-based assay. Preliminary results show that cell-free supernatants increased apparent TIA compared to non-enzymatically treated controls, indicating that components present in the supernatant can influence TIA measurements beyond simple inhibitor content. Despite this overall increase, samples treated with the high-protease-producing strain PRO64 consistently exhibited lower TIA than those treated with a low-protease-producing Bacillus subtilis strain (PRO2), suggesting that elevated extracellular protease activity partially counteracts the supernatant-induced increase in apparent TIA through proteolytic modification of trypsin inhibitors. These findings suggest that protease–inhibitor interactions during fermentation involve more complex mechanisms than direct inactivation, potentially including partial cleavage or altered inhibitor accessibility.

Ongoing work applies the standardized INFOGEST in vitro digestion model to track residual inhibitor activity across the oral, gastric and intestinal phases and to relate these changes to protein hydrolysis and peptide profiles. Protein digestibility is assessed using the degree of hydrolysis, SDS-PAGE and LC-MS-based peptidomics. This digestion-resolved framework links fermentation- and heat-induced modifications to gastrointestinal outcomes and highlights the importance of distinguishing inhibitor inactivation from microbial proteolysis and structural modification of the protein matrix.

Keywords

Plant-based protein digestion, Protease–inhibitor interactions, Thermal processing vs fermentation, INFOGEST

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MATRIX-DEPENDENT DIGESTION AND BIOAVAILABILITY OF ALKYLRESORCINOLS: EVIDENCE FROM INFOGEST AND A HUMAN ILEOSTOMY STUDY

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Abstract

Alkylresorcinols are lipophilic phenolic compounds found in the bran of cereals, especially wheat and rye, with an aliphatic side chain which length ranges from C15 to C25. Reportedly, 50-60% of dietary ARs are absorbed in the small intestine and act as markers of whole grain wheat and rye intake. However, the stability of ARs during digestion and their digestive behavior have not yet been thoroughly studied. This study aimed to understand the effect of individual foods, diets, and alkyl chain length on the behavior of ARs in the human gastrointestinal tract using a combination of in vitro and in vivo digestion experiments. The INFOGEST 2.0 static in vitro model was employed to digest 4 rye-based food matrices, followed by ultracentrifugation to separate the micellar phase for bioaccessibility estimation. In vivo, ileostomates in an acute double-blind randomized crossover study (NCT05845229) were given two diets, differing in the source and AR levels, and ileal fluids were collected at 2h, 4h, 6h, and 8h. ARs quantification from all the matrices was performed using LC-QToF-MS operated in single ion monitoring acquisition mode. C17 and C21 were quantified using a matrix-matched calibration curve of pure standards, while C19, C23, and C25 were semi-quantified using a matrix-matched calibration curve of C21. Among raw materials, the highest concentration of ARs was observed in rye bran, followed by crisp, pumpernickel, and rye bread. This trend suggests that fiber content positively correlates with AR levels in food matrices. In vitro digestion revealed that the dietary matrix significantly affects the bioaccessibility of ARs, with rye bread showing the highest value, followed by rye bran. Chain length significantly affected the bioaccessibility with short chain homologues being more bioaccessible compared with long chain homologues. Ileostomal data revealed that the apparent absorption of total alkylresorcinols (sum of all homologues) ranged from 30-50% for the high AR diet and 40-70% for the low AR diet. Among homologues, ARs with long alkyl chain showed more apparent absorption than those with short side chain. ARs' excretion kinetics from the ileostomy suggested slower release from the high AR diet where a peak was observed at 6h, compared to the low AR diet with the peak appearing at 4h. Finally, it is concluded that behavior of alkylresorcinols is affected by the composition of dietary matrix and alkyl chain length. By integrating in vitro digestion with ileal recovery, our study highlights the importance of optimizing in vitro conditions when assessing bioaccessibility of lipophilic dietary phenolics.

Keywords

Alkylresorcinols, BAdd the keywords separated bioavailability, Bioaccessibilty, LC-QToF-MS, INFOGEST 2.0, Ileostomy

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LYSINE-LOADED CONCENTRATED DOUBLE EMULSIONS: UNDERSTANDING PHYSICAL STABILITY AND LYSINE BIOACCESSIBILITY UNDER SIMULATED DIGESTION CONDITIONS

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Abstract

Concentrated double emulsions (CDEs) are w/o/w systems characterised by high volume fractions, resulting in the close packing of oil droplets in a semi-structured network. CDEs can be promising amino acid delivery systems, as they may resist gastric breakdown and enable modulated release of amino acids in the small intestine, improving the temporal imbalance of amino acid absorption. The aim of this study is to investigate the digestive behaviour of lysine loaded CDEs. The CDEs, comprising an inner aqueous lysine phase stabilised by polyglycerol polyricinoleate, an oil phase of soybean oil and hydrogenated palm oil, stabilised by OSA-modified starch and xanthan gum, were prepared via a two-step hot emulsification process. The CDEs were submitted to static in vitro digestion and characterised by their particle size, zeta potential, microstructure, and encapsulation efficiency before and during digestion. Before digestion, the CDEs yielded a D 4,3 of 9.81 ± 1.22 μm and an encapsulation efficiency of 39.98 ± 2.1 %. The emulsion progressively increased its particle size as it transits through the gastric and intestinal phase (D 4,3 = 28.76 ± 2.17 and 125.97 ± 10.34, respectively) and shifts to forms multimodal distribution. Zeta potential measurement highlights that the surface charges become increasingly negative down the digestive tract. Confocal imaging suggests the retention of double emulsion structure in the gastric phase and extensive coalescence in the intestinal phase. The bioaccessibility of lysine in the gastric phase (50.89 ± 2.14%) significantly increased to 95.74 ± 6.59 % in the intestinal stage, indicating the potential of the encapsulation system to confer targeted release of lysine in the intestinal phase. Overall, this study demonstrates the potential of CDEs as a delivery vehicle for achieving controlled release of amino acids during digestion.

Keywords

Concentrated Double emulsion, In vitro digestion, controlled release, lysine bioaccessibility

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ASSESSMENT OF PROTEIN HYDROLYSIS AND BIOACCESSIBILITY OF VITAMINS D3 AND B12 IN STRAWBERRY YOGURTS USING ADULT AND ELDERLY IN VITRO DIGESTION CONDITIONS

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Abstract

This study aimed to evaluate the protein hydrolysis and the bioaccessibility of vitamins D3 and B12 in strawberry yogurts. The vitamins were encapsulated in freeze-dried liposomes. The enriched yogurts were submitted to in vitro static digestion using both adult and elderly INFOGEST protocols. Regarding protein release, it was lower in the intestinal digestion of elderly, regardless of the presence of strawberries, due to the lower trypsin activity. In adult digestion, the protein release was higher in the yogurts with strawberries, probably due to the presence of low molecular weight carbohydrates (fructose and glucose). Since proteolysis occurs in the soluble portion of the medium, the presence of these carbohydrates may have influenced the protein partition coefficient (including trypsin) and significantly affected the proteolysis rate. In the case of elderly digestion the lower enzymatic activity may have diminished the influence of this phenomenon, as protein release from the yogurt protein network would be lower, and therefore, the alteration of the protein partition coefficient would not have the same significant effect observed in adult digestion. As for the protein hydrolysis, an increase in the concentration of free amine groups was observed after the intestinal digestion for both protocols, compared to the gastric phase. The liposomes or the strawberry did not lead to significant differences in gastric protein hydrolysis. For each digestion condition of the different yogurts it was possible to notice the presence of strawberry did not influence on the molecular weight profiles after the gastric step, independent of the addition of liposomes or the digestion conditions. A different situation occurred at the end of the intestinal step - the elderly digestion condition led to a higher amount of lower molecular weight peptides. Regarding free amino acids at the end of intestinal phase, some specific amino acids seemed were released in a higher amount (%) in elderly than in adult digestion conditions. It is important to notice that among the essential amino acids, four of them (leucine, isoleucine, lysine and valine) were the most abundant in the intestinal digesta. Results obtained for leucine are quite interesting, as it was consistently the AA in the major percentage at the end of intestinal step in elderly digestion condition. As for the vitamins, the tendencies of their releases were different, probably due to their different location in the liposomes. The presence of strawberry and the elderly conditions significantly reduced the bioaccessibility of vitamin D3, but did not affect markedly the release of vitamin B12. The results obtained showed the high importance of advancing on the comprehension of the role of the food matrix in the digestion in order to explain the bioaccessibility data provenient from the digestion of macro and micronutrients.

Keywords

liposomes,cholecalciferol,cobalamin,enriched yogurt

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TOMATO PUREE ENRICHMENT WITH TOMATO BY-PRODUCT: IMPACT ON TECHNOLOGICAL PROPERTIES AND CAROTENOIDS IN VITRO BIOACCESSIBILITY

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Abstract

Tomato is one of the most widespread crops in the world. In 2024 the global annual production exceeded 180 million tons, of which 25% underwent processing. During tomato processing, peels, seeds, and a small amount of pulp, named tomato pomace (TP), are separated from the pulp. The TP accounts for approximately 3-5% (w/w) of fresh fruit. Currently, TP is generally disposed or used for animal feeding, compost production, or discharged in landfills. Several studies have shown that TP contains significantly high levels of valuable bioactive compounds, such as fibre, phenolics, flavonoids, lycopene, beta-carotene, ascorbic acid and minerals, which can have nutritional and healthy beneficial effects to humans.

A number of extraction methods (e.g., solvent; ultrasounds-, microwaves-assisted; supercritical fluid solvent extraction) have been identified to recover these bioactive compounds from TP, to obtain ingredients to be used in the productive sector, including the food products. However, their application for this purpose at the industrial level remains limited due to their health, safety, environmental and high-cost concerns. An alternative approach to the valorisation of TP may be represented by its direct incorporation into food products, but, at present, only a few studies deal with this approach.

Our study aimed at investigating the possibility of enriching tomato puree with TP to enhance its health-promoting properties while contributing to minimizing food residues discarded along the supply chain with the minimum required effort.

TP, which was obtained during tomato puree processing, was dried and ground into 250 µm powder and then incorporated into the tomato puree at the same quantity as fresh whole tomato. Finally, the mixture was high-speed homogenized. Cooked (15 min at 95 °C) and uncooked TP-enriched samples, along with cooked and uncooked tomato puree without TP added (control samples), were analysed for chemical and physical properties. Samples were subjected to the Infogest simulation of gastrointestinal digestion, and lycopene and beta-carotene bioaccessibility was determined.

Addition of TP to tomato puree caused a significant increase in lightness and redness compared with the controls. Cooking had a limited further impact on the colour parameters of the samples. Consistency of tomato puree increased significantly by the addition of TP. Cooking of tomato puree added with TP produced further matrix thickening and reduced shear dependent response. Although the contents of beta-carotene and lycopene in enriched tomato purees were not significantly different from those in the control samples, the bioaccessibility of both carotenoids was significantly higher in the cooked TP enriched samples. These findings suggest that the use of TP as a functional ingredient in tomato puree not only improves the nutritional profile but also contributes to sustainability by reducing industrial waste and creating value-added products.

Keywords

Tomato by-product; Tomato puree fortification; Carotenoids in vitro bioaccessibility; Waste prevention.

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IMPACT OF PROCESSING AND ULTRASOUND EXTRACTION ON PROTEIN DIGESTIBILITY AND NUTRITIONAL QUALITY OF ARTHROSPIRA PLATENSIS AND PHAEODACTYLUM TRICORNUTUM

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Abstract

This study compared the in vitro protein quality of three microalgae products made from two species, Arthrospira platensis and Phaeodactylum tricornutum: raw biomass, an industrial by-product after extraction (residual biomass), and protein-rich concentrates using ultrasound-assisted extraction. The total in vitro protein digestibility was assessed using the INFOGEST protocol followed by three down-stream analyses: total nitrogen (Dumas), free amino groups (OPA assay) and total amino acid (TAA) analysis. The digestible indispensable amino acid ratio (DIAAR) values were calculated, with the lowest value defined as the digestible indispensable amino acid score (DIAAS) to identify the limiting amino acid. The protein digestibility varied with processing treatment and cell wall structure. For A. platensis, the raw biomass exhibited the highest digestibility (91.0%), followed by the residual biomass (84.0%) and the ultrasound-treated protein concentrate (83.0%), indicating that the successive processing altered protein structure and affected digestibility. In contrast, the P. tricornutum raw biomass showed a low protein digestibility (56.1%), similar to its residual biomass (54.4%), whereas the protein-rich concentrate displayed a marked increase (90.2%). Intact cell walls in raw and residual biomasses limited enzymatic access and reduced their digestibility, while protein extraction enhanced the digestibility by removing this barrier. The nutritional quality also showed significant differences. P. tricornutum raw biomass and by-products exhibited DIAAR values below the FAO threshold 75% for adequate nutritional quality, primarily due to the lack of histidine. The protein concentrate showed improved quality, with most indispensable amino acids meeting or exceeding FAO reference values (≥75%), although lysine remained limiting (65%). A. platensis samples generally demonstrated good to excellent protein quality for adults, with DIAAR values ranging from 75% to over 100%. However, the protein concentrates showed a slight reduction in quality, with lysine as the limiting amino acid (68%). Structural and physicochemical changes during digestion were characterized by SDS-PAGE, size-exclusion chromatography and confocal laser scanning microscopy, revealing modifications associated with enhanced protein accessibility and hydrolysis. Overall, these findings support the valorisation of P. tricornutum and A. platensis industrial residues as sustainable sources of functional and nutritional protein ingredients and improve understanding of factors governing microalgal protein digestibility.

Keywords

Microalgae, proteins, digestibility, residue, characterisation

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

PRODUCTION OF BIOACCESSIBLE ANTHOCYANIN METABOLITES DURING INFOGEST IN VITRO DIGESTION OF NATIVE SPANISH AND BRAZILIAN BERRIES

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Abstract

Berry consumption has increased substantially due to their anthocyanin contents (ACNs), which exhibit antioxidant and anti inflammatory activities associated with the benefits over several diseases like obesity, diabetes, cancer, neurodegenerative and cardiovascular disorders. Incorporating native berries into local food systems may enhance public health, promote sustainability, preserve biodiversity, and reduce dependence on monoculture crops. The health effects of ACNs depend not only on their concentration but also on the metabolic transformations occurring during gastrointestinal digestion. Therefore, this study aimed to characterize the formation of bioaccessible ACN metabolites during INFOGEST in vitro digestion of spanish cereza picota (Prunus avium), and brazilian black pitanga (Eugenia uniflora) and grumixama (Eugenia brasiliensis), while evaluating the matrix effects over metabolism. Samples were collected, and their whole pulps were freeze-dried, and their ACNs were extracted exhaustively. Whole pulp and extracts were submitted to INFOGEST in vitro gastrointestinal digestion coupled to UHPLC-TQD-MS-MS analysis. Mass spectrometry analysis of anthocyanins and their metabolites was conducted in MRM mode of 20 different compounds. MRM transitions were defined by selecting specific mass-to-charge (m/z) ratios of precursor and product ions characteristic of each anthocyanin compound.

Results identified up to 12 ACNs. The main original ACNs in undigested samples were Cyanidin-3-O-glucoside, Delphinidin-3-O-glucoside, Pelargonidin-3-O-glucoside and Peonidin-3-O-hexoside. Following digestion, these initial ACNs decreased for all three fruits, 70 – 100% reduction, regardless whole pulp or extracts. In the meantime, 9 different metabolites were found for all three fruits, some of them appeared in high amounts only in digested samples (Delphinidin aglycone and Glycated chalcone C3G), while Cyanidin 3-O-Glucoside carbinol pseudobase – a metabolite chemical derived from Cyanidin-3-O-glucoside - increased substantially. The ACNs aglycone forms increased greatly in all three fruits due to amylase activity of digestive enzymes, while the carbinol and chalcone forms are mainly derived from pH changes during digestion. In conclusion, all three fruits and treatment (whole pulp vs extracts) presented similar patterns of metabolites with only slight differences, meaning that the chemical and enzymatic rules that govern ACNs metabolism during digestion are little affected by fruit matrix. Our investigations will enhance the understanding of the health-promoting properties of ACNs, and support their potential application in the development of functional foods. Additionally, the identification and valorization of native berries offers a sustainable approach to diversifying local diets while promoting biodiversity conservation.

Keywords

Metabolomic, Polyphenols, Bioactive Compounds, Fruits, Bioacessibility

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EFFECT OF SURFACTANT-TO-OIL RATIO ON THE DIGESTIBILITY AND BIOACCESSIBILITY OF B-CAROTENE-LOADED CHICKPEA PROTEIN NANOEMULSIONS

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Abstract

The growing consumers demand for food with synthetic-free ingredients has led to a search for plant-based emulsifiers, with legume proteins emerging as natural alternatives for stabilizing emulsion-based delivery systems of bioactive compounds. Among them, chickpea protein stands out as a promising emulsifier due to its favourable nutritional profile and amphiphilic nature. However, its relatively low solubility limits its technological application. To overcome these constraints, optimising the surfactant-to-oil ratio (SOR) to ensure adequate interfacial coverage and droplet stability is crucial. This study investigates the emulsifying capacity of chickpea protein under different SORs and evaluates their effect on the bioaccessibility of β-carotene loaded in the prepared nanoemulsions. These were formulated with 10% (w/w) β-carotene-enriched corn oil and varying concentrations of chickpea protein as an emulsifier to archive SORs of 0.05, 0.10, and 0.25 at pH 7. Then, the resulting dispersions were submitted to high-speed homogenization followed by microfluidization. Particle size, microscopy, ζ-potential, oil digestibility and β-carotene bioaccessibility were evaluated. In vitro digestion was performed according to the standardized INFOGEST protocol. SOR markedly influenced emulsions properties. The smallest oil droplets were obtained at SOR 0.1 (0.36 µm. However, nanoemulsions prepared at SOR 0.25 exhibited the most negative ζ-potential (≈ −66 mV) while maintaining a small average size (0.42 µm), suggesting enhanced electrostatic stabilization. During the gastric phase, all formulations exhibited aggregation, attributable to the sharp pH decrease. Nevertheless, the emulsion prepared at SOR 0.1 exhibited reduced aggregation, resulting in a smaller apparent particle size at the beginning of the intestinal phase, possibly because smaller droplets provide greater colloidal stability. Subsequently, the emulsions redispersed during the intestinal phase, facilitating enzyme accessibility and micelle formation. Although oil digestibility at the end of the intestinal phase was similar among all formulations (≈70%), β-carotene bioaccessibility ranged from 13.98 ± 0.85 % to 23.75 ± 0.53 %, with the highest values observed for the SOR 0.25 formulation. These results indicate that the bioavailability of lipophilic compounds is not solely determined by the extent of lipolysis. Variations in SOR, together with a more negative ζ potential, may influence β-carotene bioaccessibility, possibly related to protein-lipid interactions and differences in micellisation efficiency under intestinal conditions. In conclusion, chickpea protein-stabilised nanoemulsions proved to be effective delivery systems for β-carotene under simulated gastrointestinal conditions. Notably, the formulation prepared at SOR 0.25 lead to the greatest bioaccessibility, highlighting the critical role of SOR in determining the functional properties of the emulsions.

Keywords

chickpea protein, nanemulsions, bioaccessibility, β-carotene

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PROCESSING MATTERS: HOW CELL DISRUPTION AND DRYING SHAPE YEAST PROTEIN QUALITY

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Abstract

Background: Yeast-based protein is commonly evaluated using crude compositional metrics and processed to optimize techno-functional yields. The effects of downstream processing, such as cell disruption and drying, on nutritional composition, in vitro digestibility, and potential health relevance remain poorly understood.

Methods: In this study, a combined compositional and in vitro digestion approach was applied to evaluate processing-related effects on the digestibility of yeast protein concentrate. Yeast cream from a selected Saccharomyces cerevisiae strain underwent four downstream routes: (1) direct spray drying, (2) direct drum drying, (3) lysis followed by centrifugation to obtain the pellet fraction, and (4) the supernatant fraction, both subsequently spray dried. Proximate composition, including lipid, protein, dietary fiber, and nucleotides, was determined according to AOAC standard methods. In vitro gastrointestinal digestion was performed using the standardized INFOGEST 2.0 and INFOGEST Quant static protocols. Protein digestibility was assessed based on solubilized nitrogen content and the release of primary amino groups.

Results: Compositional analysis revealed that lysis and subsequent phase separation led to important nutrient redistribution. The pellet fraction was enriched in dietary fiber (32% dry matter) compared to the supernatant fraction (8%), whereas the supernatant fraction showed higher protein (57%) and nucleotide (11%) content compared to the pellet fraction (protein 31% and nucleotide 4%). These profiles differed clearly from directly dried samples, both spray and drum-dried, which showed intermediate profiles (dietary fiber 21%, protein 44%, and nucleotides 9%), with no significant compositional shifts between drying methods. These differences are expected to influence in vitro protein digestibility, providing insight into how processing modulates sample digestibility.

Conclusion: This work bridges the gap between compositional characteristics and digestibility of yeast-based protein, supporting a more functional evaluation of single-cell protein beyond crude compositional metrics. The results suggest that the choice of processing routes may represent important determinants of protein bioaccessibility. Such insights could provide a basis for guiding industrial processing to enhance the nutritional quality of sustainable single-cell protein sources for food and feed applications.

Keywords

yeast protein concentrate, downstream processing, nutritional quality, Saccharomyces cerevisiae

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VALORIZATION OF TOMMY ATKINS MANGO (MANGIFERA INDICA L.) PULP AND BY- PRODUCTS FOR FOOD APPLICATIONS

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Abstract

The valorisation of mango processing by-products as sources of bioactive compounds represents an attractive strategy for sustainable food development. In this study, flours obtained from Tommy Atkins mango pulp and byproducts were characterised and applied in bakery products. Five mango flours were produced: three from pulp using different drying methods (lyophilisation, conventional hot air drying, and foam mat drying) and two from by-products (kernel and peel). Cupcakes were formulated by replacing wheat flour at a 35% substitution level. Mango flours and cupcakes were analysed bioactive composition, including phenolic compounds and carotenoides An in vitro static gastrointestinal model that simulates digestion was used for the cupcake samples, following the INFOGEST 2.0. The Bioaccesibilility (%) was calculated as follows: (A/B) × 100. Where A is the total content of bioactive compound in the supernatant (phenolic compounds) or in the micellar fraction (carotenoids and tocopherols) after in vitro digestion, and B is the total content of bioactive compounds in the cupcake before digestion, expressed in the same units. Regarding bioaccessibility, phenolic acids were the most bioaccessible compounds, followed by mangiferin, galloyl derivatives, and flavonols. Cupcakes made with mango pulp flour demonstrated a higher bioaccessibility of phenolic acids and mangiferin than those with by-product mango flours, likely due to differences in matrix composition and processing. Carotenoids were not detected in cupcakes containing kernel flour, and peel-based cupcakes exhibited lower bioaccessibility compared to pulp-based ones. Although the peel flour cupcakes initially had a higher tocopherol content, their bioaccessibility was reduced after digestion. In contrast, foam mat-dried pulp flour yielded the highest tocopherol bioaccessibility, highlighting the impact of the drying method on the release of bioactives.
However, we emphasise the feasibility of bioactive compounds comprised in mango by-products to be envisaged for innovative functional food developments.

References

Blancas-Benitez, F.J. et al (2015). Bioaccessibility of polyphenols associated with dietary fiber and in vitro kinetics release of polyphenols in Mexican ‘Ataulfo’ mango (Mangifera indica L.) by-products. Food Functional 6:859-68.

Brodkorb, A. et al (2019). Infogest static in vitro simulation of gastrointestinal food digestion. Nature Protocols. 14, 991-1014.

García-Mahecha, M., Soto-Valdez, H. et al (2023). Bioactive Compounds in Extracts from the Agro-Industrial Waste of Mango. Molecules, 28, 458.

Sagar, N.A. et al (2018). Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization. Comprehensive Reviews in Food Science and Food Safety, 17, 512–531.

Werner, S., Bohm, V. (2021). Bioaccessibility of carotenoids and vitamin E from Pasta: evaluation of an in vitro digestion model. Journal of Agricultural and Food Chemistry. 59, 4, 11.

Keywords

Mango (Mangifera indica L.) by-products, bioactive compounds, mangiferin, bakery products, bioaccessibility.

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EVALUATION OF BIOACTIVE PROPERTIES OF RAMON (BROSIMUM ALICASTRUM) PROTEIN DIGESTATES GENERATED THROUGH INFOGEST SIMULATED DIGESTION: ANTIOXIDANT POTENTIAL

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Abstract

Ramon (Brosimum alicastrum) is a traditional Mayan food source native to the tropical forests of Mexico and Central America. While raw and roasted Ramon fruits and seeds are seasonally consumed by local communities, their nutritional and health applications, including bioaccessible proteins and bioactive peptides, remain poorly explored. This work evaluated the effects of heat treatment and level of processing on the digestibility and bioactivity of Ramon proteins and the release of antioxidant peptides during gastrointestinal digestion (INFOGEST) by means of in vitro assays of green seed flour (GSRF), roasted seed flour (RSRF), green seed protein concentrate (GSPC), and roasted seed protein concentrate (RSPC). Soluble protein and peptide results revealed that GSPC and RSPC digestates have markedly higher contents (29 -151 mg/mL) than GSRF and RSRF (5 – 33 mg/mL), with values steadily increasing throughout the digestion process (up to 151 mg/mL at the end of the intestinal phase). However, peptide levels were found to be higher in GSPC (32–151 mg protein/mL) compared to RSPC (21–82 mg protein/mL), suggesting that roasting affected the digestibility and peptide release during digestion. The highest ABTS scavenging activity was observed for RSRF digestates (61 %) at 1 mg/mL, followed by the GSRF intestinal digestates (52 % at 1 mg/mL), which is greater than their respective protein concentrates. Metal-chelating activity measured in raw-flour digestates (~72 %) at the end of the gastric phase was significantly greater than that of the protein-concentrate digestates (~43 %) at 1 mg/mL. In addition, reducing-power assays indicated that GSPC showed the lowest value at the end of the intestinal phase (0.46 at 1 mg/mL), which may reflect loss of phenolic compounds during the extraction process. The enhanced antioxidant activity of RSRF digestates may be attributed to roasting-induced Maillard reaction products and improved release of antioxidant compounds, such as melanoidins, which have the potential to chelate metals and scavenge oxygen radicals, whereas the reduced activity in RSPC likely results from the loss of non-protein antioxidants during protein extraction and heat-induced protein aggregation that limits antioxidant peptide release. In addition, peptidomic analysis revealed that GSPC digestates generated the highest number of peptides during the gastric phase (2800) and declined dramatically during the intestinal phase. This could be due to further enzymatic hydrolysis producing predominantly free amino acids and very short peptides that fall below the detection threshold. These findings demonstrate that processing conditions modulate protein hydrolysis, peptide release, and antioxidant activity of Ramon-derived ingredients during simulated gastrointestinal digestion, underscoring the importance of digestion phase–specific evaluation when assessing their bioactive potential.

Keywords

Ramon seed, Brosimum alicastrum, protein concentrate, simulated gastrointestinal digestion, antioxidant activity

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

DIGESTION RESISTANT DIETARY PEPTIDES IN HUMAN URINE IDENTIFIED BY UNTARGETED PEPTIDOMICS

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Abstract

Monitoring in vivo the products of dietary protein metabolism is essential for understanding digestion, absorption, and bioavailability of food-derived peptides. Urinary analysis represents a valuable non-invasive tool in clinical and nutritional research, as it enables direct investigation of the metabolic fate of dietary proteins without invasive procedures. In this study, we developed and optimized an analytical method for the characterization of food-derived peptides in human urine, overcoming challenges related to peptide extraction and purification from a complex biological matrix. The workflow is based on a multistep clean-up strategy including sequential ultrafiltration and size-exclusion chromatography, followed by high-resolution mass spectrometry (MS)-based untargeted peptidomics.

Bread was selected as a model food to investigate wheat peptides resistant to digestive proteases. After a two-day washout period, eleven healthy adults (6 females, 5 males; 28–55 years) consumed 100 g of bread baked with wheat flour (Triticum aestivum). Urine samples were collected over eight hours post-meal and pooled.

Untargeted peptidomic analysis revealed wheat-derived peptides, mainly from gliadin and glutenin proteins, in the urine of all participants. These peptides were undetected in the urine samples collected at baseline. Although peptide number and sequences varied among individuals, all eleven subjects shared a common peptide corresponding to the 32–50 region of α-gliadin, which was therefore selected as a biomarker of gluten exposure. The α-gliadin 32–50 peptide was quantified using a targeted proteomics approach with an isotopically labelled internal standard, enabling absolute concentration determination. Detected levels were in the 33.1-83.3 nanogram per millilitre range (ppb), demonstrating the high sensitivity of the method.

These results show that specific wheat peptides can resist gastrointestinal digestion, cross the intestinal barrier, and be detected and quantified in urine, supporting urinary analysis as a promising non-invasive strategy for monitoring dietary exposure. This approach is particularly relevant for peptides resistant to enzymatic hydrolysis, with applications in nutrition (e.g., bioactive peptides) and clinical research, including toxic or immunogenic sequences such as allergens.

In the context of wheat proteins, the α-gliadin 32–50 peptide may serve as a urinary biomarker to monitor gluten intake and assess adherence to a gluten-free diet in patients with celiac disease.

The proposed methodological framework can also be extended to other dietary proteins, broadening its application in studying the metabolic fate of food-derived peptides in humans.

Keywords

in vivo digestion; urine; peptidomics; quantitative analysis; wheat proteins

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

STUDIES ON THE METABOLIZATION OF DIETARY 3-DEOXYGLUCOSONE IN THE INTESTINAL EPITHELIUM

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Abstract

3-Deoxyglucosone (3-DG) is a key intermediate formed during the Maillard reaction, both in foods and within the human body. Compounds like 3-DG, which contain 1,2-dicarbonyl (DC) groups, are believed to contribute to the development of age-related diseases and complications associated with diabetes. Since foods can contain significant amounts of 3-DG, it is important to understand how this compound is absorbed and metabolized in the body. Previous studies have shown that an increased intake of 3-DG leads to renal excretion of significant amounts of its metabolite, 3-deoxyfructose (3-DF), besides small amounts of 3-DG. [1,2]. The mechanism underlying this biotransformation of 3-DG to 3-DF is unclear.

Caco-2 cells, 21 days postconfluence, were used as an in vitro model of the small intestinal epithelium. Cell viability was determined via neutral red assay [4]. Therefore, Caco-2 cells were treated for 21 days with varying 3-DG concentrations (0.75, 1, 2.5 and 5 mM). The ability of Caco-2 cells to metabolize 3-DG was studied by treatment with different 3-DG concentrations (0.25-6 mM) over several treatment periods (0-180 min). The formation of 3-DF was measured via GC-MS/MS [2]. A Transwell system was utilized to model the intestinal epithelial barrier, after exposure to 1.2 and 0.6 mM 3-DG for 2 hrs. Transepithelial electrical resistance (TEER) were performed to assess barrier function. Metabolite formation was assessed apical and basolateral to evaluate the DC uptake.

Acute exposure for 2 h to 3-DG (up to 10 mM) did not alter cell viability, indicating that short-term dietary exposure is unlikely to exert cytotoxic effects. In contrast, chronic exposure to 5 mM 3-DG over a 21d period led to noticeable reductions in cell viability (37%), highlighting the risk of long-term carbonyl stress. 3-DG treated Caco-2 cells showed a linear increase in 3-DF formation with exposure time (3.33%±0.02 3-DF is formed from the used 3-DG). This trend indicates a robust metabolic activity towards 3-DG under the analyzed conditions, without apparent substrate saturation within the tested range. With increasing 3-DG incubation time, 3-DF concentrations increased linearly, indicating a time-dependent formation of the metabolite. The results of the Transwell studies show that 3-DG treatment has no influence on epithelial integrity and that 3-DG is also reduced to 3-DF in this assay. Independent of 3-DG treatment concentration 37 µM 3-DG and 17,28±2,58 µM 3-DF could be located basolateral.

Taken together, 3-DG is not toxic to the intestinal epithelial cell model at food-associated concentrations. Differentiated Caco-2 cells are capable of metabolizing 3-DG to 3-DF, and both dicarbonyl compounds can be transported across the intestinal barrier.

References
[1] Degen. J Agric Food Chem. 2012. [2] Rückriemen. Eur Food Res Technol. 2018 [3] Hidalgo. Gastroenterology. 1989. [4] Repetto. Nat Protoc. 2008.

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INFLUENCE OF AGE ON PLANT PROTEIN DIGESTIBILITY, EPITOPE FORMATION, AND ENTEROENDOCRINE HORMONAL RESPONSES

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Abstract

Protein digestion and absorption are dynamic processes influenced by physiological factors, such as age and the nature of the protein source. In later life, age-related changes in gastrointestinal function, including reduced enzyme secretion, altered motility, and increased gastric pH, can impair protein digestion. Moreover, protein digestion products can stimulate the secretion of enteroendocrine hormones regulating intake and glucose homeostasis, such as glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK) (Santos-Hernández et al., 2023; Vivanco-Maroto et al., 2023). In this context, understanding how different proteins are degraded along the gastrointestinal tract is crucial for optimizing nutritional strategies that enhance their utilization, gut physiological response, and safety. This study investigates the influence of age-digestive conditions on the digestion of three plant proteins differing in digestion susceptibility, their potential allergenicity, and the ability of the resulting products to induce CCK and GLP-1 secretion. To this end, simulated gastrointestinal digestions were performed following the tailored INFOGEST protocols under adult and elderly conditions (Menard et al., 2023) using as substrates: Rapeseed Protein Isolate (RPI), Corn Protein Meal (CPM), and Pea Protein Isolate (PPI).

RPI showed efficient protein hydrolysis under both adult and elderly gastrointestinal conditions, whereas the gastric digestibility of PPI was markedly reduced in the elderly model. CPM generated the highest amount of insoluble material after digestion, indicating its limited susceptibility to enzymatic degradation. Across all protein sources, elderly gastrointestinal conditions led to the appearance of longer digestion-resistant peptides. The number of digestion-resistant peptides homologous to known epitopes was higher in all substrates under elderly conditions compared to adult conditions. In addition, hormonal secretion in the enteroendocrine cell line STC-1 also exhibited age- and protein source-dependent variations. The strongest age-related effect was found for CPM, whose gastrointestinal digests elicited lower CCK secretion in the elderly condition. Additionally, both CCK and GLP-1 secretion were diminished in elderly gastric digests relative to adult digests. In contrast, the effect of age on hormone secretion for RPI and PPI was minimal. Overall, these findings demonstrate that both age and protein source significantly influence protein digestion, the generation of digestion-resistant peptides with potential allergenic epitopes, and the secretion of enteroendocrine hormones, highlighting the importance of considering age-specific digestive conditions when designing nutritional strategies for adults and the elderly.

References

De Martinis et al. (2019). https://doi.org/10.3390/ijms20225580

Menard et al. (2023). https://doi.org/10.1039/D3FO00535F

Santos-Hernández et al. (2023). https://doi.org/10.1016/j.foodchem.2022.134225

Keywords

Elderly gastrointestinal digestion, plant protein isolates, epitope formation, hormonal response

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THE INFLUENCE OF PH AND MYROSINASE ACTIVITY ON GLUCOSINOLATE HYDROLYSIS AND ISOTHIOCYANATE FORMATION DURING THE IN VITRO DIGESTION OF MUSTARD BY-PRODUCTS.

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Abstract

This study examines the hydrolysis of three glucosinolates—sinigrin, glucotropaeolin, and sinalbin—from the green tissues of two mustard species (Brassica carinata and Sinapis alba) into their corresponding isothiocyanates (ITCs) (allyl-, benzyl-, and 4-OH-benzyl-ITC, respectively) throughout the successive stages of human digestion (oral, gastric, and intestinal). The assayed conditions of the INFOGEST digestion method with slight modifications were used to estimate glucosinolate and isothiocyanate bioaccessibility The results show that the three isothiocyanates tend to form during the oral phase (5.80, 9.64, and 20.31 µmol/g, respectively), undergoing progressive degradation in the subsequent phases of the digestive process (gastric and intestinal) with values in the intestinal phase of 3.72 and 5.11 µmol/g for allyl- and benzyl- ITCs, respectively. Their instability was particularly significant for 4-OH-benzyl-ITC, which was no longer detectable at the end of the intestinal phase. Interestingly, under the stomach's acidic pH conditions, the enzyme myrosinase retained up to 50% of its activity, enabling glucosinolate hydrolysis and, thus, the formation of isothiocyanates during the gastric phase. In addition, glucosinolates were mainly found in the bioaccessible fraction, whereas isothiocyanates tended to concentrate in the non-bioaccessible fraction due to the molecules' lipophilic characteristics.

Keywords

Allyl-isothiocyanate, benzyl-isothiocyanate, 4-OH-benzyl-isothiocyanate, bioactive compounds, myrosinase activity

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

ASSESSING THE BIOACCESSIBILITY AND BIOAVAILABILITY OF ALTERNARIA MYCOTOXINS IN TOMATO-BASED PRODUCTS DURING IN-VITRO GASTROINTESTINAL DIGESTION

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Abstract

Introduction: In recent years, climate change has increased the presence of Alternaria species in tomato and other cultivation fields, raising concern about the presence of related mycotoxins and their potential impact on human health. Currently, no specific regulations govern Alternaria toxins in food. In 2011, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain assessed dietary exposure in Europe, leading to the European Commission proposal (2022/553) introducing requirements for monitoring toxins in processed tomato products. The main Alternaria toxins detected in food are alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN) and tenuazonic acid (TeA), which have shown mutagenic and carcinogenic properties in vitro and in vivo [1].

Objectives: This study evaluated the impact of food matrix and in vitro digestion on the bioaccessibility and bioavailability of Alternaria toxins in tomato, a major dietary source of exposure.

Methods: Naturally contaminated and spiked tomato purees were subjected to in vitro digestion following the INFOGEST protocol with slight modifications to meet analytical constrains [2]. After centrifugation, soluble (bioaccessible) and insoluble fractions were collected. Lyophilized samples underwent solid–liquid extraction and SPE purification. Intestinal bioavailability was assessed using a Caco-2/HT-29MTX co-culture model. Apical and basolateral compartments were collected after 2 hours and analyzed by HPLC-MS.

Results: Preliminary data suggest that AOH, AME and TEN remain unaltered in the soluble duodenal fraction digest. The different chemical nature of TeA required a more acidic (pH=4,5-4,7) environment to be extracted, indicating its limited solubility under physiological intestinal conditions. The intestinal compartment was analyzed to assess toxin bioaccessibility and transepithelial transport, revealing compound-specific differences without evidence of metabolic transformation (e.g., glycosylation or sulfation).

Conclusions: The INFOGEST digestion model allowed monitoring and assessment of bioaccessibility of mycotoxins during digestion. The implementation of the model with the successive step based on the Caco-2/HT-29MTX monolayers highlighted compound-specific differences in intestinal. Evidences indicates that these compounds are recovered as parent molecules, suggesting limited metabolic biotransformation.
Further studies are needed to clarify digestion, absorption and health risks, particularly considering co-occurrence and widespread dietary exposure.

[1]H. Louro et al., 'Hazard characterization of Alternaria toxins to identify data gaps and improve risk assessment for human health', Arch. Toxicol., vol. 98, no. 2, pp. 425–469, Feb. 2024, doi: 10.1007/s00204-023-03636-8.
[2]A. Brodkorb et al., INFOGEST static in vitro simulation of gastrointestinal food digestion', Nat. Protoc., vol. 14, no. 4, pp 991–1014, Apr. 2019, doi: 10.1038/s41596-018-01

Keywords

LC-MS, INFOGEST, intestinal epithelial cells, Bioaccessibility and bioavailability

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

COMPARATIVE ANALYSIS OF PROTEIN DIGESTIBILITY AND FUNCTIONAL BIOACTIVITY IN HMO ENRICHED INFANT FORMULAS

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Abstract

The first six months of life are critical for immune and microbiome development. Since breastfeeding is not always possible, there is a clear need for high-quality breast milk alternatives. In this context, HMO-enriched formulas aim to mimic the functionality of human milk, making it essential to validate their quality and bioactivity under neonatal conditions. This study evaluates six commercial infant formulas (IF) with different combinations of HMO and protein profiles, linking their digestive processes with functional impact on the gut. A static in vitro gastrointestinal digestion model (0-6 months) was optimized following the INFOGEST consensus1, with specific adjustments2. The peptide profile of the bioaccessible fraction was analyzed by size exclusion chromatography (SEC). Protein digestibility was determined by quantifying NH2-groups (OPA) and total amino acids. Digestible Indispensable Amino Acid Ratio (DIAAR) and Score (DIAAS)3 were determined to establish protein quality of the products. Going a step further, the digestas were used to evaluate cellular functionality, analyzing the induction of reactive oxygen species (ROS) in Caco-2 cells. In parallel, the potential anti-inflammatory action of the HMOs present in the IF was studied using a pro-inflammatory intestinal epithelium model (Caco2/HT29-MTX). Following sequential induction with IFN-γ (16 h) and a TNF-α/IL-1β cocktail (24 h), the expression of key barrier, mucin secretion and inflammatory response genes were quantified via RT-qPCR. FPLC analysis proved that the digestive process can generate small, potentially absorbable, and bioactive fragments, highlighting the uniqueness of formula IF05, composed by partially hydrolyzed proteins. Results showed a high protein digestibility (>79%) for all IF, but the DIAAR assessment identified significant variations in the limiting amino acids. Specifically, IF01 achieved a DIAAS of 122.8, classifying it as an “excellent” protein source, whereas IF05 failed to meet the minimum threshold for health claims. This highlights that a higher degree of initial protein hydrolysis does not necessarily correlate with superior nutritional quality scores4. Regarding bioactivity, pre-incubation with these bioaccessible fractions reduced ROS by 30% in Caco2 cells. These findings, combined with our ongoing analysis on gene expression in the inflammation model, emphasize that IF functionality must be evaluated through integrated techniques that capture the complex interaction between digestive transformation and intestinal health.

1Menard et al., 2018. Food Chem., 240, 338-345.
2Brodkorb et al., 2019. Nat. Protoc. 14, 991-1014.
3FAO, 2013. Dietary protein quality evaluation in human nutrition.
4Sousa et al., 2023. Food Res. Int. 166,112569.

Keywords

infant formulas, HMOs, protein digestibility, DIAAS, DIAAR, antioxidant activity,anti-inflammatory action.

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

GLS – ITC PAIR BIOACCESSIBILITY FROM WHITE MUSTARD SEEDS. INFLUENCE OF SUPPLEMENTATION WITH BY – PRODUCTS DERIVED FROM THE GREEN PARTS OF THE PLANT.

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Abstract

In this study, the hydrolysis process of sinalbin, the main glucosinolate (GLS) present in white mustard seeds (Sinapis alba) into its corresponding isothiocyanate (ITC) (4-OH-benzyl-ITC) was studied throughout the different phases of the digestive process. The supplementation of these seeds with extracts from green parts (leaves and stems) of the same plant, in which another glucosinolate-isothiocyanate pair (glucotropaeolin – benzyl-ITC) is also present, but absent in the seeds, was also studied. The use of these stems and leaves as both an ingredient and a source of bioactive compounds improved the nutritional quality of seeds. Additionally ,this aligns with the current consumer demands for health-promoted foods and promotes sustainability through the application of a biocircular economy approach in the food industry.

The initial content of sinalbin present in seeds of Sinapis alba was 50.2 mg/g (or 108 µmol/g). This glucosinolate was quickly hydrolyzed to 4-OH-benzyl-ITC (85%) in the oral phase of the bioaccessibility assay. Only 4.30 µmol/g of sinalbin (4% of total) resisted enzymatic hydrolysis, with this glucosinolate concentration remaining practically steady throughout the subsequent phase (gastric) and then decreasing up to 0.20 µmol/g at the end of intestinal phase. Despite this high efficiency in the production of the corresponding ITC, the concentration of 4-OH-benzyl-ITC fell dramatically, by up to 9.84 µmol/g, at the end of the intestinal phase.

The initial content of glucotropaeolin and sinalbin present in stems and leaves of Sinapis alba were 4.14 and 25.4 mg/g, respectively. Extraction process of these glucosinolates were performed with hot water at 60 – 90 °C during 30 min. The best extraction efficiency was achieved at 80 °C. After extraction process, mustard seeds were supplemented with glucotropaeolin extract at doses of 1 – 5 µmol/g. For most doses studied, glucotropaeolin was efficiently hydrolyzed by myrosinase present in seeds, to benzyl-ITC. Finally the extracts were microencapsulated by vibrational microencapsulation technology (300, 450 and 750 µm) and benzyl-ITC bioaccessibility were also tested.

Keywords

Sinalbin, Glucotropaoelin, Benzyl-isothiocyanate, OH-benzyl-isothiocyanate, By-products

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

TEMPEH FERMENTATION FROM INDONESIA TO THE NORDICS: IN VITRO DIGESTION, NUTRIENT RELEASE, AND STRUCTURAL CHANGES IN FABA BEAN–OAT TEMPEH

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Abstract

We explored how fermentation enhances the nutritional value of faba bean and oats from Sweden. The effects of pre-treatments (soaking with Lactiplantibacillus plantarum and boiling) and subsequent solid-state fermentation (SSF) with Rhizopus oryzae to produce a tempeh-like food were investigated through comprehensive nutritional composition analysis, static in vitro digestion, microscopy before and after in vitro digestion, and multivariate modeling. Changes were followed from raw material to pre-treated and fermented samples, while in vitro digestion was performed before and after tempeh fermentation.

Fermentation significantly enhanced protein concentration, with faba bean tempeh reaching 44.5 g/100 g DM, compared to 36.0 g/100 g DM in the raw material. Free amino acids increased markedly, especially in LAB-soaked faba–oat tempeh. Resistant starch and insoluble dietary fiber decreased post-fermentation, while total starch was substantially reduced. Total fiber in tempeh samples was around 6 g/100 g of fresh product. Anti-nutritional factors were dramatically lowered after fermentation: phytic acid decreased by up to 93%, vicine by 77–91%, and convicine by 96–98% after only 40 h of SSF. All tempeh products qualified as “rich in fiber” (≥6 g/100 g fresh weight).

In vitro digestion using the standardized INFOGEST protocol revealed clear functional advantages of fermentation. The highest protein digestibility (degree of hydrolysis) was observed in T4, reaching approximately 48% DH, aligning with elevated free amino acids and protein content. Starch degradation was measured after in vitro digestion, and fermented samples showed significantly lower glucose and maltose release than pre-treated controls, demonstrating reduced starch susceptibility and suggesting lower glycemic potential. Digesta viscosity was also investigated; however, no significant differences were observed.

Confocal laser scanning microscopy of fresh food products provided direct evidence of structural changes. Moreover, post–in vitro digestion microscopy was performed, and the results were outstanding, clearly showing differences between plant and fungal cell wall structures. Pre-treated samples retained intact cell walls encapsulating starch and proteins. In contrast, fermented matrices exhibited extensive cell wall degradation, increased porosity, protein body disruption, and greater intracellular exposure of nutrients. Post-digestion imaging revealed near-complete starch disappearance in tempeh samples and profound protein matrix breakdown. Notably, fungal structures (sporangiophore-like elements) remained visible after digestion, suggesting partial resistance of fungal biomass to gastrointestinal enzymes—an important structural finding.

Multivariate PCA (PC1 = 65.4%) and PLS modeling (63.3% explained variance) robustly confirmed treatment-driven separation from before and after Tempeh fermentation.

Keywords

Tempe, Rhizopus oryzae, digestion, plant-based, lactic acid bacteria

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

INVESTIGATION OF RELEASE AND BIOACTIVITY OF EXTRUSION-ENCAPSULATED VITAMIN A AFTER SEMI-DYNAMIC IN VITRO DIGESTION

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Abstract

Background

Vitamin A, particularly retinyl esters such as retinyl palmitate, is highly sensitive to light, oxygen, and heat [1], undergoing isomerization and oxidative degradation during processing and storage. Extrusion-based encapsulation may protect vitamin A against degradation processes and enables targeted release in the small intestine [2]. The aim of the present study was to compare a conventional (CON) and an optimized (OPT) extrusion method with respect to storage and thermal stability of retinyl palmitate, with emphasis on its gastrointestinal release and phase-specific cellular bioactivity following semi-dynamic in vitro digestion.

Methods

Retinyl palmitate (RP) was extruded into CON- and OPT-capsules, the latter formulated with glycerol and ascorbic acid. Capsule RP contents were analyzed after storage (4 months, with and without light/oxygen exposure) and after thermal treatment (0.5–2 h, 120 °C, under controlled air/inert atmosphere conditions) using HPLC. Gastrointestinal digestion was performed using a semi-dynamic INFOGEST-based model with automated pH control (Titrando), covering oral, gastric, and intestinal phases [3, 4]. Samples from each phase were centrifuged and HEK293-RARα reporter cells (RAR, retinoic acid receptor) were incubated for 24 h at 37 °C with the supernatants to assess cell viability (WST assay) as well as vitamin A bioactivity (RARα luciferase assay). For calculation of RP bioaccessibility, RP and retinol (ROL) were quantified by HPLC in supernatants and pellets from the intestinal phase.

Results

After storage, RP losses in CON capsules reached up to 32%, whereas OPT capsules showed reductions of only up to 9%. Under heat stress, CON capsules exhibited almost complete RP degradation (up to 98%), while OPT capsules remained markedly more stable (up to 12% reduction). Exposure of HEK293-RARα reporter cells to subtoxic concentrations of in vitro digested OPT samples resulted in 10% higher RARα induction compared to CON samples (p 0.001). After semi-dynamic in vitro digestion, OPT intestinal supernatants exhibited higher RP (36.5 µg/g) and ROL contents (59.0 µg/g) than the respective CON samples (RP: 22.1 µg/g; ROL: 50.5 µg/g). Pellets from digested OPT capsules also retained more ROL (53.7 µg/g) than those from CON capsules (42.0 µg/g), resulting in a higher calculated overall RP bioaccessibility in OPT (8%) compared to CON samples.

Conclusion

The optimized extrusion approach enhances stability, in vitro release in the gastrointestinal model, and cellular bioactivity of vitamin A. These findings suggest that the optimized extrusion process may represent a promising strategy for stabilizing and delivering sensitive lipophilic micronutrients.

References

[1] Barua & Furr (1998) Mol. Biotechnol., 10(2), 167-182

[2] Patil et al. (2024) Food Sci. Biotechnol., 33:2937-2951

[3] Brodkorb et al. (2019) Nat. Protoc. 14:991-1014

[4] Mulet-Cabero et al. (2020) Food Funct., 11, 1702-1720

Keywords

extrusion, encapsulation, retinyl palmitate stability, in vitro digestion, bioaccessibility, cellular bioactivity

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IMPACT OF ULTRASOUND AND TRANSGLUTAMINASE-INDUCED STRUCTURAL MODIFICATIONS ON IN VITRO DIGESTIBILITY OF PEA PROTEIN ISOLATES AND CONCENTRATES

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Abstract

Pea protein is increasingly recognised as a promising sustainable plant-based protein owing to its balanced amino acid profile. Nevertheless, its nutritional quality ultimately depends on protein digestibility and amino acid bioaccessibility. Pea proteins exhibit relatively compact globular conformations and tend to form aggregates during processing, which may reduce solubility, restrict enzyme accessibility during digestion, and hinder gel formation. Pea protein isolate (PPI) generally has a higher protein content but may exhibit greater denaturation and aggregation compared to pea protein concentrate (PPC). PPC retains more non-protein components, such as starch and dietary fibre, and a higher proportion of proteins remaining in their native state. These compositional and structural differences may consequently influence network formation and digestion behaviour. Ultrasound treatment can disrupt protein aggregates and improve protein solubility, whereas transglutaminase (TGase) treatment induces covalent crosslinking which strengthens protein network formation. This study explored the influence of protein-to-TGase ratio and ultrasound pretreatment conditions on gel network properties and in vitro protein digestibility of PPI and PPC. The degree of crosslinking was determined using OPA assay, and mechanical gel properties (Young's modulus and fracture properties) were determined using uniaxial compression tests. Confocal laser scanning microscopy (CLSM) was employed to visualise gel microstructure. In vitro protein digestion was assessed using the INFOGEST 2.0 protocol with minor modifications. Preliminary results suggest that ultrasound pretreatment increased protein solubility and reduced aggregate size, especially for PPI. It also increased the degree of crosslinking and Young’s modulus of the resulting PPI and PPC gels. Excessive ultrasound pretreatment tended to reduce in vitro digestibility. The addition of TGase increased the degree of crosslinking and Young’s modulus and was generally accompanied by a decrease in protein digestibility of PPI and PPC gels. Further data analysis is currently ongoing, and the results will be presented at the conference.

Keywords

Pea protein, Ultrasound, Transglutaminase, Gel structure, In vitro digestion

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

DIGESTION BEHAVIOUR AND RELEASE OF ANTIOXIDANT AND ANTIDIABETIC BIOACTIVE PEPTIDES FROM RAPESEED MEAL PROTEIN INGREDIENTS DURING GASTROINTESTINAL DIGESTION

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Abstract

Plant-protein–derived peptides are attracting interest as sources of bioactive compounds. Agricultural by-products such as rapeseed meal (Brassica napus) are therefore promising reservoirs of nutrients owing to their high protein content. This study compared the digestive behaviour and bioactivity of three rapeseed ingredients with different processing levels: sieved defatted rapeseed meal (SDRM), rapeseed meal protein concentrate (PC), and rapeseed protein isolate (PI). Digestates were collected during simulated gastrointestinal digestion (INFOGEST 2.0) at defined time points and analysed for soluble protein, peptide content and degree of hydrolysis (DH); furthermore, different in vitro antioxidant and antidiabetic properties were assessed. Finally, a peptidomic analysis was performed to identify the peptides released during gastrointestinal digestion simulation. Following INFOGEST digestion, SDRM and PC showed progressively increasing soluble-protein concentrations (240.4 and 261.6 mg/mL, respectively) throughout the intestinal phase. Peptide concentration increased for all three samples during digestion from 9.3 to 147.3 mg/mL, consistent with progressive proteolysis. Furthermore, the degree of hydrolysis for SDRM, PC and PI were increased to 37.2%, 34.3% and 47.9% at the end of intestinal phase, respectively. Antioxidant assays (copper and iron chelation) indicated higher activity at the intestinal stages than at gastric stages, while ABTS scavenging activity and reducing power showed higher values during gastric stage when measured at the screening concentrations (0.06, 0.125, 0.25 and 0.5 mg/mL). PC demonstrated the strongest copper-chelating activity after 240 min of intestinal digestion (65.5% at 0.5 mg/mL), while PI showed the highest iron-chelating activity at the end of digestion (12.3 % at 0.5 mg/mL). Overall, antioxidant endpoints tended to increase in a dose dependent manner, with maximal activities observed at 0.5 mg/mL. Antidiabetic properties showed that PC exhibited the greatest inhibition of α-amylase (23.1%) and α-glucosidase (39.0%) during the intestinal phase (150–240 min) at 0.03 mg/mL, outperforming SDRM and PI. Both PC and SDRM displayed potent DPP-IV inhibition that rose during intestinal digestion, peaking at 78.2–79.6% at 0.03 mg/mL in intestinal digestates (120 minutes). Notably, some enzyme-inhibition assays such as α-amylase and α-glucosidase showed stronger effects at the lower tested concentration (0.03 mg/mL) than at higher concentrations (0.12 mg/mL), indicating a non-linear dose–response that suggests further investigation. Moreover, peptidomic analysis revealed that at the end of gastrointestinal digestion, PC digestates produced the highest quantity of peptides (2400) compared to SDRM (900) and PI (400). In summary, these results demonstrate that rapeseed-derived protein fractions release digestion-derived peptides with antioxidant and antidiabetic activities.

Keywords

Plant protein, valorisation, agricultural by-products, INFOGEST 2.0, bioactivities peptide

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECTS OF GERMINATION AND ULTRASOUND TREATMENT ON THE THERMODYNAMICS, NUTRITIONAL AND STRUCTURAL QUALITY OF HIGHLAND BARLEY FRACTIONS

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Abstract

Current research investigates the effect of ultrasonication (US) (20/40/60 kHz, 220 W, 30 min), germination (65 ◦C, 6 h), and their combined treatment (US + G) on gamma aminobutyric acid (GABA) enhancement and quality profile of barley flour and bran. The results showed the highest improvements in ultrasound-assisted germinated barley flour. GABA levels increased significantly, correlating with enhanced GAD and GABA-T enzyme activities. Similarly, TPC, TFC and antioxidant potential were improved, associated with upregulated expression of mPAL, mC3H, mCHS, and mC4H genes in WB and BB tissues, enhancing phenolics biosynthesis. Surface disruptions, increased porosity, and cellular disintegration were observed in ultrasonicated samples. XRD patterns showed significant molecular arrangements and increased amorphous regions in ultrasound-treated fractions. Furthermore, FTIR spectra reveal protein unfoldings in the amide I region, suggesting enhanced protein functionality in ultrasound-assisted germinated flour. Hence, ultrasound-assisted germination can be proposed as a sustainable approach for nutritional enhancement of barley fractions to improve their suitability for functional implications.

Keywords

Highland barley Ultrasound Germination GABA Phenolics Gene expression

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

DIGESTIBILITY AND NUTRITIONAL QUALITY OF PROTEINS IN PROCESSED SOY-BASED FOODS

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Abstract

Worldwide, there is an increasing demand for sustainable protein sources. While food processing can improve shelf life, safety, and sensory attributes, it may also alter protein structure and thereby influence protein bioaccessibility. Legumes, particularly soybeans, are widely recognized as a high-quality plant protein source and play an important role in many countries' diets.

This aim of this study was to evaluate the protein digestibility and nutritional quality in soy-based processed foods using a dynamic in vitro digestion model (tiny-TIMsg). Two commercially available soy-based products — a soy drink and a soy-based sausage—were investigated. Following simulated gastrointestinal digestion, the digestibility of proteins was quantified, and the protein nutritional quality was assessed using the Digestible Indispensable Amino Acid Score (DIAAS).

The overall protein digestibility of the soy drink was 82.6 ± 1.4%, while the soy sausage exhibited a slightly lower digestibility of 80.8 ± 0.5%. The DIAAS analysis revealed sulfur-containing amino acids as the first limiting amino acids in both products. The soy drink achieved a DIAAS value of 100% (high protein quality), while the soy sausage showed a lower DIAAS of 86% (good protein quality). These results suggest that the nutritional quality of proteins varies between different processed soy-based foods.

These findings demonstrate that food processing can differentially affect protein digestibility and the nutritional protein quality in soy-based products. Combination dynamic in vitro digestion models and DIAAS assessment provides valuable insights into nutritional protein quality that go beyond total protein content.

Keywords

in vitro protein digestibility, bioaccessibility, DIAAS, soy-based food

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

TECHNOLOGICAL AND DIGESTIBILITY PROPERTIES OF ICE CREAM WAFER MADE WITH LENTIL PROCESSING BY-PRODUCTS

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Abstract

The by-products from the pulse processing industry, mainly composed of seed coats, germ, and fragmented cotyledons, are wasted or partially intended for the feed industry. This study aimed to develop an ice cream wafer sheet using Altamura green lentil flour (LF) processing by-product (LW). This was constituted essentially by the lentil skins, and proximate analysis showed that they are rich in fibre, starch, and proteins.
The chemical-physical, thermal, and technological properties of the flours were also evaluated, which allowed to optimize the formulation of the wafer in terms of technological, sensory and nutritional properties. Wafer batter included, respectively: LF or LW, water, sugar, eggs, oil, salt, vanilla flavour. The wafers were then characterised for their chemical-physical, nutritional (comprising anti-nutritional factors), and mechanical properties. LF and LW wafer showed both excellent nutritional profiles, particularly a low glycaemic index (45.9 and 43.7), high protein (25.5% and 22.3%) and fibre content (22.4% and 34.6%), respectively. LW exhibited significantly higher water holding capacity (WHC) and oil holding capacity (OHC) ($p \leq 0.05$), while LF showed a significantly greater enthalpy variation related to starch gelatinisation.
The moisture content and pH were influenced by the flour type, whereas the water activity was similar across all samples. Nutritionally, LW wafer sheet had significantly lower total starch content than LF. The hardness values obtained were lower in wafer sheets made with LW flour due to the fibre and moisture content.
Protein digestibility was evaluated using the static INFOGEST protocol, including an intestinal absorption step based on Caco-2 cell monolayers. Cell viability was assessed by an MTT assay to identify the appropriate concentration of digested samples for cell exposure, showing no cytotoxic effects. Peptide transport across the intestinal epithelium was investigated using Caco-2 cell monolayers differentiated for 21 days. Intestinal barrier integrity in the presence of digested samples (150 µg/mL) was monitored by transepithelial electrical resistance (TEER). After 2 h of exposure, no significant differences were observed between LF and LW and the control samples. However, preliminary western blot analysis of tight junction proteins revealed significant differences (p 0.05) in occludin expression compared with the control. Moreover, peptides generated after digestion and absorption were further characterized by LC–MS-based peptidomics.
In conclusion, technological assays showed good digestibility values for the wafer products. All samples exhibited low levels of protease inhibitors, very low haemagglutination activity, and reduced phytic acid content compared with raw flours. No adverse effects of digested LF and LW on intestinal barrier integrity were observed. Overall, these results indicate the potential of lentil-derived flour for application in bakery products.

Keywords

Pulses, lentil processing by-product, technological properties, lentil protein digestibility, LC MS peptidomics

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

BEYOND BIOACCESSIBILITY: REPURPOSING THE INFOGEST MODEL TO UNDERSTAND ENDOGENOUS N-NITROSAMINE FORMATION

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Abstract

Understanding the fate of food during digestion in the oral-gastrointestinal tract has advanced through the development and harmonisation of physiologically relevant in vitro digestion models such as the INFOGEST protocol, which simulates the oral, gastric, and small intestinal phases under controlled conditions (Minekus et al., 2014). The protocol is widely used to investigate mechanisms of food digestion, including protein and lipid hydrolysis, nutrient release, and the bioaccessibility of bioactive compounds, providing insights into how food structure and composition influence digestion and human health.

Beyond its conventional application, the INFOGEST protocol can also be used to investigate chemical reactions occurring during digestion, including the endogenous formation of potentially harmful compounds (Niklas et al., 2023). One example is nitrite derivatives reacting with endogenous precursors forming N-nitrosamines associated with colorectal cancer. Nitrosation occurs when nitrite, derived from dietary nitrate or added directly, is protonated under acidic gastric conditions to form reactive species that react with amines. Human exposure occurs via endogenous formation and via intake of foods where N-nitrosamines have formed during processing, yet the conditions governing these reactions remain poorly characterised.

In previous work, N-nitrosamines were shown to form during sausage processing and in vitro digestion after adding sodium nitrite and/or spinach emulsion using the INFOGEST protocol. Addition of sodium nitrite in the oral phase to mimic salivary input increased formation, while spinach-derived bioactives showed protective effects, highlighting the importance of matrix composition and digestive conditions (Niklas et al., 2023).

Building on this, the new project (Dual origins, common threats: mapping endogenous and exogenous N-nitrosamine formation and chronic disease pathogenesis, funded by NHMRC, Australia) will apply the INFOGEST protocol to investigate mechanisms behind endogenous N-nitrosamine formation across different nitrate and nitrite sources and gastrointestinal conditions. This represents a novel application of the framework, shifting its use from assessing bioaccessibility to capturing nitrosation chemistry and identifying drivers of chemical risk formation. The project aims to establish pathways and modulating factors governing endogenous formation and to determine long-term health effects of exposure to both exogenous and endogenous N-nitrosamines, generating insights to improve exposure assessment and inform mitigation strategies.

References

Minekus et al., 2014. Food & Function 5, 1113–1124.

Niklas et al., 2023. Food Research International 166, 112595.

Keywords

INFOGEST, Endogenous formation, Volatile N-nitrosamines, Non-volatile N-nitrosamines, Pro-carcinogens

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

FORMATION OF SELECTED NEUROACTIVE COMPOUNDS IN ORGANIC, FERMENTED VEGETABLE BEVERAGES OBTAINED BY LACTIC ACID FERMENTATION

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Abstract

Fermented foods are considered potential functional foods due to their content of bioactive metabolites and their capacity to modulate the gut microbiome, which may contribute to gut–brain axis interactions (Casertano et al., 2022). While dairy-based fermented products have been widely investigated, plant-based fermented beverages are less studied as potential psychobiotic foods and their neuroactive metabolite profiles remain incompletely characterized. In addition, plant-based fermentations may involve diverse lactic acid bacteria (LAB) communities, potentially leading to distinct metabolic outcomes (Shawky et al., 2025). The aim of this study was to quantify selected neuroactive compounds – dopamine, acetylcholine and γ-aminobutyric acid (GABA) in three organic, fermented vegetable beverages and evaluate their formation during lactic acid fermentation.

Organic beetroot, carrot, and parsley–celery beverages produced from vegetables cultivated under controlled organic farming conditions were subjected to controlled lactic acid fermentation using a selected starter culture developed as part of a doctoral research project. Samples were collected at pre-defined stages and fermentation progress was followed by pH and lactic acid. Acetylcholine and GABA were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) while dopamine by high-performance liquid chromatography with UV-Vis diode-array detection (HPLC-UV/Vis-DAD).

The results demonstrated the presence of all three neuroactive compounds in the fermented products, with substrate-dependent differences observed among tested vegetable types. The formation dynamics suggested an association between active lactic fermentation and increased levels of selected metabolites. Dopamine concentration increased most during beetroot fermentation, reaching peak levels at late fermentation stages. The highest GABA concentrations were observed in parsley–celery and carrot fermentations, whereas acetylcholine was detected at lower but measurable levels across all matrices.

These findings suggest that controlled lactic acid fermentation can enrich organic vegetable beverages in neurotransmitter-like metabolites reported in lactic acid fermented foods and consistent with microbial neurotransmitter pathways [Casertano et al., 2022; Shawky et al., 2025]. Further studies are required to determine their stability and bioavailability during gastrointestinal digestion and their functional relevance in the context of gut-brain axis interactions required targeted in vitro/in vivo studies.

1. Casertano M., Fogliano V., Ercolini D., 2022. Psychobiotics, gut microbiota and fermented foods can help preserving mental health. Food Research Internetional, 152, 110892
2. Shawky E., Surendran S., El-Khair R., 2025. Fermented Vegetables as a Source of Psychobiotics: A Review of the Evidence for Menthal Health Benefits. Probiotics and Antimicrobial Proteins, http://doi.org/10.1007/s12602-025-10592-5

Keywords

fermented vegetable beverages, functional foods, lactic acid fermentation, neuroactive compounds, HPLC, LC-MS/MS

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

PROTEIN DIGESTIBILITY OF EDIBLE SEAWEEDS ASSESSED BY THE STANDARDIZED INFOGEST IN VITRO DIGESTION MODEL

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Abstract

The growing demand for sustainable protein sources has intensified interest in edible seaweeds as alternative ingredients for human nutrition. Although several species display well-balanced amino acid profiles, their effective nutritional contribution depends on the proportion of protein that becomes bioaccessible and available for absorption during gastrointestinal digestion. In seaweeds, proteins are entrapped within complex and highly organized cell walls, mainly composed of non-digestible polysaccharides, which may restrict enzyme accessibility and limit protein release upon digestion. Furthermore, these polysaccharides often present high liquid absorbing capacity and modify strongly the viscosity of the digestion medium. Therefore, structure and functional properties must be carefully considered when applying standardized in vitro digestion models to these structurally complex food systems.

The objective of this study was to assess protein and amino acid digestibility in different edible seaweeds using the harmonized INFOGEST static in vitro digestion protocol, with adaptations to account for structural and functional particularities of seaweeds, including the use of a tailored protein-free substrate. Green (Ulva lacinulata), red (Porphyra dioica), and brown (Saccharina latissima) species were selected to represent the biochemical and structural diversity of macroalgal cell walls. After simulated gastrointestinal digestion, the extent of protein hydrolysis was quantified, and the digestible indispensable amino acid ratio (DIAAR) and digestible indispensable amino acid score (DIAAS) were calculated. Digestion products were further analyzed in terms of structural and physicochemical features using confocal laser scanning microscopy, FT-IR spectroscopy and transmission electron microscopy, providing deeper insight into matrix disintegration throughout the digestion process.

All species exhibited comparable amino acid compositions, with indispensable amino acids accounting for approximately one-third of total amino acids. However, pronounced interspecies differences in protein digestibility were observed. In particular, Porphyra dioica showed the highest digestibility, indicating greater protein accessibility, whereas Ulva lacinulata and Saccharina latissima presented substantially lower values, likely due to their more recalcitrant, polysaccharide-rich cell wall architectures.

These results demonstrate that, despite comparable amino acid profiles, the hierarchical structural organization of the cell wall matrix is a key determinant of protein bioaccessibility in seaweeds. The study also highlights the importance of critically applying and, when required, adapting the standardized INFOGEST digestion protocol

for alternative food matrices, and supports the development of targeted processing strategies to enhance the nutritional quality of seaweed-derived proteins.

Keywords

marine seaweeds; INFOGEST; in vitro digestion; protein bioaccessibility; DIAAS; matrix structure

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECTS OF COOKING WITH OLIVE OIL ON INTESTINAL PARTICLE SIZE DISTRIBUTION AND CAROTENOID RELEASE OF ORANGE-FLESHED SWEET POTATO DURING INFOGEST DIGESTION

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Abstract

Orange-fleshed sweet potatoes (OFSP) are a relevant dietary source of carotenoids, bioactive compounds with recognized health benefits. However, their nutritional potential depends on their release from the food matrix during digestion and subsequent incorporation into mixed micelles prior to absorption (Failla et al., 2009). As OFSP are mainly consumed cooked and lipid addition can enhance carotenoid solubilization, understanding how oil influences intestinal particle size distribution and carotenoid behavior is of considerable interest (Bengtsson et al., 2010; Salvia-Trujillo et al., 2017). Therefore, this study aimed to evaluate the effect of olive oil (OO) on intestinal particle size distribution and carotenoid profiles in cooked OFSP.

Cooked OFSP samples, with and without OO, were subjected to the standardized static INFOGEST in vitro digestion protocol (Brodkorb et al., 2019). Particle size distribution in the intestinal phase was determined by laser diffraction, and carotenoid profiles were analyzed by UPLC-DAD before and after digestion.

Cooking markedly reduced all-trans-β-carotene content (from 96.25 ± 3.3 µg/g raw OFSP to 27.65 ± 1.0 µg/g cooked OFSP) and led to the formation of 13-cis-β-carotene (3.5 ± 0.1 µg/g), which was not detected in raw samples. The addition of OO to cooked OFSP increased all-trans-β-carotene (108.35 ± 2.1 µg/g) and 13-cis-β-carotene (9.33 ± 0.4 µg/g) contents compared to oil-free samples (p less than 0.001). β-cryptoxanthin content decreased after cooking (from 5.30 ± 0.1 µg/g raw OFSP to 1.40 ± 0.0 µg/g cooked OFSP). Following in vitro digestion, OFSP cooked without oil exhibited a monomodal intestinal particle size distribution (d50 = 0.790 µm), whereas samples containing OO showed a multimodal distribution, including a distinct fraction of larger particles (d90 = 27.22 µm). OFSP cooked with OO showed higher all-trans-β-carotene concentrations in the intestinal bioaccessible fraction (5.29 ± 0.78 µg/g) compared to samples cooked without oil (3.25 ± 0.25 µg/g, p less than 0.05). β-Cryptoxanthin was not detected in the bioaccessible fraction of samples cooked without oil, whereas it was present in samples cooked with OO (6.54 ± 0.10 µg/100 g). Moreover, all-trans-β-carotene concentrations in the gastric phase of OFSP cooked with OO were markedly lower than in the corresponding intestinal phase.

The addition of OO modifies intestinal particle size distribution in cooked OFSP and may be associated with differences in β-carotene release during in vitro digestion compared to OFSP without oil. These findings highlight the importance of food processing and lipid presence in modulating digestion-related characteristics relevant to carotenoid bioaccessibility. Future studies will further explore how oils with different lipid composition influence both intestinal particle size distribution and carotenoid bioaccessibility.

Keywords

INFOGEST, Orange-fleshed sweet potato, Olive oil, Carotenoids, Bioaccessibility, Particle size distribution

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

FARM-TO-GUT: CULTIVAR VARIATION MODULATES STARCH AND PROTEIN DIGESTION IN OATS.

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Abstract

Background & Aim: Oats are renowned for EFSA-approved cardiovascular and glycaemic benefits via their soluble fibre β-glucan, yet most consumers fall short of these targets with a typical daily porridge serving. For commercial oats to have a positive health impact, up to three daily servings are often required, a gap amplified by breeding programs prioritising agronomic yield over nutritional quality. Overlooking the vast compositional diversity across cultivars limits consumer choice and risks the crop's functional potential not being adequately highlighted to inform commercial oat breeding initiatives. This study aims to contribute to the closing of this gap by profiling the nutritional composition of 95 Irish-grown oat cultivars and evaluating the digestion behaviour of a representative subset to explore the links between compositional diversity and digestive profile.

Method: Nutritional profiles, including protein, starch, fat, ash, β-glucan, and fibre-rich fractions, were quantified across 95 cultivars. Six cultivars with divergent profiles were selected and digested in vitro as porridge using the INFOGEST semi-dynamic protocol. Digesta were collected throughout the oral, gastric, and intestinal phases to determine carbohydrate and protein release and hydrolysis. Structural changes were assessed at each stage using light, scanning electron (SEM), and confocal laser scanning microscopy (CLSM).

Key findings: Cultivars showed wide nutritional variation: protein 11.5-22.9%, starch 47.2-65.3%, fat 2.4-10.0%, ash 1.4-2.9%, β-glucan 2.8-6.6%, and fibre-rich fraction 8.5-26.9% (dry matter basis). Carbohydrate release peaked within the first 15 minutes of the gastric phase, with a second peak at the end of the intestinal phase for some cultivars. Two distinct starch release patterns were observed among cultivars, whereas no distinction was evident for released oligosaccharides. Protein release and hydrolysis rates also varied by cultivar, mirroring compositional differences and suggesting variety-specific matrix breakdown. Microscopy confirmed that flake structure is largely maintained during porridge preparation, while intact plant cells encapsulating starch granules remained at the end of in vitro digestion.

Conclusions: The large compositional variability across cultivars indicates that nutrient density, and thus potential health benefits, vary substantially between varieties. Consumers would need to eat roughly twice as much of a low-protein, low-β-glucan oat to match the intake provided by a nutrient-dense cultivar. These compositional differences also modulate starch and protein digestion kinetics. The findings underscore breeding opportunities for health-optimised cultivars beyond yield, guiding selection for products that may enhance nutrient bioavailability, metabolic outcomes and added premium potential for farmers. The impact of these findings on postprandial glycaemic responses will be investigated in a human study.

Keywords

cereals, starch, Avena sativa L., glycaemic response, functional breeding, in vitro digestion

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

SUSTAINED EFFECTS OF ADDING LOW ACYL GELLAN GUM TO WHITE RICE ON GLYCEMIC AND APPETITIVE RESPONSES: A RANDOMISED, CONTROLLED, CROSS-OVER STUDY

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Abstract

Objective

Starchy foods are main sources of carbohydrate, and their digestibility affects the postprandial metabolic responses, which in the long term may be associated with an increased risk of type 2 diabetes. We have shown that addition of hydrocolloid low acyl gellan gum (LAGG) during the cooking process of jasmine rice reduced its starch digestibility in vitro [1]. We have also shown that it reduced in vivo postprandial glycemic responses after an acute feeding intervention in healthy volunteers (HVs) [2].

The aim of this study was to determine if the effect of adding LAGG to white rice on glycaemic responses was maintained over a sustained feeding intervention.

Methods

Eight HVs participated in a randomised, controlled, crossover study. On the first study day they consumed a 232kcal dish of jasmine rice cooked with (rice+LAGG) or without (rice control) 3% w/dry rice weight LAGG. Fingerprick blood glucose was measured, and appetite questionnaires were collected for 3.5h. The HVs then took home a rice cooker and pre-dosed pots of rice with/without LAGG to sustain the rice intervention once daily for 7 days. They then returned to the study centre to repeat the test protocol used in the first day (clinicaltrials.gov identifier NCT05713227).

Results

All participants completed the protocol. The first, acute rice+LAGG intervention reduced blood glucose iAUC2h by 21% compared to rice control (from 159±35mmol/L·min to 126±20mmol/L·min, P=0.1812). One week of sustained intervention maintained the reduction in blood glucose iAUC2h for rice+LAGG (124±19mmol/L·min), but remarkably rice control increased it to 188±38mmol/L·min (P=0.1209). Two-way RM-ANOVA analysis over the whole time course showed a significant interaction of rice meal type x time both on the first study day (P=0.0023) and after 1 week intervention (P=0.0010). Following this, Sidak's post-hoc test showed significant difference between blood glucose means for rice+LAGG and rice control on the first study day, at time T=45 min (P=0.0014) and T=60 min (P=0.0440). Similarly, significant differences were found after 1 week of sustained intervention at time T=45min, P=0.0039 and T=60 min, P=0.0021. Peak blood glucose for rice control rose to 8.2±0.5mmol/L after 1 week of intervention and the mean value was 1.2 mmol/L higher than that for the corresponding value for rice+LAGG, P=0.0219. Differences in appetite scores were not statistically significant.

Conclusion

The addition of LAGG to jasmine rice cooking was effective in reducing glycemic responses and this response was sustained after 1 week of intervention with a 34% reduction in blood glucose iAUC2h compared to rice control. This is a simple, tasteless and inexpensive modification of the rice cooking process. It could provide an effective intervention to help reducing glycemic responses following white rice consumption.

References:

1. S Muttakin et al. Food Res Int 174, 2023
2. NA Alshammari et al. Food Res Int 196, 2024

Keywords

Starch digestibility, hydrocolloids, blood glucose

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

COOKING-INDUCED STRUCTURAL CHANGES MODULATE PROTEIN DIGESTIBILITY IN RED SEAWEED GRACILARIA BURSA-PASTORIS

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Abstract

Seaweeds are increasingly explored as sustainable food resources because of their low environmental footprint and high nutritional potential. Among them, red seaweeds represent promising alternative protein sources, yet their contribution to human nutrition remains limited by poor protein bioaccessibility. Previous studies suggest that the complex hierarchical architecture of seaweed cell walls can hinder enzymatic access and reduce protein digestibility, but how food processing modulates this barrier remains largely unknown. Since seaweeds are typically consumed after cooking, understanding the impact of culinary treatments on their nutritional performance is essential. However, the relationship between cooking-induced structural transformations and protein digestibility in seaweeds has not been established.

This study aimed to investigate the structural changes induced by three different cooking methods on the red seaweed Gracilaria bursa-pastoris and evaluated their impact on nutritional quality and protein digestibility. For this purpose, steaming, boiling, and sous-vide cooking were applied to the seaweed biomass, followed by compositional and multiscale structural characterisation. Protein digestibility was assessed using the harmonized INFOGEST in vitro gastrointestinal digestion method. The results demonstrate that culinary processing induced minor changes in macronutrient composition, preserving the essential amino acid profile (>41% of total amino acids). In contrast, it induced marked structural rearrangements, which translated into differences in protein digestibility. Moderate cooking treatments weakened structural carbohydrates, reduced cell wall crystallinity, and promoted agar redistribution, leading to enhanced in vitro protein digestibility (≈72%) compared with the native seaweed (≈62%). Conversely, high-temperature steaming preserved rigid cell wall organisation by increasing the fraction of crystalline carbohydrates, which resulted in reduced digestibility (≈38%). Histidine was the limiting amino acid in all samples, and the DIAAS of sous-vide cooked seaweed (96.40) was comparable to values reported for other plant proteins. These findings reveal how cooking parameters govern nutrient bioaccessibility in seaweeds through structural remodeling, establishing a framework for designing seaweed-based foods with optimized nutritional performance and unlocked protein potential.

Keywords

seaweed, alternative proteins, structure, in vitro digestibility

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

ASSESSING THE NUTRITIONAL QUALITY AND PROTEIN DIGESTIBILITY OF PLEUROTUS OSTREATUS FUNGAL EXTRACTS

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Abstract

The need for sustainable, healthy and accessible protein sources has driven interest in edible fungi as alternative ingredients for human nutrition. The edible fungus Pleurotus ostreatus constitutes a particularly attractive protein alternative due to its favourable amino acid profile and high protein content, as well as the presence of health-promoting -glucans. However, the nutritional quality of fungal proteins strongly depends on extraction efficiency, compositional changes, and digestibility, which remain insufficiently characterized.

Therefore, the aim of this work was to characterize the protein quality of Pleurotus ostreatus biomass and derived by-products, and to assess how different extraction strategies influence protein recovery, amino acid composition, in vitro digestibility and protein nutritional quality.

Protein extracts were obtained from the whole fungal biomass and the discarded stems using optimized extraction protocols, including aqueous extraction and pH-shifting. Extracts were characterized in terms of gross composition, as well as a more exhaustive characterization of the protein and polysaccharide fractions. Protein digestibility was also determined using the standardized INFOGEST in vitro gastrointestinal digestion protocol, and nutritional quality was assessed through calculation of the Digestible Indispensable Amino Acid Score (DIAAS), according to FAO guidelines. Furthermore, the molecular profile and multi-scale structure of the digestion products were also analysed to evaluate structural changes during digestion.

The results show that both extraction techniques, particularly the pH-shifting protocol, effectively increase the protein concentration (40–50%) compared to the initial biomass (10–20%), as well as the essential amino acid content by up to 50%. High in vitro digestibility values (90–95%) were observed for both the whole fungal biomass and the respective extracts; however, the stems presented a lower digestibility (57%), which was then increased in the extracts (85-90%). Notably, DIAAS values increased from approximately 50% in the initial biomass to up to 90% in the pH-shifting extracts, indicating a substantial improvement in protein nutritional quality.

These findings demonstrate the value-adding of fungal biomass as sustainable ingredient for the development of functional foods and show that Pleurotus ostreatus represents a viable source of high-quality, and highly digestible protein.

Keywords

Alternative proteins, Fungal biomass, Nutritional quality, Digestion, DIAAS.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IMPACT OF PUFFING TREATMENT ON THE IN VITRO DIGESTIVE BEHAVIOR OF PROTEINS IN THE DEFATTED SOYBEAN POWDER

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Abstract

Changes in the protein structure and in vitro digestive behavior were investigated following puffing treatment of defatted soybean powder. Defatted soybean meal was mixed with rice and placed in the chamber of a gun puffing machine. Rice was incorporated as a thermal-stabilizing filler to reduce localized overheating and prevent premature carbonization of the soybean meal during pressurization. The chamber temperature was raised to approximately 230 °C, and internal pressure was adjusted to 200, 300, or 400 kPa. Once the target pressure was reached, the chamber door was rapidly opened to induce instantaneous decompression and expansion. Untreated soybean meal served as the atmospheric-pressure control. Both control and puffed samples were ground using a laboratory mill and stored in airtight polyethylene bags at room temperature until further analysis.

Protein structure was evaluated using the protein extract of soybean powder in the phosphate buffer. Intrinsic tryptophan fluorescence intensity decreased after puffing treatment, accompanied by a red shift in the wavelength of maximum emission. Surface hydrophobicity increased with rising pressure, suggesting unfolding and exposure of hydrophobic regions. In addition, the content of reactive sulfhydryl groups increased. Secondary structure analysis using circular dichroism spectroscopy revealed a decrease in relative α-helix content and a corresponding increase in β-sheet content as pressure increased. These changes indicate that pressure and high temperature induced denaturation and structural rearrangement of soybean proteins. Protein solubility was markedly reduced in puffed soybean powders compared with the untreated control. SDS-PAGE analysis confirmed the inactivation and structural disruption of major soybean proteins, including β-conglycinin (α′, α, and β subunits) and glycinin (acidic and basic subunits).

Protein digestibility was evaluated using the INFOGEST in vitro digestion protocol. During the gastric phase, all puffed samples exhibited a decrease in α-amino group content compared with the untreated control, indicating limited proteolysis. However, after the intestinal phase, puffed samples showed higher α-amino group content than the untreated sample, suggesting enhanced proteolytic susceptibility during intestinal digestion. Confocal laser scanning microscopy revealed pronounced particle aggregation in treated samples in the acidic gastric phase. In contrast, during the intestinal phase, disruption of the fibrous matrix structure was observed, which likely facilitated enzyme access and improved protein hydrolysis.

Consequently, puffing treatment of defatted soybean powder induced significant denaturation of protein structures, reduced solubility, and disrupted the fibrous matrix. These structural modifications ultimately enhanced intestinal protein digestibility by improving susceptibility to proteolytic enzymes.

Keywords

soybean, puffing, in vitro digestion, protein digestibility

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

NUTRITIONAL QUALITY OF FUNGAL MYCELIUM AND ITS POTENTIAL AS A WHOLE-CUT MEAT ALTERNATIVE

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Abstract

Background: Fungal mycelium can be manipulated through innovative processing to produce whole-cut meat alternatives. The aim of this project was to nutritionally evaluate a mycelium-based steak alternative in comparison to plant- and beef equivalents. The impact of heat treatment required for RNA reduction in mycelial biomass on protein quality was also investigated.

Methods: A recently established INFOGEST gastro-intestinal digestion workflow was used to determine amino acid digestibility and to calculate protein quality using the digestible indispensable amino acid scores (DIAAS). Mycelial biomass (pre- and post-heat treatment) and mycelial steak were benchmarked against selected plant- and beef steak equivalents for protein, amino acid (LC-MS/MS) and mineral content (ICP-MS) as well as in vitro amino acid digestibility and in vitro DIAAS.

Results: Protein content was calculated using a nitrogen-to-protein conversion factor (NCF) of 6.25 and showed good agreement with total amino acid data for plant and beef samples. In mycelial biomass, the standard NCF overestimated protein content, reflecting the contribution of non-protein nitrogen. A revised NCF was therefore derived for mycelium, yielding values in the range of 4.3 - 4.8. Amino acid digestibility of raw and cooked samples was comparable for plant and beef, while mycelial biomass showed matrix-dependent variation between raw and cooked formats (93% and 75%, respectively). Corresponding DIAAS estimates for cooked products were 45% (plant), 70% (beef), and 90% (mycelial steak). Iron contents were comparable across all samples.

Conclusions: Fungal mycelium is a promising source of essential amino acids and minerals. While heat treatment influenced amino acid digestibility, the excellent amino acid profile compensated for this to maintain a high protein quality score. Future studies should focus on optimising heat treatments for RNA reduction and evaluating the bioavailability of minerals.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

INNOVATIVE FOLLOW-ON FORMULA INCORPORATING OIL BODIES FROM OILSEEDS: IN VITRO DYNAMIC DIGESTION AT A 6-MONTH-OLD INFANT STAGE

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Abstract

Refined vegetable oils are widely used in follow-on formula (FOF) as the primary lipid source. However, their use raises concerns regarding sustainability, particularly due to organic solvent-based extraction processes. Moreover, their nutritional adequacy is questioned, as the resulting emulsion structures are not biomimetic of human milk. In contrast, oil bodies (OB) from oilseeds have gained increasing interest for their techno-functional and nutritional advantages. They provide a naturally encapsulated lipid structure stabilized by a monolayer of proteins and phospholipids.

In this study, innovative FOF incorporating OB as an alternative to refined vegetable oils, in combination with dairy cream, was developed. The aim was to evaluate the digestive behavior and the impact on potential allerginicity. One innovative FOF combining vegetable cream with OB from oilseeds and dairy cream (VC/DC), and two control FOF based on refined vegetable oils alone (RVO) or blended with dairy cream (RVO/DC), were produced. Each FOF was spray-dried (inlet/outlet temperatures of 180/80°C) and subjected to dynamic in vitro digestion (DIDGI) adapted to the digestive conditions of a 6-month-old infant. Digestas were sampled at regular intervals and analyzed for structural evolution using confocal microscopy and laser light scattering. Digestion kinetics were evaluated by monitoring protein (SDS-PAGE, OPA, protein digestibility) and lipid hydrolysis (gas chromatography, TLC). Digestion-resistant proteins were investigated by high-resolution mass spectrometry (HRMS).

In vitro digestion showed marked structural differences during the gastric phase among FOF. The VC/DC formula exhibited strong initial aggregation and limited changes in particle size. In contrast, RVO formula showed pH-induced aggregation followed by enzymatic size reduction. Protein digestibility was not significantly different ($p > 0.05$) among the three FOF and reached an average of 83.4 ± 1.6%. The same observation was made for the degree of protein hydrolysis, with no significant difference and a final average degree of hydrolysis of 53.8 ± 0.9%. However, digestion-resistant proteins around 20 kDa were detected in the VC/DC formula by SDS-PAGE. HRMS analysis identifies an 11S seed storage protein among the undigested proteins, which is recognized to have an allergenic potential in other vegetable species. Further investigations are underway to explore the latter issue more in-depth.

Lipid digestion showed clear differences in kinetics between FOF. Hydrolysis was slower during the gastric phase but faster in early intestinal digestion for FOF containing OB and dairy cream (VC/DC and RVO/DC) compared to the RVO formula. This is likely due to differences in aggregation and enzyme–interface interactions.

OB integration into FOF appears to be a promising strategy for developing more sustainable and biomimetic products. Further studies should address allergenicity risks associated with OB components.

Keywords

Oil body, follow-on formula, allergenicity, lipid, protein

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

CAN LUPINE ALLERGENICITY BE REDUCED? EFFECTS OF PROCESSING AND GASTROINTESTINAL DIGESTION ON Γ-CONGLUTIN IMMUNOREACTIVITY

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Abstract

Lupine is a legume widely consumed as a functional food due to its high nutritional value and favorable technological properties. However, lupine intake may trigger adverse immunological reactions, representing a significant health risk for sensitized allergic individuals [1]. This study aimed to evaluate the impact of food processing combined with simulated gastrointestinal (GI) digestion on the immunoreactivity of lupine γ-conglutin.
Wheat pasta model foods containing 35% lupine flour from Lupinus albus, L. luteus, and L. angustifolius were prepared and subjected to boiling to simulate domestic food processing. Proteins were extracted with Tris–HCl buffer (100 mM, pH 8.0), quantified by UV-Vis spectrophotometry and their profiles characterized by SDS-PAGE. Simulated GI digestion of thermally treated pasta was carried out using the harmonized INFOGEST digestion protocol (version 2.0) using individual enzymes (trypsin/chymotrypsin) in the intestinal phase. The IgG-binding capacity of γ-conglutin was evaluated by immunoblotting under non-reducing conditions and by indirect ELISA using anti-γ-conglutin IgG.
The results indicate that boiling affected the immunoreactivity of γ-conglutin in a species-dependent manner. In fact, processing led to an IgG-binding reduction of 40% in L. luteus, an increase of 5000% in L. albus, while no effect was observed for L. angustifolius. Simulated GI digestion caused extensive degradation of protein structures and immunoreactivity reduction, especially in L. luteus and L. angustifolius (99% and 98%, respectively), whereas in L. albus the gastric phase did not affect the IgG binding of γ-conglutin. A more pronounced effect was detected during the intestinal phase, leading to a strong reduction of IgG affinity towards γ-conglutin and its potential presentation to immunocompetent cells.
Overall, these findings provide valuable insights into how food processing and GI digestion influence the immunoactivity of lupine γ-conglutin and may support the development of food formulations with reduced allergenic potential.

References:

[1] Villa C et al 2020, 10.1111/1541-4337.12646

Keywords

Lupine edible species, γ-conglutin allergens, In vitro digestion, Trypsin/chymotrypsin, Thermal processing

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

COMPOUND STRUCTURE AND MATRIX GOVERN INTESTINAL FATE OF TOMATO PHENOLICS

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Abstract

Tomatoes are a significant dietary source of phenolic compounds, mainly concentrated in the skin and seeds. These fractions are commonly discarded as byproducts, known as pomace, during tomato sauce production. Incorporation of pomace into sauce has been proposed as a strategy to increase phenolic content and enhance nutritional value. However, the health relevance of such enrichment depends not only on phenolic concentration but also on bioaccessibility, which is determined by compound structure, food matrix characteristics, and processing conditions.

This study investigated the effect of tomato pomace enrichment on the digestion behaviour and bioaccessibility of individual phenolic compounds in tomato sauce. Conventional sauce was compared with pomace-enriched sauce, and the enriched sauce was additionally evaluated in a meal context through co-digestion with boiled rice. All samples were subjected to static in vitro digestion following the INFOGEST 2.0 protocol. Phenolics were profiled in soluble and insoluble fractions across oral, gastric and intestinal phases using UHPLC-ESI-QTOF, while quantification and bioaccessibility were determined by UHPLC-ESI-QTRAP. This combined approach enabled detailed monitoring of compound-specific transformations and partitioning during digestion.

Pomace enrichment did not significantly increase total phenolic concentration or overall bioaccessibility compared with conventional sauce (linear mixed model, $p > 0.05$), although responses varied according to compound structure. This may be partly explained by the preserved microstructure of the pomace, which may retain phenolics within cell wall matrices and influence their release during digestion. Digestion induced marked compound-specific changes, including degradation of complex caffeoylquinic acids, formation of low molecular weight derivatives, and redistribution of phenolics between soluble and insoluble fractions. Co-digestion with rice generally led to lower soluble concentrations of phenolics, reflecting matrix interactions. An exception was quercetin-3-O-glucoside, which showed higher soluble levels in the meal context; statistical analysis for this compound indicated that differences were primarily driven by matrix composition (p 0.0001), with no significant interaction between tomato formulation and digestion phase ($p = 0.1$). For the other phenolic compounds, the interaction between formulation and digestion phase was significant.

Overall, these findings indicate that phenolic bioaccessibility from tomato products is determined by compound-specific properties, food matrix interactions, and structural characteristics influencing phenolic release during digestion.

Keywords

INFOGEST, Structure, CQA, Quercetin, Hydrolysis, Co-digestion, Soluble, Insoluble, Intestinal

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

CNC-INDUCED STRUCTURAL CHANGES ENHANCE CHICKPEA PROTEIN DIGESTIBILITY THROUGH CONCENTRATION-DEPENDENT MODULATION

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Abstract

Although chickpea proteins are important for nutrition, their digestibility is often limited by their structural compactness and aggregation. The potential of cellulose nanocrystals (CNC) to improve protein bioaccessibility through nanoscale interactions that promote unfolding and enhance enzymatic susceptibility remains largely unexplored. In this study, we systematically investigated the interactions between chickpea protein isolate (CPI) and CNC and characterized the conformational properties and secondary structures of CPI and CPI–CNC complexes after in vitro digestion to understand the role of CNC concentration in protein digestibility. Comprehensive physicochemical characterization included particle size distribution, surface charge, hydrophobicity, sulfhydryl group content, aggregation, emulsifying activity, protein solubility, and rheological properties. Protein hydrolysis was determined using the degree of hydrolysis (OPA) and electrophoretic profiling. The structural changes were further elucidated using Fourier-transform infrared (FTIR) spectroscopy. CNC incorporation induced concentration-dependent structural reorganization of chickpea proteins prior to digestion, increasing the aggregation index (≈33→87%), particle size (≈220→780 nm), viscosity, surface hydrophobicity, and free sulfhydryl content (≈15→44 μmol/g), indicating partial unfolding and the formation of protein–CNC networks via electrostatic and hydrogen-bond interactions. Secondary structure analysis consistently revealed a marked increase in pseudo β-sheet content (≈15→42–45%) and α-helix proportion, accompanied by a reduction in antiparallel β-sheet and β-turn structures, reflecting protein rearrangement toward more aggregated and intermolecularly stabilized conformations. During gastric digestion, protonation and pepsinolysis reduced the ζ-potential magnitude, aggregation, viscosity, hydrophobicity, and solubility, evidencing the disruption of supramolecular assemblies. In the intestinal phase, proteolytic fragmentation restored the negative surface charge (~−30 mV), decreased particle size, and enhanced solubility (up to ~62%), promoting dispersion. Notably, moderate CNC incorporation (1%) maximized hydrolysis (~101%), whereas excessive CNC (3%) reduced digestibility (~85%) due to steric shielding and dense protein–CNC complexation. These results demonstrate a concentration-dependent structure–digestibility relationship governed by CNC-mediated modulation of protein packing density and enzymatic accessibility.

Keywords

chickpea proteins, CNC, cellulose nanocrystals, digestibility, protein solubility, aggregation

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

INTESTINAL TRANSPORT AND CELLULAR RESPONSES TO ACHETA DOMESTICUS DERIVED PEPTIDES IN CACO-2 CELLS

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Abstract

Insects are increasingly recognized as a sustainable and nutrient-dense alternative to conventional livestock, offering advantages such as lower greenhouse gas emissions, efficient feed conversion, reduced land and water use, and minimal infrastructure requirements. They provide high-quality protein, with amino acid profiles comparable to common meats. Additionally, insects have long been associated with health-promoting properties, and recent studies attribute many of them to bioactive peptides released during gastrointestinal (GI) digestion. Understanding these bioactive components is essential for enhancing the value of insect-based foods and supporting their acceptance in Western markets. Acheta domesticus (house cricket), one of the EU-approved species for human consumption, is particularly promising due to its simple farming requirements, sensory characteristics, and nutritional profile. This study intended to explore the bioactive properties of peptides from A. domesticus by assessing their in vitro intestinal absorption and ability to modulate key proteins involved in diabetes and hypertension.

A molecular docking protocol was used to select six peptides (DVW, AVQPCF, QIVW, CAIAW, PIVCF, and IIIGW), which were obtained from simulated GI digestion of three A. domesticus proteins (acyl-CoA Delta12-desaturase, acyl-CoA Delta-9 desaturase and diuretic hormone receptor). The six peptides were evaluated for their transepithelial transport, cytotoxicity, and effects on epithelial barrier integrity using a Caco-2 model differentiated in permeable inserts. Furthermore, their capacity to modulate the gene expression of dipeptidyl peptidase-4 (DPP-4) and sodium–glucose cotransporter 1 (SGLT1), both associated with glucose homeostasis and diabetes, as well as somatic angiotensin-converting enzyme (sACE) and ACE2, which are involved in blood pressure regulation and hypertension, was also evaluated [1].

The results showed that of the six peptides examined, only DVW was able to cross intact the intestinal barrier, although at low levels and with some impact on epithelial integrity. The remaining peptides underwent extensive hydrolysis, likely driven by brush border enzymes such as DPP-4, APN, AP1, APW and sACE, thus limiting their bioavailability. Despite the limited transport and high degradation of PIVCF, both DVW and PIVCF were able to modulate SGLT1 expression, suggesting a potential role in regulating glucose absorption and warranting further investigation. None of the peptides was cytotoxic up to 2 mM. Overall, these results provide new insights into the bioactivity of insect-derived peptides and their relevance to the development of sustainable protein ingredients. Future work should focus on improving peptide stability and delivery, including encapsulation strategies, and validating these effects in vivo.

References

[1] Teixeira, C.S.S. et al. (2026). Int. J. Biol. Macrom. 347, 150750. DOI: 10.1016/j.ijbiomac.2026.150750

Keywords

House cricket, GI digestion, bioactive peptides, Caco-2 monolayer, intestinal absorption, protein expression.

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

THE DIGESTIVE FATE OF LACTIPLANTIBACILLUS PLANTARUM AND BIOACTIVE COMPOUNDS IN SYNBIOTIC BEVERAGES: IMPACT OF POMEGRANATE POMACE AND FOOD MATRIX CONSTITUTI

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Abstract

Probiotic survival mechanisms and bioactive compound stability during gastrointestinal digestion are crucial for functional food design. The aim of this study was to evaluate the impact of 40-day refrigerated storage and in vitro gastrointestinal digestion on the Lactiplantibacillus plantarum (DSM 25710) viability and the antioxidant capacity of synbiotic beverages based on dairy (whole milk) and plant-derived (spelt) matrices enriched with pomegranate pomace (PL). Beverages were formulated using a 50:50 ratio of apple juice and pulp, with either whole milk (WJ) or spelt (SJ) added. The formulations were enriched with 0.7% pomegranate pomace and inoculated with approximately 7.5 log CFU/mL of L. plantarum. The samples subsequently engaged in a 40-day storage at 4 °C. The INFOGEST 2.0 standardised in vitro digestion protocol was applied to samples before and after the storage. Probiotic viability was determined by the plate count method on MRS agar, while antioxidant presence and capacity were assessed using Folin-Ciocalteu, TEAC, and ORAC assays. Physico-chemical shifts (pH and CIELAB colourimetry) were also monitored. Our findings demonstrate that both matrices effectively support the delivery of L. plantarum, maintaining viability above the recommended probiotic threshold (6 log CFU/mL) even after the intestinal phase. Notably, integrating PL into the milk matrix (WJPL) caused a significant reduction in post-storage pH (to ~4.2; p 0.05), indicating a prebiotic-like effect that stimulated the metabolic activity of the strain. PP enrichment increased pre-GID TPC by 42–169% and pre-GID TEAC by up to 18-fold across both matrices (p 0.05). After simulated digestion, TPC increased by 23–60% in PP-enriched WJ formulations (highest: 5,921 mg GAE/L, WJPL T0), while in SJ, post-GID TPC showed a matrix-dependent response, with a marked increase at T40 (up to 4,639 mg GAE/L, SJP) but a reduction at T0. TEAC increased by 4- to 19-fold (highest: 23,216 µmol TE/L, WJP T40) and ORAC by 2.2- to 6.3-fold (highest: 127,339 µmol TE/L, WJPL T40) across both matrices (p 0.05). PP addition caused significant colorimetric shifts (Δb* +6.6–11.1; ΔE* +3.2–8.7; p 0.05) yet created a superior antioxidant environment stable throughout digestion. Key findings of this research reveal that food matrix composition and pomegranate enrichment significantly influence L. plantarum viability and antioxidant stability during gastrointestinal digestion. These results provide valuable insights for developing shelf-stable, synbiotic beverages that can deliver bioaccessible antioxidant compounds and viable probiotics to the lower gastrointestinal tract.
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Keywords

Probiotics, bioaccessibility, Lactiplantibacillus plantarum, INFOGEST, pomegranate pomace, food matrix interaction

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

PROTEIN DIGESTIBILITY OF HYBRID LEGUME-SEAWEED FOOD PROTOTYPES

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Abstract

The transition towards more sustainable protein sources has increased the interest in seaweeds as alternative non-animal protein ingredients. Green seaweeds of the genus Ulva contain relatively high protein content (20-25%), balanced amino acid profile and significant levels of dietary fiber, minerals, and bioactive compounds. Notably, Ulva lacinulata shows an amino acid composition that can complement legume proteins, potentially improving overall protein quality in combined in food products. Moreover, partial substitution of fat-rich ingredients with seaweed biomass may reduce total caloric content and enhance nutritional value. However, the incorporation of seaweed into legume-based matrices may affect protein accessibility and digestibility due to complex polysaccharides and cell wall components that influence product structure and funcionality. Therefore, evaluating the impact of seaweed addition on protein digestibility in real food systems is essential for their successful application as ingredients.

The aim of this study was to develop legume-based food prototypes enriched with U. lacinulata (with loadings of 1–7% with regards to the total prototype weight) and to evaluate the impact on the nutritional composition, structure and in vitro protein digestibility. Two different widely consumed legume-based food products were produced: tempeh (soybean-based, fermented product) and hummus (chickpea-based, non-fermented), allowing the comparison between structurally and biochemically distinct systems. To enhance dispersion and functionality within the food matrices, the seaweed biomass was subjected to mechanical pre-treatments, including ultra-turrax homogenization and ultrasound-assisted processing. These treatments reduced particle size, disrupted cell wall structures, and increased protein solubility, improving bioaccessibility during digestion as compared to the native seaweed.

Subsequently, protein digestibility was assessed using the Infogest static in vitro gastrointestinal digestion protocol, simulating oral, gastric, and intestinal phases. Incorporation of the seaweed at low levels (1% and 3%) did not significantly affect protein digestibility in either tempeh or hummus compared to their controls, indicating that moderate seaweed enrichment does not impair protein bioaccessibility. In contrast, higher i levels (5–7%) led to evident changes in digestibility, suggesting the influence of seaweed-derived polysaccharides and matrix interactions that may limit enzyme diffusion or promote protein–fiber associations. Overall, low-percentage incorporation of U. lacinulata enables nutritional diversification, amino acid complementation, and potential caloric reduction in legume-based foods without compromising protein digestibility. These findings support the strategic use of green seaweed biomass as a sustainable functional ingredient and provide insight into matrix-dependent effects relevant to next-generation plant-based protein pro

Keywords

Seaweeds; alternative proteins; novel foods; nutritional quality; food structure

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

BEYOND IN VITRO DIGESTIBILITY: WHAT PROTEOMICS AND METABOLOMICS ADD TO OUR UNDERSTANDING OF PROCESSED PLANT PROTEINS

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Abstract

Plant proteins generally have lower digestibility and a less complete indispensable amino acid (IAA) profile than animal proteins, and are therefore often considered to have poorer nutritional quality. The food matrix of plant protein sources, including starch, fibers, and antinutrients such as protease inhibitors, can limit protein digestibility. Processing can improve protein digestion and amino acid bioaccessibility, but high temperatures may induce structural changes and reactions such as oxidation and Maillard reactions, which can reduce IAA bioavailability. These modifications are often overlooked on assessments of in vitro protein digestibility. To better understand how processing affects plant proteins, a deeper analysis of protein structural changes and amino acid modifications is needed. In this work, proteomics and metabolomics were applied to examine how different processing stages of plant protein-based foods influence protein and metabolite profiles, and whether these changes are reflected on protein digestibility outcomes. Peas (Pisum sativum L.) were selected as a model legume due to their increasing relevance in Europe, low allergenicity, sustainability, and high protein content. Samples with distinct food matrices and antinutrient levels were included: pea protein ingredients produced by wet fractionation (protein isolate, PI) and dry fractionation (protein-rich fraction, PRF), as well as heat-treated ingredients and food models. In the first stage, we assessed the effect of heating (90°C and 140°C, 20 min) on protein profiles (LC–MS/MS) and in vitro digestibility of PI and PRF using the INFOGEST method. In the second stage, PI and PRF were processed at 140°C under mechanical shear and incorporated into food models with identical macronutrient composition, which were cooked at 200°C and further digested (INFOGEST). Peptide and metabolite profiles were analyzed post digestion. Heating PI at 140°C caused more pronounced methionine oxidation than in PRF, likely because PI was already oxidized before treatment. As expected, PRF contained higher levels of trypsin inhibitors, and heating reduced their activity by up to 87%. Correspondingly, PRF heated at 140°C showed a 55% increase in intestinal-phase digestibility compared to the non-heated sample, while PI showed no significant change. After 4 h digestion, however, both ingredients showed similar overall digestibility, masking processing effects. These differences became evident only when examining gastric and intestinal phases separately. Changes detected by proteomics were also not reflected in total digestibility, despite protein oxidation occurring in both samples. A similar trend was observed in the second stage: processing and cooking did not significantly affect total digestion of PI or PRF, although clear changes in metabolite profiles were detected.

Keywords

Protein digestibility, pea protein, metabolite profile, proteomics, INFOGEST

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IN VITRO EVALUATION OF NOVEL DIETARY PROTEINS: DIGESTION, SATIATING POTENTIAL, GUT BARRIER INTERACTION AND ABSORPTION

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Abstract

Currently Europeans are over reliant on meat and milk for dietary protein intake. Proteins from plants, algae and insects offer sustainable alternatives to animal proteins. However, these alternative proteins must be evaluated in terms of digestion, absorption and bioactivity. This in vitro study selected the alternative protein ingredients; Honey Chlorella vulgaris powder, fava bean isolate and concentrate, cricket powder and microbial biomass Xanthobacter spp protein concentrate, for evaluation. Ingredients were digested using the in vitro static adult gastrointestinal method, INFOGEST. The bioaccessible fraction was characterized for the degree of protein hydrolysis, the distribution of peptide sizes and the concentration of free amino acids. Satiating potential was assessed by treating enteroendocrine cells (STC-1) with digested ingredients and measuring secreted level of the satiety hormone, GLP-1. Human intestinal cell lines, Caco-2 and HT29-MTX, were co-cultured for 21 days to create an in vitro gut barrier. The apical side of this barrier was treated with digested ingredients for 2 hours to mimic in vivo absorption times. Barrier integrity was assessed by measuring transepithelial electrical resistance (TEER). Absorption of digested ingredients was tracked by quantifying free amino acids and peptides on the basolateral side.

Size exclusion chromatography revealed that, 92.6±3.2% of the peptides at the end of the intestinal phase were 1kDa in size, regardless of protein ingredient. Digested Honey Chlorella vulgaris significantly increased secretion of active GLP-1 levels (3388.4 ± 246.7pg/mL) from STC-1 cells, compared to all other protein ingredients including the benchmark protein, whey protein isolate (P0.05). Caco-2/HT29-MTX barrier was not disturbed with any ingredient treatment, with TEER values remaining > 800Ω*cm2, similar to control cells treated with HBSS alone (P>0.05). Concentration of free amino acids in the Caco-2/HT29-MTX basolateral chamber was highest when treated with digested cricket (367.6±92 nmol) compared to all other ingredient treatments, including whey protein isolate (P0.05). In addition, mRNA transcript levels of the amino acid transporter SLC6A14 in Caco-2/HT29-MTX monolayers was significantly higher with cricket treatment (P0.05). Our results demonstrate that these novel proteins are (a) easily digested, (b) do not compromise gut barrier health and (c) deliver free amino acids to the bioavailable side. Honey chlorella vulgaris induces the highest GLP-1 response by STC-1 cells, implying it may be the most satiating protein source. Further work will continue to evaluate these promising ingredients for health and wellness outcomes.

Keywords

dietary proteins, Caco-2, protein absorption, satiety, GLP-1

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

NANOSTRUCTURED LIPID CARRIERS TO IMPROVE THE ORAL BIOAVAILABILITY OF FLAVONOLS

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Abstract

Introduction

Plants contain various compounds reported to possess beneficial health properties, such as flavonols. Flavonols have potential as dietary supplements, as their consumption has been associated with the prevention and/or management of several diseases, including type 2 diabetes and neurodegenerative diseases. However, poor bioavailability limits the physiological impact of flavonols. In this project, flavonols have been encapsulated in nanostructured lipid carriers, which are hypothesized to improve bioavailability by keeping flavonols solubilized, protecting them against the digestive environment, and facilitating an alternative intestinal absorption route through intact particle endocytosis and/or mixed micelle formation.

Methods

A solid lipid was melted at 70°C and mixed at a 70:30 ratio with a liquid lipid suitable for the solubilization of flavonols. An emulsion was established through 10 minutes of hot high-shear homogenization at 10,000 rpm, followed by 10 minutes of ultrasonication. The emulsion was then cooled back to room temperature under slight agitation to allow solidification of the solid lipid, establishing nanostructured lipid carriers. Dynamic light scattering was used to determine the size and zeta-potential of the nanocarriers. Encapsulation efficiency was determined spectrophotometrically by indirectly measuring absorbance of free flavonols after ultrafiltration to remove the nanocarriers. Iron chelating capacity was assessed using the ferrozine assay after incubation of free and encapsulated flavonols with iron sulfate in a molar ratio 1:1 for 24 hours.

Results

Empty and quercetin-filled nanocarriers had diameters of 228.8±1.2 nm and 215.8±4.2 nm, respectively, which make them suitable for uptake by endocytosis. The zeta-potential of the empty and quercetin-filled nanocarriers was -29.5±6.3 mV and -21.5±7.3 mV, respectively, indicating moderate colloidal stability due to electrostatic repulsion. Encapsulation efficiency was 96.7±1.8%, indicating that most of the flavonol in the formulation was incorporated into the nanocarriers. After incubation with encapsulated and free flavonols, a reduction of 32.7% and 43.9%, respectively, in free ferrous iron was observed in the iron sulfate solution, suggesting preserved iron chelation activity after encapsulation.

Conclusion

In conclusion, flavonol nanostructured lipid carriers had a diameter suitable for endocytosis, were colloidally stable, and effectively incorporated flavonols that were still functional in iron chelation. These physicochemical properties provide the nanostructured lipid carriers with promising characteristics that could contribute to enhancing intestinal absorption and bioavailability of bioactive flavonols. Moving forward, stability of the nanocarriers and flavonol release during digestion will be determined using the INFOGEST model, while intestinal absorption will be investigated using in vitro absorption models (e.g. Caco-2, HT29-MTX).

Keywords

Nanostructured lipid carriers, nanoencapsulation, flavonols, bioavailability, intestinal absorption

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

TECHNOLOGICAL PROSPECTING AND PATENT LANDSCAPE OF STERCULIA STRIATA (CHICHÁ) AS A STRATEGIC INGREDIENT FOR FUNCTIONAL FOODS

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Abstract

Introduction: The search for sustainable food systems has increased interest in Unconventional Food Plants (UFP) due to their resilience and unique nutritional profiles. Sterculia striata A.St.-Hil. & Naudin, a species native to the Brazilian Cerrado, has emerged as a promising resource. Recent evidence indicates that its almonds possess a high content of unsaturated fatty acids and significant antioxidant potential, which is remarkably enhanced after gastrointestinal digestion. Studies using in vitro digestion models have demonstrated that the bioaccessibility of phenolic compounds in S. striata increases significantly compared to conventional seeds, while screening has confirmed the absence of cytotoxicity in these matrices (Prates et al., 2024). These biological benefits align with mechanisms discussed by Czigle and Bittner Fialová et al. (2022) regarding the action of plant constituents in gastrointestinal disorders.

Methods: This study performed a technological prospecting and mapping of the patent landscape related to S. striata to identify innovation trends. A systematic search was conducted across Google Patents, Espacenet, and Patentscope databases, focusing on the International Patent Classification (IPC) code A23L (foods). The analysis was structured into three dimensions: Main Innovation and Practical Application (Oslo Manual, 2018); and Level of Evidence (TRL scale, NASA, 2012), following WIPO (2015) standards for patent landscapes.

Results and Discussion: A total of 32 patent documents were identified. Results show that technological efforts are concentrated on oil extraction, nutrient stabilization, and plant-based analogues. The analysis reveals a convergence between scientific findings on bioaccessibility and the industrial pursuit of processes that preserve the nutritional integrity of UFP. Although international databases returned fewer records, prospecting via Google Patents and Patentscope confirmed Brazilian technological exclusivity over the use of S. striata in complex matrices, such as cereal bars. This concentration in national filings (INPI) suggests that the global protection of Brazilian biodiversity is in its early stages, reinforcing Brazil's role as the primary holder of applied knowledge on this species.

Conclusions: S. striata shows an increasing patent density and technological maturity to serve as a sustainable alternative to Arachis hypogaea L. The identified exclusivity represents a strategic opportunity to export technologies based on Cerrado biodiversity to global "Novel Foods" markets.

1. CZIGLE, S.; BITTNER FIALOVÁ, S. et al. Treatment of Gastrointestinal Disorders—Plants and Potential Mechanisms of Action. Molecules, 27(9), 2881, 2022.

2. PRATES, S. M. S. et al. Cytotoxicity Screening of Sterculia striata and Arachis hypogaea Before and After in Vitro Digestion. Journal of Medicinal Food, 27(10), 2024.

Keywords

Unconventional Food Plants, Sterculia striata, Industrial Property, Food Sovereignty, Technological Maturity

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

ANTIHYPERTENSIVE POTENTIAL OF ACHETA DOMESTICUS PEPTIDES: IN SILICO AND IN VITRO ASSESSMENT OF SACE INHIBITION.

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Abstract

Entomophagy has emerged as a valuable alternative to conventional protein sources, such as meat and plants, due to its nutritional value and lower environmental impact. Beyond their macronutrient composition, insects can release small peptides during gastrointestinal (GI) digestion that mediate health-promoting effects by interacting with specific molecular targets. The somatic angiotensin-converting enzyme (sACE) is a key pharmacological target in the management of hypertension, a condition affecting 1.28 billion adults worldwide and a leading cause of premature mortality. To date, the EU has authorized four insect species as novel foods. One of them is Acheta domesticus (house cricket), a species that is easy to farm and produces a nutritious flour with a favourable sensory profile.

This study aims to assess the ability of A. domesticus to generate peptides during GI digestion, capable of inhibiting sACE and, consequently having antihypertensive properties.

An in silico simulation of the GI digestion of A. domesticus proteins was performed, followed by a molecular docking protocol to evaluate the peptide binding interactions with sACE [1]. The formation of the selected peptides was validated after in vitro GI digestion using LC-MS/MS, and their sACE-inhibitory activity, along with the bioactivity of the bioaccessible digesta fraction, was assessed in vitro [2].

The in silico protocol identified seven peptides with high docking scores towards the catalytic domains of sACE, indicating a strong binding affinity and potential inhibitory activity against the target [1]. Among them, six peptides (AVQPCF, CAIAW, IIIGW, QIVW, PIVCF, and DVW) demonstrated ACE-inhibitory activity in vitro, with IC_{50} values from 3.69 to 195.5 µM. The peptides AVQPCF, PIVCF, and CAIAW showed the greatest inhibitory capacity, with IC_{50} values of 3.69 ± 0.25, 4.63 ± 0.16, and 6.55 ± 0.52 µM, respectively, and were successfully identified in the digesta by LC-MS/MS [2]. These results validated the in silico protocol as an effective tool for identifying sACE-inhibitory peptides from known protein sequences.

This study establishes for the first time that whole A. domesticus insects, subjected to GI digestion without prior protein extraction or concentration, exhibit significant sACE-inhibitory activity (IC_{50} = 77.1 ± 11.8 µg protein/mL extract). It is also demonstrated herein that A. domesticus is not only a valuable source of protein, comparable to other commercially established alternatives, but also, a promising source of bioactive peptides with potential health benefits through the mediation of antihypertensive effects. Further research will be conducted to confirm the in vivo antihypertensive effects of the most promising peptides.

References

[1] C. S. S. Teixeira, et al. (2023) Food Res. Int., vol. 169, doi: 10.1016/j.foodres.2023.112847.

[2] C. S. S. Teixeira et al. (2024) Foods, vol. 13, no. 21, doi: 10.3390/foods13213462.

Keywords

Acheta domesticus, gastrointestinal digestion, bioactive peptides, antihypertensive, angiotensin converting enzyme

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

ASSESSMENT OF PROTEIN QUALITY AND AMINO ACID DIGESTIBILITY IN MEAT, HYBRID, AND MYCOPROTEIN-BASED PRODUCTS USING THE INFOGEST PROTOCOL

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Abstract

The global transition towards more sustainable diets has prompted the food industry to develop hybrid products that combine traditional animal proteins with alternative protein sources. However, a comprehensive understanding of how these new formulations impact protein quality and the bioavailability of individual amino acids (AA) is essential to ensure they meet human nutritional requirements. In light of this background, the aim of this work was to evaluate and quantify the in vitro digestibility of both protein and individual amino acids (AA) in various commercial and experimental formulations comprising off-the-shelf meat products (cooked chicken breast, pork hot dog and chicken hot dog), and experimental hybrid formulations (chicken, turkey and soy hot dog, and a hybrid hot dog including chicken, turkey, soy and 20% w/w mycoprotein). Pure mycoprotein (Fusarium venatum mycelium) was also included for comparison. All samples were subjected to the INFOGEST standardized gastrointestinal digestion protocol. Protein digestibility was assessed by quantifying free amino groups using the ortho-phthalaldehyde (OPA) method, as well as by total amino acid analysis, and the in vitro DIAAS (Digestible Indispensable Amino Acid Score) was determined for each product. The results showed that all meat and hybrid products reached protein digestibility values close to 100%, whereas the pure mycoprotein exhibited a lower digestibility, ranging between 85-87%. Regarding protein quality metrics, the traditional meat products are classified as having excellent protein quality (DIAAS > 100%) according to FAO criteria. Specifically, the cooked chicken breast (Val:127%) and the pork hot dog (Val:125%) presented the highest protein quality scores among all samples. In the case of hybrid formulations, the addition of mycoprotein to the poultry and soy hot dog resulted in a significant increase in protein quality, raising the DIAAS from Val:93 to Val:99. This improvement could be attributed to the higher digestibility of specific essential AAs in mycoprotein such as Ile, Lys, Thr, and Val which effectively complements the AA profile of standard poultry products. Finally, the pure mycoprotein achieved a DIAAS of 90 (SAA), and can be classified it as good protein quality (DIAAS between 75-99), reflecting its potential as a high-quality sustainable alternative protein source. In conclusion, while conventional meat products have good to excellent protein quality, the strategic incorporation of mycoprotein into hybrid products can significantly enhance their nutritional value. These findings demonstrate that mycoprotein not only serves as a sustainable functional ingredient but can also serve to complement the indispensable amino acid profile, offering a viable strategy for developing high-quality, sustainable protein products.

Keywords

Alternative proteins, protein nutritional quality, in vitro DIAAS, meat products, hybrid products

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

NUTRITIONAL QUALITY, DIGESTIBILITY, AND GLP-1 REGULATORY EFFECTS OF ALTERNATIVE PROTEINS DURING IN VITRO DIGESTION

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Abstract

Over 10% adults suffer from overweight and obesity, and the global prevalence of diabetes and obesity continues to rise (Lancet. 2025). Insulin secretion, crucial for maintaining glucose balance, is stimulated by glucagon-like peptide-1 (GLP-1), which is secreted by enteroendocrine L cells in the gut. Although GLP-1 receptor agonists are used for glycaemic control, their clinical application is limited by high cost and gastrointestinal side effects, creating an urgent need for safe and sustainable dietary strategies. Research has indicated that digestion products and bioactive peptides from animal or plant proteins can enhance GLP-1 secretion (Santos-Henandez et al., 2023; Zhang M et al., 2025). However, the potential of alternative protein sources to modulate GLP-1 secretion remains largely unexplored, despite their increasing relevance as sustainable protein sources in food science and preventive medicine.

The objectives of this study were: i) to investigate the protein quality and digestive kinetics of alternative proteins derived from microalgae and insects, in comparison with whey and soy proteins; ii) to evaluate the potential of their digestive products to contribute to glucose homeostasis by regulating GLP-1 metabolism. In vitro gastrointestinal digestion was performed on all four protein sources, followed by chemical characterization of the proteins and their digesta. After the intestinal phase, the digesta were applied to an L-cell model to assess GLP-1 secretion and proglucagon gene transcription.

Protein quality assessment showed that microalgae and insect proteins exhibited higher essential amino acid scores than soy protein. Moreover, microalgae protein demonstrated a higher protein efficiency ratio than soy protein. Digestion experiments revealed marked differences in digestion kinetics and bioavailability among protein sources. Microalgal protein displayed lowest degree of hydrolysis among other proteins, while insect protein showed highest amino acid availability. The amount of amino acids in microalgal protein was a little lower than in whey and soy protein. Notably, microalgal protein produced the most bioactive peptides during digestion, suggesting potential health benefits. Evaluation of GLP-1 metabolic regulation indicated that simulated digestion products from all four protein sources exhibit GLP-1 secretion and proglucagon gene transcription. Although alternative proteins demonstrate lower GLP-1 metabolic regulatory activity than whey protein, microalgae protein induced a higher level of proglucagon gene transcription.

In conclusion, microalgal and insect proteins are promising alternative protein sources with favorable nutritional quality and potential metabolic health benefits in glucose homeostasis. This study provides a theoretical basis for the development of sustainable protein resources and their application in the discovery of bioactive compounds for preventing and controlling metabolic diseases.

Keywords

Alternative proteins, Microalgae, Insects, Amino acid availability, Bioactive Peptides, GLP-1

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

LIPOSOMES OUTPERFORM OIL DISPERSIONS UNDER SIMULATED GASTROINTESTINAL DIGESTION

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Abstract

The oral bioavailability of functional compounds is critically dependent on the capacity of colloidal delivery systems to endure gastrointestinal processing while maintaining structural features that facilitate uptake. This study systematically compares the digestive fate of two phospholipid-based oral formulations of high industrial relevance: nanoscale liposomes and a phospholipid-stabilised triglyceride oil dispersion. A validated in vitro digestion model encompassing sequential oral, gastric, and intestinal phases was applied to monitor particle dynamics under physiologically representative enzyme and bile salt conditions. Structural evolution during simulated digestion was characterised by dynamic light scattering, cryogenic transmission electron microscopy, and quantification of lipolysis.

The liposomal system demonstrated pronounced resistance to digestive stress, preserving a narrow size distribution (below 200 nm) and intact microstructure throughout all stages of simulation, including the presence of phospholipase activity and bile salts. In contrast, the oil dispersion exhibited extensive restructuring during the intestinal phase, marked by significant lipid hydrolysis, reduction in particle size, and persistent heterogeneity. These distinct behaviours underscore the pivotal role of formulation architecture in determining gastrointestinal stability.

Overall, nanoscale phospholipid liposomes produced via scalable manufacturing approaches exhibited superior structural robustness relative to mixed lipid oil dispersions during simulated digestion. Their sustained nanoscale dimensions post-digestion suggest favourable interactions with intestinal transport barriers, supporting their potential as effective oral carriers to enhance the uptake of sensitive nutraceutical and bioactive compounds. These findings inform formulation strategies aimed at improving bioaccessibility in functional foods, dietary supplements, and specialised nutritional products.

Keywords

phospholipid, liposome, commercial formulations, oral administration, in vitro digestion

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

LAB PROTEASES IN PLANT-BASED FOODS: REDUCING PROTEASE INHIBITORS, IMPROVING PROTEIN BIOACCESSIBILITY

Joanna Ivy Irorita Fugaban [1 2] , Vince Balázs [1] , Guillermo-Eduardo Sedo Molina [3] , Lise Friis Christensen [4] , Claus Heiner Bang-Berthelsen [2] , Egon Bech Hansen [1]

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Abstract

The increased demand and market presence of plant-based and alternative foods raise questions about their overall impact on consumer health. A key concern is the presence of antinutritional factors that limit amino acid bioaccessibility, including protease inhibitors that make plant proteins difficult to digest and restrict access to amino acids. This work focuses on proteases from lactic acid bacteria (LAB) and their role during food fermentation in releasing peptides for amino acid catabolism and hydrolysing protease inhibitors in plant-based foods. To isolate protease-specific effects, individual proteases from previously characterised starter Leuconostoc spp. were engineered into an isogenic Lactococcus lactis background. These strains were then used to ferment soy milk in a yoghurt-like model system, and peptide profiles and protease inhibitor turnover were characterised by proteomics. In parallel, we developed a minimal medium in which trypsin inhibitor was the main protein source and observed growth of selected strains, indicating that these LAB proteases generate amino acids that can be directly used for microbial metabolism. Proteomics-based analysis identified specific groups of LAB proteases (clades A and B) that efficiently hydrolysed these antinutritional factors. The observed hydrolysis patterns indicate that LAB proteases preferentially target protein regions with highly unstructured conformations. Together, these findings suggest that selecting LAB strains with suitable protease profiles can help reduce protease inhibitors in plant-based foods and improve protein bioaccessibility.

Keywords

Protease, bioaccessibility, amino acids, plant-based foods

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

BIOACCESSIBILITY AND INTESTINAL TRANSPORT OF POLYPHENOLS FROM GRAPE POMACE-BASED BEVERAGES AFTER ADULT AND ELDERLY IN VITRO DIGESTION

Maria Rosaria Carillo [1] , Loredan Tararran [1] , Alessio Buono [2] , Laura Cavallarin [1] , Carmela Gerardi [3] , Giovanna Giovinazzo [3] , Francesco Romaniello [2] , Simona Lucia Bavaro [1]

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Abstract

Grape pomace is one of the most abundant by-products of the wine supply chain, with approximately 20 kg generated per 100 L of wine. Although considered a waste material, it retains a high content of bioactive phytochemicals, particularly polyphenols such as anthocyanins, flavonoids and tannins. Within a circular economy perspective, the recovery and valorization of these compounds represent a valuable opportunity for the development of polyphenol-enriched foods.

In this study, pomace from two Italian red wines (Lambrusco and Negroamaro) and one white wine (Fiano) was dried and used to prepare water-based beverages. Their antioxidant properties were first evaluated by spectrophotometric assays (TEAC and DPPH) and further characterized by HPLC-UV analysis. All samples showed a relevant antioxidant capacity, with Fiano exhibiting markedly higher values than the red varieties. HPLC-UV analysis confirmed these results, with total polyphenol contents (expressed as gallic acid equivalents, AUC) of 677.75 mg/L for Fiano, 293.51 mg/L for Lambrusco and 169.99 mg/L for Negroamaro. The main polyphenolic constituents were identified by UHPLC-HRMS (Q-Exactive Plus Orbitrap) through HRMS and MS/MS experiments.

The beverages were subsequently subjected to simulated gastrointestinal digestion using the INFOGEST static in vitro protocol, applying both adult and elderly digestion models. Changes in antioxidant activity were monitored in the resulting digestates. To assess the biological relevance of the digested samples, intestinal transport was investigated using a 2D in vitro intestinal co-culture model of differentiated human epithelial and mucus-secreting cells. Cell viability was preliminarily evaluated by MTT assay to determine non-cytotoxic concentrations (LC_{50}) for permeability studies. Polyphenols transported across the intestinal barrier were subsequently identified by UHPLC-HRMS .

This integrated approach allowed a preliminary assessment of the bioaccessibility and potential bioavailability of grape pomace-derived polyphenols. Overall, the results support grape pomace as a sustainable source of bioactive compounds and a promising functional ingredient for antioxidant foods, reinforcing its valorization within a circular economy framework and providing a basis for further in vitro and in vivo investigations.

Keywords

Polyphenols, In vitro digestion, Adult, Elderly, Intestinal barrier, UHPLC-HRMS (Q-Exactive Plus Orbitrap),

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

BIOACTIVE PEPTIDES WITH ANTICANCER POTENTIAL GENERATED DURING GASTROINTESTINAL DIGESTION OF BUCKWHEAT PROTEIN (FAGOPYRUM ESCULENTUM)

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Abstract

Colorectal cancer is the third most common malignant neoplasm worldwide, and its incidence is projected to increase by approximately 60% by 2030. Nutritional epidemiology has increasingly highlighted the strong relationship between diet and cancer morbidity and mortality. Several dietary patterns with anticancer potential are associated with protein sources of plant, animal, or microbial origin, which can release bioactive peptides during gastrointestinal digestion. These peptides may exert biological activity by modulating molecular pathways involved in carcinogenesis, making the evaluation of their bioaccessibility and biological effects essential. Plant-derived peptides have shown promising in vivo bioactivity and in situ anticancer effects against colon cancer. Buckwheat (Fagopyrum esculentum) is a pseudocereal characterized by high fiber content and an important protein content (8–18%), along with essential vitamins and bioactive molecules. In this study, buckwheat proteins were extracted and subjected to simulated gastrointestinal digestion using the standardized INFOGEST protocol in a dynamic digestion model that mimics oral, gastric, and intestinal phases. Peptide fractions (3 kDa, 3-10 kDa and >10 kDa), obtained from each digestion phase were evaluated using cell-based bioassays. Anticancer activity was assessed in human colorectal cancer cell lines HT-29 and Caco-2 by determining cell viability and cytotoxicity. In addition, the anti-inflammatory potential of the peptide fractions was evaluated in macrophages RAW 264.7, while their antioxidant capacity was assessed using cellular antioxidant activity assays. Peptide identification and characterization were performed by liquid chromatography–high-resolution mass spectrometry (LC-HRMS). The results demonstrated the generation of bioactive peptides with antioxidant and anti-inflammatory activities during gastrointestinal digestion, with a higher abundance and biological activity observed in the intestinal phase. These findings suggest that bioactive peptides derived from buckwheat proteins may represent a promising nutritional strategy and therapeutic complement for colorectal cancer prevention and management.

Keywords

Buckwheat, bioactive peptides, anticancer, anti-inflammatory, antioxidant

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

METABOLIC STABILITY, BIOACCESSIBILITY, AND VASCULAR PROTECTIVE EFFECTS OF A DIGESTED NEGROAMARO GRAPE POMACE BEVERAGE AFTER SIMULATED ELDERLY DIGESTION

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Abstract

Grape pomace is a sustainable source of polyphenols with recognized potential for preventing vascular ageing and age-related cardiovascular disease. Current research is increasingly focused on the development of polyphenol-enriched foods derived from grape pomace to counteract ageing processes. This study evaluates the metabolic stability, bioaccessibility, and bioactivity of a Negroamaro cv. grape pomace–based beverage before and after simulated gastrointestinal (GI) digestion, with a specific focus on elderly digestion conditions. A multidisciplinary strategy combining 1H NMR-based metabolomics, in vitro elderly digestion based on the standardized INFOGEST protocol, and bioactivity on vascular endothelial cell models was applied to assess molecular composition, digestive stability, and cellular bioactivity. The 1H NMR profiling of the undigested beverage revealed a complex bioactive metabolite composition, including polyphenols (flavonoids, hydroxycinnamates, anthocyanins), sugars, amino acids (AAs), and organic acids (OAs), confirming its high nutraceutical value. Post-digestion analysis showed the persistence of discriminant metabolites relative to digestion controls, with stable signals for key compounds such as catechin/epicatechin, caftaric acid, and anthocyanins. Multivariate analysis highlighted the differences between the control (digestion solution) and GPD metabolic profiles, confirming the stability of major polyphenolic classes after digestion and indicating high bioaccessibility of functional metabolites. The vascular effects of undigested and digested samples were investigated using an in vitro model of cultured human endothelial cells. The potential cytotoxicity of digested grape pomace (GPD) was evaluated by MTT assay. In addition, the effects of GPD on endothelial dysfunction induced by tumor necrosis factor-α (TNF-α) were assessed. Our results show that GPD effectively attenuated TNF-α–induced endothelial inflammation by downregulating the expression of endothelial adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), supporting the anti-inflammatory and endothelial-protective properties of grape pomace even after simulated GI digestion in an elderly subject.

Collectively, these results demonstrate that GI digestion remodels but does not abolish grape pomace polyphenol bioactivity, preserving, anti-inflammatory, and vasculoprotective functions. The Negroamaro cv. grape pomace beverage therefore represents a promising nutraceutical platform for vascular aging chemoprevention and functional food development for healthy aging.

Keywords

Grape Polyphenols, In vitro digestion, Elderly, Endothelium, Inflammation, NMR profiling

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

CAN FEATHER KERATIN SERVE AS A NUTRACEUTICAL? ASSESSMENT OF IN VITRO BIOACTIVITY, BIOACCESSIBILITY AND TOXICITY BEFORE AND AFTER GASTROINTESTINAL DIGESTION

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Abstract

Feather keratin is an abundant by-product of the poultry industry with potential as a sustainable source of bioactive peptides. We previously demonstrated that chicken feather keratin isolate and its enzymatic hydrolysates exhibit antioxidant activity, which can be further enhanced through the Maillard reaction. Building on these findings, the present study aimed to evaluate the bioaccessibility, multifunctional bioactivity and toxicological safety of selected keratin preparations at different processing stages, before and after simulated gastrointestinal digestion (SGID).

Keratin isolate (KI) was prepared by reductive extraction with L-cysteine, hydrolysed with subtilisin (KI-S), and further glycated with xylose (KI-S-X). All preparations were subjected to SGID using the static INFOGEST protocol. Molecular weight distribution was analysed by size exclusion chromatography, combined with ABTS derivatisation to identify fractions exhibiting antiradical activity. Total antioxidant activity, including ABTS radical scavenging, Fe^{2+} chelation and Folin-Ciocâlteu reagent reducing activity, was determined together with cytotoxicity in Caco-2 cells (MTT assay) and mutagenicity in Salmonella Typhimurium TA98 and TA100 (Ames test), with and without metabolic activation. In addition, inhibitory activities against angiotensin-converting enzyme (ACE) and dipeptidyl peptidase IV (DPP-IV), as markers of antihypertensive and antidiabetic potential, were evaluated before and after SGID.

Intact KI exhibited the lowest overall antioxidant activity. KI-S had significantly enhanced radical scavenging, Fe^{2+} chelating and reducing properties. KI-S-X showed even higher radical scavenging and reducing activity than KI-S, but impaired Fe^{2+} chelating potency. Antiradical activity was primarily associated with compounds below 1 kDa. SGID increased the proportion of low molecular weight peptides in all preparations and modulated antioxidant activity in a processing-dependent manner, with KI-S-X retaining the strongest overall post-SGID effect. KI showed weak ACE and DPP-IV inhibition, whereas KI-S and KI-S-X demonstrated stronger and comparable inhibitory activities, which generally increased after SGID. None of the preparations exhibited cytotoxic, direct mutagenic or indirect mutagenic effects under physiologically relevant concentrations.

In conclusion, enzymatic hydrolysis, controlled glycation and SGID modulated peptide profile, bioaccessibility and multifunctional bioactivity of feather keratin preparations while maintaining a favourable in vitro safety profile. Enrichment of low molecular weight peptides appears crucial to both antioxidant and enzyme-inhibitory activities, supporting the potential of processed keratin as a safe source of bioactive peptides for nutraceutical applications.

Keywords

Bioactive peptides, INFOGEST, Feather keratin, Maillard reaction, Toxicity, Waste valorisation

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

NUTRITIONAL CHARACTERIZATION, BIOLOGICAL ACTIVITY ASSESSMENT AND IN VITRO DIGESTIBILITY OF SPRAY-DRIED WINEMAKING LEES

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Abstract

Winemaking lees (WL), the sediment formed during wine fermentation, represent the second largest by-product of the wine industry, accounting for approximately 25% of total winery waste. Despite their richness in proteins, polysaccharides, lipids, vitamins, and phenolic compounds, WL remain largely underexploited. Within the framework of circular economy principles and sustainable agro-industrial valorization, this study investigates the nutritional composition and amino acid profile of spray-dried WL powders obtained from four grape varieties: Syrah (SY), Muscat Ottonel (MO), Sauvignon Blanc (SB), and Riesling Italian (RI) sourced from the "Apoldia Maior" winery, Sibiu County, Romania (2025 vintage). WL biomass was processed by spray drying and subjected to proximate analysis, antioxidant activity assays (DPPH, ABTS methods), total phenolic and total flavonoid content determination, and static in vitro gastrointestinal digestion following the standardized INFOGEST protocol. Free amino acid profiles were determined by LC-MS both prior to and following simulated digestion, enabling the assessment of amino acid bioaccessibility across the four varietal WL powders. Prior to digestion, Riesling Italian exhibited the highest total free amino acid content (17.90 mg/g dry weight), followed by Syrah (10.82 mg/g), Sauvignon Blanc (7.93 mg/g), and Muscat Ottonel (6.20 mg/g). Notably, RI displayed the richest profile of nutritionally essential amino acids, including leucine (2.68 mg/g), valine (1.89 mg/g), isoleucine (0.94 mg/g), methionine (0.62 mg/g), threonine (0.31 mg/g), and tryptophan (0.39 mg/g). Following in vitro digestion, total free amino acid content increased substantially across all varieties, confirming high gastrointestinal bioaccessibility of the WL protein fractions. Riesling Italian again demonstrated the highest post-digestion total (90.50 mg/g dry weight), followed by Syrah (69.00 mg/g), Muscat Ottonel (35.69 mg/g), and Sauvignon Blanc (32.08 mg/g). Among essential amino acids, leucine, valine, lysine, and tryptophan were particularly well-released during digestion. A remarkably high post-digestion arginine content was observed in the RI fraction (22.13 mg/g), suggesting significant protein hydrolysis and release of this conditionally essential amino acid under simulated gastrointestinal conditions. These findings demonstrate that spray-dried WL powders, particularly from Riesling Italian, represent a nutritionally valuable and bioaccessible source of free amino acids, supporting their potential integration as functional food or feed ingredients. This work contributes to the growing body of evidence for the feasibility of large-scale winemaking by-product valorization within a circular economy framework.

Keywords

winemaking lees, spray drying, amino acid bioaccessibility, circular economy, functional food ingredient

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

WHAT IS THE IMPACT OF PROCESSING A FOOD MATRIX ON THE PRODUCTION OF A RAW MATERIAL? THE CASE OF PUPUNHA (BACTRIS GASIPAES) FLOUR

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Abstract

Peach palm (Bactris gasipaes), also known as pupunha, is an underutilized tropical fruit with potential for ingredient innovation in bakery foods. However, scientific research on this fruit remains limited, particularly regarding how processing conditions influence its technological and nutritional properties. Therefore, this study evaluated how cooking and drying strategies affect the chemical composition, bioactive profile, and nutritional functionality of peach palm flour. The fruits were processed under four conditions: (1) freeze-dried raw fruit (FDF), (2) freeze-dried cooked fruit (CFDF), (3) oven-dried raw fruit (CDF), and (4) oven-dried cooked fruit (CCDF). The flours were evaluated for proximate composition, total phenolic content, antioxidant capacity, cytotoxicity against normal and cancerous cells (ISO 10993-5), and protein hydrolysis (Church et al., 1985) after in vitro digestion (Brodkorb et al., 2019). Processing conditions significantly influenced all characteristics analyzed. Regarding proximate composition, total fiber was higher in cooked flours (CFDF: 6.62 g/100g; CCDF: 7.19 g/100g), suggesting structural changes associated with starch modification during processing. The CDF sample showed higher phenolic contents (4.13 mg GAE/g) and better results for antioxidant capacities, suggesting that temperature may, to some extent, favor the release of these compounds from the food matrix. On the other hand, although the CDF sample showed a higher bioactive content, the phenolic extract also showed pronounced cytotoxicity toward normal cells (1.18 mg/mL). Regarding protein hydrolysis during in vitro digestion, cooking the pupunha fruit prior to oven drying the flour increased the release of free amino groups (FDF: 276.53a ± 13.86, CFDF: 259.98ab ± 33.24, CDF: 227.75b ± 13.87, CCDF: 283.59a ± 4.34 mg of NH2/g of protein). Notably, the CCDF treatment showed a balanced functional profile, combining high protein hydrolysis (>250 mg NH_2/g protein) with selective cytotoxicity toward cancer cells (2.84a ± 0.06 mg/mL) compared with normal cells (3.34b ± 0.20 mg/mL). These findings demonstrate that processing strategies do not simply enhance or reduce flour quality but modulate nutritional and biological functionality in distinct ways. Overall, the results highlight the potential of strategically tailored processing to improve nutrient bioaccessibility and support the application of peach palm flour as a functional and sustainable food ingredient.

ISO 10993-5, & I. O. for S. (2009). International Standard - Tests for in vitro cytotoxicity
Brodkorb, A. et al. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nature Protocols, 14(4), 991–1014.
Church, F. C., et al. (1985). An o-phthalaldehyde spectrophotometric assay for proteinases. Analytical Biochemistry, 146(2), 343–348.

Keywords

Amazonian fruit, pupunha, in vitro digestion, cytotoxicity, bioactive compounds, food ingredient

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

CALCIUM DELIVERY FROM RETAIL PLANT-BASED CHEESES IN THE UK: COMPARISON OF LABELLED/MEASURED CALCIUM AND IN-VITRO DIALYSABLE CALCIUM ACROSS PRODUCT FORMATS

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Abstract

Background: Plant-based cheese alternatives (PBCAs) are increasingly consumed as dairy substitutes, and several products are marketed as “calcium-fortified”. However, declared or total calcium (Ca) may not reflect in-vitro estimated Ca bioaccessibility, which depends on mineral form and matrix behaviour during digestion.

Methods: UK retail dairy cheeses and PBCAs across common formats (blocks/soft blocks, grated, and soft/spread, n = 34) were characterised using label information and ingredient/format descriptors. Total Ca was quantified by ICP-MS. A standardised static in-vitro gastrointestinal digestion was performed, and the dialysable Ca fraction at the end of the intestinal phase was used as an operational estimate of bioaccessible Ca. Outcomes were expressed as (i) bioaccessibility (% of total Ca), (ii) bioaccessible Ca per 30 g serving, and (iii) “dairy-equivalent servings” required for PBCA products to match dairy comparators. Multivariate mapping (PCA) and non-parametric statistics were used to explore compositional and format-level patterns.

Results: Measured total Ca varied widely across products (near-zero to ~800 mg/100 g) and agreement between label-declared and measured Ca was inconsistent among PBCAs, particularly for fortified items. Intestinal-phase bioaccessibility ranged from 1.69% to 68.31%, indicating substantial product-to-product variation in dialysable Ca release. Serving-level estimates highlighted differences in delivered Ca: the dairy block comparator delivered 58.65 ± 1.35 mg dialysable Ca/30 g, whereas plant-based blocks typically delivered 1.80–24.00 mg/30 g, requiring 2.4–32.6 servings to match the dairy block reference. A notable example was a Ca-dense PBCA snack (818.72 ± 33.86 mg/100 g) with very low bioaccessibility (1.69 ± 0.05%), yielding only 4.20 mg/30 g dialysable Ca. Grated products showed the highest bioaccessibility trend; plant-based grated cheeses reached 43.29–68.31%, but still delivered less dialysable Ca per portion (25.65–42.90 mg/30 g) than the dairy grated comparator (73.20 ± 8.40 mg/30 g) due to lower total Ca. An intestinal-phase efficiency index (dialysable/soluble Ca) centred around ~0.5–0.6 and did not differ significantly by cheese product format ($p > 0.05$).

Conclusion: Retail PBCAs show large variability in in-vitro intestinal dialysable Ca, and total/declared Ca does not consistently predict serving-level dialysable Ca delivery. Reporting dialysable Ca per serving and dairy-equivalent servings provides an interpretable framework for comparing products and identifying formulations that merit further validation.

Keywords

plant-based cheese; calcium fortification; in-vitro digestion; dialysable calcium; bioaccessibility; ICP-MS.

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QUERCETIN, KAEMPFEROL AND ISOQUERCETIN INHIBIT SACCHARIDE DIGESTION AND INCREASE QUERCETIN AND KAEMPFEROL ABSORPTION IN DIFFERENTIATED CACO-2 CELLS

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Abstract

Background: Impaired glycemic control is a central feature of metabolic disorders such as obesity and type 2 diabetes. Plant-derived polyphenols such as quercetin, kaempferol, and isoquercetin have been proposed to inhibit carbohydrate-digesting enzymes and modulate intestinal monosaccharide and polyphenol absorption. This study evaluated the individual and combined effects of these polyphenols on α-amylase and α-glucosidase activity, and on monosaccharide and polyphenol absorption in differentiated Caco-2 cells.

Methods: α-Glucosidase and α-amylase were incubated with individual polyphenols, combinations of two polyphenols, or a mixture of all three (0–200 µM) for 20 min, and enzyme activity was determined using spectrophotometric assays. Glucose and fructose transport were quantified by measuring their concentrations in the basolateral medium after 1 h of exposure to a final apical concentration of 100 µM of each polyphenol, either individually or as a mixture, in differentiated Caco-2 cells. Polyphenol absorption was determined in the same basolateral medium by HPLC analysis.

Results: Quercetin (200 µM) inhibited α-glucosidase activity by 94 %, while α-amylase activity was modestly and non-significantly reduced (10 %). The quercetin–kaempferol combination (total concentration 200 µM) significantly decreased α-amylase activity (19 %), whereas the three-polyphenol mixture (total concentration 200 µM) significantly reduced this activity by 30 %. Quercetin (100 µM) individually and the mixture of all polyphenols (100 µM) both significantly reduced glucose absorption by approximately 65 %, without affecting fructose uptake. During single-compound exposure, quercetin reached a basolateral concentration of 10.1 µM, corresponding to a transport efficiency of 17 %. In the mixture condition, the transport efficiency of quercetin increased significantly to 29 % compared with single-compound exposure (6.53 µM basolateral concentration).

Discussion: The enhanced α-amylase inhibition induced by polyphenol mixtures may result from binding of individual compounds to distinct sites on the enzyme, leading to additive effects. The increased quercetin transport may be mediated by kaempferol-induced modulation of intestinal efflux transporters. Collectively, these findings suggest that multi-component polyphenol formulations may simultaneously modulate carbohydrate-digesting enzymes and enhance quercetin intestinal transport, supporting a multi-targeted approach to improve glycemic regulation.

Keywords

Polyphenols, polyphenol absorption, α-amylase inhibition, α-glucosidase inhibition, Caco-2, glucose absorption.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EXTRUDED BREAKFAST CEREALS: INNOVATIVE BIOACTIVE-ENRICHED PRODUCTS WITH SENSORY AND GASTROINTESTINAL STABILITY EVALUATION

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Abstract

Background: The development of innovative food products enriched with bioactive compounds (BC) is a promising strategy to enhance nutritional and functional properties while promoting human health. In this context, this work aimed to develop chocolate cereal breakfasts enriched with BC, incorporating yacon or carob flours as a source of phenolic compounds and prebiotic fructo-oligosaccharides (FOS), which occur naturally in yacon and are enzymatically produced from carob.

Methods: Flours from two yacon varieties (Morado and Hualqui) and carob were produced and characterized by LC-MS and HPLC-IR to evaluate FOS and phenolic content. Extrusion formulations and processing parameters were optimized to obtain a final prototype with the desirable physical and sensorial attributes, including crunchiness and expansion. The developed prototypes were evaluated through sensory analysis (100 consumers) and BC composition, including their quantification in the final product and gastrointestinal stability, using a standardized in vitro digestion model (INFOGEST 2.0).

Results: Both yacon flours are mainly composed of phenolic acids, with total phenolic contents of 236-385 mg/100 g, and FOS content of 22-33 g/100 g, depending on the yacon variety. In contrast, carob flour showed a more diverse phenolic profile, including gallic acid and quercetin derivatives (total of 94 mg/100 g) and FOS content of 13.5 g/100 g. All prototypes were successfully extruded and displayed positive key sensory attributes, with higher acceptability than the control prototype. Morado yacon-flour prototype achieving the highest overall liking score of 6.0 on a 9-point hedonic scale. HPLC and LC-MS analysis after extrusion confirmed the presence of BC derived from the base flours, including FOS and phenolic compounds, particularly caffeoylquinic and tricaffeoylatraric acid isomers from yacon and gallic acid from carob. Total phenolic contents were highest in the Hualqui yacon-flour prototype (54 ± 5 mg/100 g), followed by Morado yacon-flour (47 ± 6 mg/100 g) and carob flour (32 ± 3 mg/100 g), representing a significant increase over the control (16 ± 2 mg/100 g). Both extruded prototypes also exhibited higher antioxidant activity compared to the control prototype. Preliminary in vitro digestion results show that some BC, including theobromine and caffeine (from cocoa powder), as well as caffeoylquinic acids and FOS (from yacon and carob flours), were preserved after the intestinal phase, indicating stability under simulated gastrointestinal conditions.

Conclusion: Chocolate breakfast cereal prototypes based on innovative flours were successfully developed by extrusion, exhibiting desirable sensory properties and higher consumer acceptability. Phenolic and prebiotic compounds were preserved during extrusion, and some of them remained stable after simulated digestion, demonstrating the potential of these breakfast cereals as functional foods.

Keywords

Extruded Cereals Breakfast; Bioactive Compounds; Gastrointestinal Stability.

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

STRUCTURAL CONSTRAINTS GOVERNING PEA STARCH DIGESTIBILITY: EFFECTS OF PARTICLE SIZE, FRACTIONATION, AND HEAT TREATMENT

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Abstract

Pea starch is considered a slowly digestible carbohydrate source, yet the influence of traditional processing parameters on its digestibility and glycaemic response remains insufficiently understood. This study aims to elucidate how particle size, fractionation and thermal processing affect in-vitro starch digestibility, estimated glycaemic index (EGI), and amylolysis kinetics in diverse pea-based systems. Dehulled yellow peas were ground into defined particle sizes (4.35 – 0.3mm, determined through sieving). The same peas were further used to produce fractions of starch-, protein-, and fiber-rich components, which were combined to formulate a simulated pea matrix with composition comparable to that of the yellow pea flour. Gelatinisation was achieved through short (20 min) or prolonged (300 min) heating at 95oC. Pea starch digestion was simulated with a simplified in-vitro digestion protocol using porcine α-amylase and reducing sugar release was quantified by a microplate DNS assay. Hydration Indices and Estimated Glycemic Indices were derived from the obtained hydrolysis curves using white bread as a reference. The subsequent digestion profiles were fitted with linear and first-order fractional-conversion models to infer mechanistic regimes. Coarse particles displayed low digestibility and low EGIs, whereas sub-millimeter fractions showed sharply increased digestibility and EGIs, revealing a biphasic, particle size-dependent response with a critical transition zone between 1.5- and 1.0-mm. Prolonged heating increased digestibility across all samples and shifted several coarse fractions from low- to medium- or high- EGI, while fine flour and isolated starch remained highly glycaemic across all conditions. The simulated pea matrix consistently lowered digestibility and EGI relative to particle size analogues, indicating the importance of milling on starch digestibility and the potential protective role of the protein and fiber-rich domains. This study serves as a mechanistic understanding for designing pea-based functional foods with tailored glycaemic responses.

Keywords

In vitro digestion; pea starch; Thermal processing; Particle size; Estimated glycaemic index; Amylolysis kinetics

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

COMPARISON OF CARBOHYDRATE AND PROTEIN ILEAL DIGESTIBILITY OF TWO BREADS USING A DYNAMIC IN VITRO GASTROINTESTINAL MODEL.

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Abstract

Empirical observations have reported that a bread made from ancient durum wheat and sourdough (test bread) shows better digestive tolerance than conventional white bread. Beyond differences in nutritional composition due to the raw materials (ancient durum wheat vs modern soft wheat; wholemeal flour vs white flour), the sourdough fermentation process can induce changes in the composition or accessibility of carbohydrates and proteins. These variations could in turn affect the digestibility of these macromolecules, leading to different physiological effects. The aim of this study was therefore to compare, in vitro, the digestibility of carbohydrates and proteins in this test bread with that of conventional white bread.

To determine carbohydrate and protein ileal digestibility of the two breads and follow their hydrolysis kinetics during digestion, we conducted experiments using the TNO dynamic gastrointestinal model (TIM-1). This well-documented model dynamically simulates the main parameters of human digestion in the stomach and the three parts of the small intestine. Hollow fibers connected to the jejunum and the ileum continuously dialyze the intestinal contents, allowing the collection of small peptides and sugars from digestion. Non-digestible material is collected in ileal effluents. In vitro bread and blank digestions (without bread) were conducted in triplicate for 5h with a protocol simulating the fed state of a healthy adult. Total carbohydrates and total nitrogen in dialysates and ileal effluents were quantified using respectively the anthrone colorimetric method and the Dumas method with the FlashSmart™ Elemental Analyzer (Thermo Finnigan, USA). In vitro ileal digestibility was calculated as the cumulative amount recovered in jejunal and ileal dialysates relative to the total recovered in dialysates and ileal effluents.

Profiles of carbohydrates and nitrogen cumulated in jejunal and ileal dialysates during in vitro digestion were similar between breads, suggesting no difference in the hydrolysis process of starch and proteins. However, the test bread showed a tendency towards more unabsorbed carbohydrates, unlike protein where the difference remained non-significant. For the test bread, in vitro ileal digestibility of carbohydrates and proteins was 89.1 ± 0.6 % and 83.7 ± 2.7 % respectively, compared with 91.6 ± 2.1 % and 88.2 ± 5.1 % for white bread. These differences were not statistically significant.

The aim of this study was to compare, in vitro, the carbohydrate and protein ileal digestibility of two types of bread. A tendency towards a higher amount of unabsorbed carbohydrates was observed in one type of bread, although the difference was not statistically significant. This suggest that both breads are equally digestible. Thus, the better tolerance may have another origin, such as the quantity of FODMAPs or the composition of gluten proteins. A more detailed analysis of the collected samples could answer this hypothesis.

Keywords

Protein and carbohydrate digestibility Bread Digestive tolerance TNO dynamic gastrointestinal model (TIM-1)

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

CAN HYPOALLERGENIC AND ACE INHIBITORY HYDROLYSATES FROM TENEBRIO MOLITOR RESIST GASTROINTESTINAL DIGESTION?

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Abstract

Hypertension affects 1.4 billion adults aged 30-79 worldwide (33% prevalence, 2024), representing a major global health crisis linked to cardiovascular morbidity/mortality. Nutritional strategies featuring bioactive peptides (3 kDa, 2–20 amino acids) offer natural inhibitors of key molecular targets such as somatic angiotensin-converting enzyme (sACE; EC 3.4.15.1) a well-established drug target in the management of hypertension. Insect protein hydrolysates, including Tenebrio molitor, one of 4 European Union-approved insects as food, have been reported to possess antihypertensive potential following enzymatic treatment. However, insects are potentially allergenic foods through cross-reactivity in crustacean allergic individuals due to their phylogenetic closeness. The present work aimed to develop and optimize enzymatic hydrolysis treatments to produce hypoallergenic and functional insect-based ingredients with antihypertensive properties and to evaluate their resistance to gastrointestinal (GI) digestion.

Dried and ground T. molitor larvae were hydrolysed with 0.1-5% (w/w) of alcalase or neutrase under optimised conditions. The OPA/NAC assay was used to quantify the degree of hydrolysis. Proteins profile and IgE-binding capacity of hydrolysates were assessed by SDS-PAGE and immunoblotting using sera from crustacean-allergic individuals, respectively. The inhibitory activity of the hydrolysates against sACE was evaluated using a fluorimetric method [1]. In vitro GI digestion (INFOGEST 2.0 protocol) of selected hydrolysates was performed to assess the stability of bioactivity after digestion.

Enzymatic hydrolysis of T. molitor larvae using alcalase or neutrase caused extensively protein degradation and effectively reduced IgE cross-reactivity with sera from crustacean-allergic individuals in all tested concentrations of alcalase and neutrase, while generating small peptides with in vitro ACE-inhibitory activity. The bioactivity was enzyme- and dose-dependent: alcalase hydrolysates at 5% (v/v) showed the strongest inhibition (IC_{50} = 21.9 ± 2.0 mg/mL), whereas neutrase was most effective at 1% (IC_{50} = 16.0 ± 1.2 mg/mL), indicating that increasing enzyme concentration does not necessarily enhance bioactivity. Following simulated GI digestion, both hydrolysates retained high inhibitory capacity. Overall, hydrolysates produced with 1% (v/v) alcalase or neutrase represent an industrially viable approach for developing ingredients with hypoallergenic and antihypertensive potential for functional foods or nutraceuticals.

[1] Tavares et al. (2011). Int. Dairy J. 21, 926-933. DOI: 10.1016/j.idairyj.2011.05.013.

Keywords

Mealworm, Hypoallergenic formulations, Bioactive peptides, Enzymatic hydrolysis, Novel and sustainable foods

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

ETHANOL-INDUCED RECRYSTALLIZATION INCREASES THERMAL STABILITY AND IN VITRO RESISTANT STARCH CONTENT OF RICE STARCH

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Abstract

Resistant starch type III (RS3), formed by the recrystallization of debranched starch, has gained significant interest as a functional ingredient for regulating postprandial glycemic responses and improving metabolic health. Many RS ingredients lose their bioactivity during thermal food processing, making the development of RS3 with high thermal stability crucial for low-glycemic food applications. However, precise control over the formation of dense RS3 matrices remains challenging. This study explores the potential of ethanol-mediated recrystallization to modulate the digestibility and structural stability of rice starches.

Native rice starch and waxy rice starch were pre-treated with pullulanase for thorough debranching to produce linear short chains (DR and DWR). These samples then underwent a heat treatment in an ethanol-aqueous system with varying ethanol volumes to induce recrystallization. The starch digestibility composition (RDS, SDS, and RS) was quantified using the Englyst method, comparing samples before and after secondary heating to assess thermal stability. Furthermore, the standardized INFOGEST 2.0 protocol was applied to representative samples to collect residues from oral, gastric, and small intestinal stages. The structural evolution of these residues was characterized via thermal stability (DSC), crystalline structure (XRD), and chain length distribution analysis.

The results demonstrated that ethanol addition significantly promoted the formation of RS3. RS content increased progressively with increasing ethanol concentration, reaching its peak at 125 mL ethanol addition. Notably, debranched normal rice starch (DR) exhibited a significantly higher RS content and superior thermal stability compared to waxy rice starch (DWR), maintaining a high RS content even after secondary heat treatment (e.g., 64% for DR-125mL). Structural monitoring during the INFOGEST digestion revealed that the presence of different chain length distributions in DR and DWR influenced the formation and stability of the crystalline matrix. Preliminary analysis suggests that the reorganization of linear fragments during ethanol treatment effectively resisted enzymatic hydrolysis throughout the gastrointestinal phases.

In conclusion, this research developed a promising method to produce RS3 with enhanced thermal stability. The integration of Englyst nutritional classification and INFOGEST structural tracking provides a comprehensive framework to understand how starch molecular structures influence the functionality of the new RS3, facilitating the design of thermally stable, low-glycemic food products

Keywords

Resistant starch, Ethanol-induced recrystallization, Debranching, INFOGEST, Structural stability, Englyst method.

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

DOES CAROTENOID ACYLATION IMPACT MICELLIZATION DURING IN VITRO DIGESTION OF LUTEIN-LOADED EMULSIONS?

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Abstract

Lutein intake is linked to reduced risk of chronic diseases and improved cognitive function in children and the elderly. In many carotenoid-rich foods, lutein is mainly ingested in an acylated form bound to fatty acids, which has been reported to be much less bioaccessible than free lutein. To elucidate this matter, the fate of acylated carotenoids during micelle fraction (MF) recovery, a key factor for determining lipophilic compound bioaccessibility, was investigated. Marigold petals were used as a source of lutein-rich extracts. Casein-stabilized emulsions without carotenoids (control) or loaded with carotenoid extracts mainly composed of acylated or free lutein were subjected to in vitro static digestion following the INFOGEST protocol. Emulsions were prepared with 2% carotenoid-enriched oil and 98% sodium caseinate solution, processed by rotor-stator mixing and high-pressure microfluidization. MF isolation was achieved by chyme centrifugation, followed by filtration (cellulose acetate, 0.2 µm pore size) of the supernatant or aqueous fraction (AF). A systematic investigation combining LC-DAD-MS/MS, SAXS analysis and cryo-TEM of MFs was carried out. Hydrolysis extent of carotenoids was expressed as the percentage of the amount of lutein esters that disappeared after digestion (chyme) in comparison with those initially present in the emulsion. Bioaccessibility was determined based on the ratio of the carotenoid content in AF or MF to that present in the emulsion before digestion. The carotenoid retention was expressed as the percentage of carotenoid retained during AF filtration. The extent of acylated carotenoid hydrolysis was 14 ± 3 %. Bioaccessibility values of carotenoids in the AFs (26 and 78% for acylated and free lutein, respectively) were higher than those in the MFs (5 and 67% for acylated and free lutein, respectively). Filtration preferentially retained acylated carotenoids (>80%). SAXS results and cryo-TEM images confirmed matrix destabilization and micellization. By comparison with the control sample, an increase in the radius of gyration and aggregation of micelles confirmed the incorporation of free carotenoids into micelle assemblies. In contrast, no differences were observed for acylated carotenoids. Although cryo-TEM images show some colloidal structures in common regardless of the carotenoid form, only MF obtained from the digestion of acylated carotenoid-rich emulsion displayed small particles (100 µm) similar to intact oil droplets. In conclusion, although mixed micelles were present in the MF, the incorporation of acylated carotenoids into them was very limited. Moreover, acylation strongly affected retention, demonstrating that filtration is a critical step to accurately determine carotenoid bioaccessibility. Our findings also reveal that differences in carotenoid structures and experimental methods for recovery of mixed micelles, despite using the same matrices, limit the comparability of results across studies.

Keywords

Carotenoid ester, bioaccessibility, mixed micelle, LC-MS, SAXS, cryo-TEM

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

MUCOADHESIVE CHITOSAN-COATED ALGINATE HYDROGEL MICROCAPSULES FOR CONTROLLED RELEASE OF QUERCETIN

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Abstract

Background: Polyphenols such as quercetin can modulate postprandial glucose responses by inhibiting digestive enzymes and modulating glucose transporter activity and expression. However, poor water solubility, limited intestinal retention, and low bioavailability constrain their physiological efficacy. Microencapsulation in alginate hydrogels coated with mucoadhesive polymers such as chitosan is hypothesized to enhance stability and prolong intestinal epithelial residence time. This study investigated quercetin encapsulation into sodium alginate microcapsules with or without chitosan coatings, evaluating physicochemical properties, release behavior, storage stability, and mucoadhesion on a mucin-containing polyvinyl alcohol hydrogel model.

Methods: Quercetin-loaded alginate microcapsules were produced via ionic gelation and coated with 0%, 0.5%, or 1% chitosan. Encapsulation efficiency was quantified spectrophotometrically (λ = 380 nm) by measuring free and total quercetin. Bead size, circularity, and morphology were analyzed using brightfield digital imaging, ImageJ software, and stereoscopic microscopy (5× magnification). Release profiles were evaluated at pH 2.0 and 7.2, simulating gastric and intestinal conditions respectively. Storage stability was monitored over three weeks at 4 °C by quantifying quercetin released into the storage buffer. Mucoadhesion was assessed using a custom flow-over assay on mucin/polyvinyl alcohol hydrogels, with adhered microcapsules quantified via quercetin extraction using 5% sodium citrate.

Results: Encapsulation efficiency was approximately 98%, with increasing chitosan concentrations causing a small but significant 1–2% reduction. Bead diameter remained consistent (~0.98 mm) across all formulations, while higher alginate concentrations significantly improved circularity. Only 1–2% quercetin was released after three weeks of storage at 4 °C, independent of alginate or chitosan concentration. Results for the release profile test at pH 2.0 show minimal quercetin release. At pH 7.2, 3% alginate beads exhibited significantly lower quercetin release (43.5%) compared to 1.5% alginate beads. Independent of alginate concentration, the addition of 1% chitosan significantly reduced quercetin release by 58.6%. Mucoadhesion was significantly enhanced by 1% chitosan coatings relative to uncoated microcapsules.

Discussion: Quercetin was efficiently encapsulated in alginate-chitosan microcapsules with stable size and morphology. Chitosan enhanced retention on mucus-mimetic membranes and reduced quercetin release at intestinal pH while maintaining minimal release under gastric conditions. Collectively, these findings suggest that alginate-chitosan microcapsules represent a promising food-grade carrier system for targeted intestinal polyphenol delivery. Future studies should validate these results in vivo, optimize long-term stability, and evaluate the bioactivity of encapsulated quercetin.

Keywords

Quercetin microencapsulation, Mucoadhesion, Release, Encapsulation morphology, Mimetic mucus membrane

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECT OF FERMENTATION STRATEGY ON ALPHA-AMYLASE INHIBITORY ACTIVITY AND SACCHARIDE COMPOSITION OFb FERMENTED WHITE CABBAGE

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Abstract

Lactic acid fermentation of vegeta les is recognised as a preservation method that enhances the ioavaila ility and iological activity of phytochemicals. InhiEition of digestive enzymes such as α-amylase and α-glucosidase represents a validated dietary strategy to attenuate postprandial glucose excursions, offering enefits in the prevention and management of type 2 dia etes. During fermentation, acterial meta olism drives the consumption of low-molecular-weight saccharides and remodelling of the saccharide profile, potentially influencing substrate availability for digestive enzymes. However, the effect of fermentation strategy — spontaneous vs. directed (backslopping) — on α-amylase inhibitors and saccharide composition in white cabbage (Brassica oleracea L. var. capitata f. alba) remains limited.

White cabbage was subjected to spontaneous and directed lactic acid fermentation over 21 days. Ethanolic (70%) extracts were collected at defined time points (days 1, 3, 7, 10, 14, 17, and 21) and after one month of refrigerated storage. α-Amylase inhibitory activity was assessed by HPTLC-based bioautographic screening using porcine pancreatic α-amylase, starch as the substrate, and acarbose as the positive control. Starch hydrolysis products were detected after derivatisation with diphenylamine-aniline-phosphoric acid reagent and heating at 110°C, and quantified by densitometric scanning at 625 nm. The same derivatisation simultaneously revealed native saccharides in the extracts, enabling visualisation of saccharide composition changes across fermentation time points. Inhibition degree was calculated from the densitograms as described by Litewski et al. (2025) [1].

Raw cabbage extracts showed no meaningful α-amylase inhibitory activity. A progressive increase in inhibition was observed in both variants, with spontaneous fermentation extracts (FC10S) reaching activity levels comparable to the acarbose positive control earlier than directed fermentation extracts (FC14U). Concurrently, fermentation resulted in a marked reduction in simple sugar content, reflecting their utilisation by lactic acid bacteria, which may contribute to the observed shift in inhibitory potential. Inhibitory activity was retained after refrigerated storage, indicating stability of the bioactive compounds formed.

These findings demonstrate that lactic acid fermentation of white cabbage promotes α-amylase inhibitor accumulation alongside favourable changes in saccharide composition, regardless of fermentation strategy. Both approaches yielded extracts with potential anti-hyperglycaemic properties, supporting fermented cabbage as a functional food ingredient relevant to digestive health.

1. Litewski, S., Mróz, M., & Kusznierewicz, B. (2025). HPTLC-based screening method for the evaluation of α-amylase inhibitory activity in edible flowers. Scientific Reports, 15, 38909. https://doi.org/10.1038/s41598-025-22736-2

Keywords

glycaemia, α-amylase inhibition, white cabbage, lactic acid fermentation, functional food

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IN SILICO IDENTIFICATION OF ACHE AND BCHE INHIBITORY PEPTIDES FROM EDIBLE CYANOBACTERIA PROTEIN DIGESTS AS POTENTIAL ALZHEIMER THERAPEUTICS

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Abstract

Cyanobacteria are ubiquitous photosynthetic prokaryotes that can be found in diverse aquatic and terrestrial environments worldwide. They are highly nutritious microorganisms with a 50–70% protein content and have recently been identified as sustainable food sources, highlighting their well-known repositories of bioactive compounds. Currently, only spirulina (Arthrospira platensis and A. maxima) has widespread consumer acceptance and commercial availability as a food supplement. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), both members of the α/β hydrolase superfamily, hydrolyse acetylcholine into acetate and choline or butyrylcholine into butyrate and choline, respectively. Cholinergic deficits characterize Alzheimer disease (AD), with extensive research establishing AChE central role in AD pathology. Elevated BChE levels in AD brains correlate with amyloid-β plaque formation. Notably, AChE and BChE contribute independently to AD progression, presenting dual-inhibition opportunities for novel bioactive therapeutic targets. Therefore, this work intends to exploit the use of in silico tools to identify AChE and BChE inhibitory peptides from edible cyanobacteria. An in silico approach was performed to simulate gastrointestinal (GI) digestion of 450 proteins from 17 cyanobacterial species/strains totalling 366 entries: Aphanothece sacrum (7), Arthrospira sp. (113), Gloeothece tepidariorum (3), Halothece sp. (3), Limnospira maxima (17), Limnospira platensis (49), Nostoc cf. commune (5), Nostoc commune (9), Nostoc sp. (1), Nostoc sphaericum (2), Parasynechococcus marenigrum (2), Picosynechococcus sp. (37), Synechococcus elongatus (38), Synechococcus sp. variants (45), Synechocystis sp. (2) and Thermosynechococcus vestitus (1). The workflow identified novel peptides capable of selective or dual AChE/BChE inhibition via molecular docking against human AChE, Torpedo californica AChE and human BChE. The use of AChE and BChE structures provided a comparative framework for dual target binding modes. Simultaneously, human AChE was essential because ligand binding varies across species. This model offers an unobstructed gorge access and the narrow active site entrance (Y341/W286 1.5 Å closer than T. californica). Both enzymes feature ~20 Å active site deep gorges, containing esteratic (hAChE: S203/H447/E334) and anionic subsites (W86/Y337), which were targeted to investigate the binding affinity and structural complementarity of the digestion derived peptides [1,2]. Top-scoring peptides underwent toxicity profiling, intestinal barrier permeability assessment, and evaluation of blood-brain barrier crossing. The peptides demonstrating selective or dual inhibitory potential of AChE and/or BChE will be experimentally validated in vitro, establishing cyanobacteria as promising sources of dual cholinesterase inhibitors for AD therapeutics.

[1] Viayna E, et al. J. Med. Chem. 2020;64:812-39
[2] Cheung J, et al. J. Med. Chem. 2012;55:10282-6

Keywords

Cyanobacteria, Acetylcholinesterase, Butyrylcholinesterase, Alzheimer, Bioactive peptides, molecular docking

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

A NOVEL BACTERIAL PROTEIN SOLEIN® - PHYSICO-CHEMICAL AND DIGESTIVE CHARACTERISTICS USING THE SEMI-DYNAMIC IN VITRO DIGESTION MODEL

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Abstract

The growing population and environmental challenges have driven the need for sustainable food systems. Solein®, a bacterial protein powder derived from Xanthobacter sp., can convert H2 and CO2 into high-protein biomass. Its production route is independent of arable land and climatic conditions, making it a versatile alternative protein ingredient. The yellow colour and smooth mixture enable incorporation into a wide range of food. This bacterial protein powder also exhibits a high protein content with a balanced amino acid profile. However, there is limited data on its digestive behaviour, leaving a critical knowledge gap for assessing its nutritional performance and ensuring a safe dietary use.

Prior to digestion, a nitrogen-to-protein conversion factor of 5.58 was determined based on the amino acid composition and non-protein nitrogen content to guarantee more accurate protein quantification. Size exclusion chromatography (SEC) revealed the presence of high-molecular-weight protein aggregates of approximately 2,000 kDa, likely formed during the spray drying process.

To evaluate the digestive behaviour of this substrate, a standardised semi-dynamic in vitro model was employed to a 4 % (w/w) protein suspension. This model incorporated gradual gastric fluid addition and five gastric emptying at 13.5 min interval to mimic human gastric condition. Progressive acidification during the gastric phase induced protein coagulation. As the pH declined to 3 after 54 min, the rate of protein hydrolysis increased markedly, coinciding with enhanced pepsin activity and resulting in peptides generated predominantly below 10kDa. Phenylalanine, cysteine and tyrosine were identified as the most pronounced amino acids released in the gastric phase. SDS-PAGE analysis revealed a high abundance of proteins, indicating by multiple intense bands, while a large fraction of protein remained insoluble in the gastric digesta.

Each gastric emptying underwent a subsequent intestinal digestion following the INFOGEST static method. Intestinal digestion induced more drastic increase of protein hydrolysis and solubility, with approximately 13 % of total amine groups released in the bioaccessible fraction, indicating the fraction potentially for absorption. Despite the extensive digestion induced by pancreatic enzymes, SEC identified protein fractions at 20-40 kDa resistant to gastrointestinal digestion. Meanwhile, free amino acids were released in this phase, particularly arginine, leucine and phenylalanine, yielding a promising essential-to-non-essential amino acid ratio of 1.44. Proteomics analysis indi-cated an overall protein digestibility between 70 – 85 %.

Overall, these results highlight the nutritional potential of the bacterial protein powder and provide the pioneering insights into its gastrointestinal digestibility, offering guid-ance for food manufacturers and informing consumers on its safe and effective incor-poration into future sustainable diets.

Keywords

semi-dynamic digestion model; bacterial protein; nitrogen conversion factor

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

STARCH PROPERTIES AFFECT IN VITRO DIGESTION AND DIAAS OF PLANT PROTEINS IN HIGH MOISTURE EXTRUDATES

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Abstract

The urgent transition toward a more sustainable and health-oriented food system is resulting in a growing demand for plant-based meat alternatives. Pea and hemp proteins have emerged as primary candidates, as alternative sources to soy, with lower allergenicity and robust nutritional profile. However, the successful formulation of these protein ingredients requires a comprehensive understanding of their interactions with other components of the food matrix, particularly starch, which not only would affect protein structuring but would also have profonde consequences in the digestion kinetics of the protein. In this work, high-moisture extrusion (54% moisture content) was utilized to generate fibrous anisotropic structures from pea and hemp protein isolates, using a protein-dominant formulation (90% protein) supplemented with 10% starch as a structuring agent. The impact of three different maize starches [high-amylose (70%), normal amylose (30%), and waxy (~0% amylose) starches] with different amylose content, swelling kinetics, and fine structures were evaluated on protein structuring, and subsequent oro-gastrointestinal digestion and DIAAS (Digestible Indispensable Amino Acid Score) using INFOGEST 2.0 protocol.

Results revealed that although most extruded matrices underwent substantial protein hydrolysis at the intestinal phase, modulation of protein release was observed dependent on the starch type. Notably, formulations which contained normal amylose maize starch showed the lowest protein digestibility among all samples, in both pea and hemp protein matrices. This reduced hydrolysis correlated with the structural characteristics identified by Low Field 1H-NMR, where protein extrudates containing normal maize starch exhibited serum water of lower proton relaxation times, suggesting the presence of a more restrictive matrix that could limit the enzymatic accessibility. By contrast, high-amylose and waxy starches altered water distribution to a minor extent, and their extrudates exhibited similar hydrolysis, amino acid release, and in vitro DIAAS. These findings demonstrate that starch incorporation promotes matrix structuring and the development of fibrous architectures; however, the specific starch type must be carefully selected to preserve high protein digestibility and avoid compromising nutritional quality.

Keywords

Pea protein; hemp protein; INFOGEST; food matrix; food structure; protein-starch interactions

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

TECHNOLOGICAL PROSPECTING OF PLANT-BASED VACCINES AND BIOACTIVE MATRICES: STABILITY, PATENT TRENDS, AND BIOACCESSIBILITY CHALLENGES

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Abstract

Introduction: The pursuit of sustainable food systems has significantly increased scientific interest in Unconventional Food Plants (UFP) due to their inherent resilience and unique nutritional profiles. However, the gastrointestinal barrier remains a primary obstacle for both functional nutrients and oral vaccine antigens, which are frequently degraded before they can be effectively absorbed. Understanding bioaccessibility is essential to overcoming the "green paradox," where plant-based constituents are neutralized by gastric pH and enzymatic activity. In the current intellectual property landscape, it is becoming increasingly vital to incorporate simulated digestion protocols to verify the exact percentage of an antigen that remains intact and available following oral ingestion.

Methods: The methodology for this study involved a macro-level technological prospecting and a systematic mapping of the patent landscape related to plant-based vaccines and the INFOGEST protocol. A systematic search was conducted across the Google Patents, Espacenet, and Patentscope databases, focusing on the International Patent Classification (IPC) code A23L. The analysis was structured according to three distinct dimensions: Main Innovation and Practical Application, as defined by the Oslo Manual; and the Level of Evidence based on the NASA Technology Readiness Level (TRL) scale, following World Intellectual Property Organization (WIPO) standards for patent landscape reports. For experimental validation, the harmonized INFOGEST in vitro digestion model was utilized to evaluate the biochemical resilience of the chimeric protein BOT, a vaccine candidate against Ascaris suum.

Results and Discussion: The results of the patent analysis revealed that, although there is broad industrial interest in nutrient stabilization, a significant gap exists in the rigorous methodological validation of these innovations. Out of the documents analyzed, only four patents utilized the standardized INFOGEST protocol to verify the stability of their claims, suggesting that most current technologies remain at a lower maturity stage (TRL 2-3). This scarcity highlights the high degree of innovation and the competitive advantage of employing such a harmonized method for oral vaccines incorporated into food matrices. Experimental evidence, to which I contributed, demonstrated that 63.94% of the antigen remains bioaccessible after the sequential stages of digestion. This stability is directly correlated with biological efficacy, as the bioavailable fraction, representing approximately 16µg, was sufficient to induce a 66.2% reduction in lung larval recovery in murine models (Castro, 2023).

Conclusions: Aligning patent trends with INFOGEST bioaccessibility data is key for Novel Foods. This integration ensures the integrity of plant-based matrices and the efficacy of oral vaccines, providing a robust technical foundation for international commercialization and biodiversity innovations.

Keywords

UFP, Bioaccessibility, INFOGEST, Patent Trends, Plant-Based Vaccines

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

FROM INFOGEST TO THE HUMAN GUT: INTEGRATING MATRIX PERSISTENCE, PROTEIN AND STARCH DIGESTION FROM AN IN VITRO AND A HUMAN ILEOSTOMY STUDY

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Abstract

Background

The INFOGEST digestion protocols have become a benchmark for simulating gastrointestinal transit of complex foods. However, our understanding of how closely these models mimic the structural and biochemical breakdown of cereal-based foods and their metabolic outcomes in humans remains limited, highlighting the need for further investigationn.

Objective

To integrate findings from INFOGEST semi-dynamic in vitro digestions and a human ileostomy study of cereal-based foods to provide a mechanistic understanding of food matrix resistance, wheat protein breakdown, starch digestibility and postprandial glycaemia.

Methods

First, three gluten-containing wheat foods (bread, pasta and an extruded breakfast cereal) were digested using the INFOGEST semi-dynamic protocol to investigate structural breakdown, protein hydrolysis and the persistence of potentially immunogenic gluten peptides (R5-antibody reactive). The cereal was then selected to simulate a low level gluten contamination within a cereal-based meal in a human ileostomy study. In this study (NCT04489810), carried out in Cork (Ireland), eleven participants consumed a standardised breakfast (oat porridge plus wheat cereal) and ileal effluent was collected hourly for 8 h. Analyses focused on: 1) microstructural features (SEM, CLSM); 2) biochemical determinations (soluble nitrogen, free amines, immunoreactive gliadin peptides and resistant starch); and 3) postprandial glycaemia.

Main Findings

In vitro, the breakfast cereal maintained heterogeneous particles throughout digestion, and, despite showing the fastest protein release among the tested foods, it exhibited slow protein hydrolysis and high persistence of potentially immunogenic gluten peptides.In vivo, ileal effluents presented amorphous protein and starch agglomerates. In addition, parenchyma-like plant fragments, similar to those observed in vitro, were visible between 4 h and 8 h after breakfast. Total ileal effluent output showed a relatively high interindividual coefficient of variation (CV) (40.2%). However, potentially immunogenic gliadin fractions exhibited a markedly higher variability (CV = 69.4%, range: 3.1 to 65.7 mg of gliadin equivalents) than total nitrogen, protein or free amines, even after normalization by the gliadin-to-soluble-protein ratio. These persistent fractions were predominantly water soluble rather than ethanol soluble. Continuous glucose monitoring and ileal RS measurements are being integrated with these observations.

Conclusions

The high interindividual variability in gliadin excretion points to the upper gastrointestinal tract as the main origin of interindividual differences in gluten hydrolysis, reflecting person-specific digestive capacities. Despite differences between in vitro and in vivo protein hydrolysis patterns, the remarkable concordance in cereal matrix persistence reinforces the potential of INFOGEST protocols to investigate structure–digestion relationships.

Keywords

Wheat, Digestion, Gliadin, Starch, Glycaemic Response, Coeliac disease, Non-coeliac gluten sensitivity

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

FROM SIMULATED DIGESTION TO GUT–LIVER MODULATION: ANTI-INFLAMMATORY EFFECTS OF BRAZILIAN ORGANIC PROPOLIS

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Abstract

Propolis is a resinous substance produced by bees and widely recognized for its remarkable biological properties, including antioxidant, antimicrobial, and anti-inflammatory activities. However, its biological effects depend on gastrointestinal digestion, intestinal absorption, and systemic metabolism (Saliba et al., 2023). This study investigated whether a Brazilian organic propolis extract (OPExt) modulates inflammatory pathways along the gut–liver axis using integrated in vitro and in vivo approaches.

OPExt was subjected to standardized in vitro gastrointestinal digestion (Brodkorb et al., 2019) and applied to a Caco-2/HepG2 co-culture system to simulate intestinal transport and hepatic exposure (Castell-Auví et al., 2010). The basolateral fraction obtained after epithelial transport was used to treat HepG2 cells, followed by RNA sequencing and differential gene expression analysis.

Phenolic acids, flavonoids, terpenes, and lignans were identified in the bioaccessible fraction. OPExt-derived compounds significantly downregulated genes associated with inflammatory response, including CXCL5, CX3CL1, and IL34 (p 0.05). Functional enrichment analysis revealed negative modulation of inflammatory signaling pathways.

For in vivo validation, experimental colitis was induced in mice using dextran sulfate sodium (DSS). Treatment with OPExt (10 mg/kg) reduced pro-inflammatory cytokine levels, including IL-6, TNF-α, and IL-1β, compared with untreated colitis animals. Moreover, ExtOP prevented DSS-induced colon shortening, thereby preserving intestinal structural integrity.

Together, these findings demonstrate that OPExt exerts coordinated anti-inflammatory effects along the gut–liver axis. After simulated digestion and epithelial transport, bioaccessible compounds modulated hepatic inflammatory gene expression, while in vivo administration attenuated intestinal inflammation and preserved colon morphology. Brazilian organic propolis emerges as a promising natural modulator of gut–liver inflammatory crosstalk.

All animal procedures were approved by the Institutional Animal Care and Use Committee (CEUA/UNICAMP, protocol no. 6424-1/2024).

References:

Brodkorb, A. et al. (2019) ‘INFOGEST static in vitro simulation of gastrointestinal food digestion’, Nature Protocols, 14(4), pp. 991–1014. Available at: https://doi.org/10.1038/s41596-018-0119-1.

Castell-Auví, A. et al. (2010) ‘Organotypic co-culture system to study plant extract bioactivity on hepatocytes’, Food Chemistry, 122(3), pp. 775–781. Available at: https://doi.org/10.1016/j.foodchem.2010.03.052.

Saliba, A.S.M.C. et al. (2023) ‘Simulated gastrointestinal digestion/Caco-2 cell transport: Effects on biological activities and toxicity of a Brazilian propolis’, Food Chemistry, 403, p. 134330. Available at: https://doi.org/10.1016/J.FOODCHEM.2022.134330.

Keywords

INFOGEST, Co culture, bioactivity; phenolic compounds

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECT OF OHMIC HEATING ON THE BIOACCESSIBILITY AND BIOACTIVITY OF AÇAÍ PHENOLIC COMPOUNDS

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Abstract

Açaí is a fruit native to the Amazon region, characterized by small, round, dark-purple berries. Its pulp, with a distinctive flavor, is widely consumed in Brazil and worldwide. The fruit is recognized for its high antioxidant capacity, attributed to phenolic compounds, especially anthocyanins, with cyanidin-3-rutinoside (C3R) and cyanidin-3-glucoside (C3G) as the predominant forms. However, the stability of these compounds during processing and their bioaccessibility after digestion are key determinants of their biological functionality. The present study aimed to evaluate the effects of ohmic heating on the stability, bioaccessibility, and bioactivity of phenolic compounds and anthocyanins in açaí, and to investigate the influence of different electric field intensities during simulated gastrointestinal digestion. Samples were subjected to ohmic heating at 50 V, 150 V, and 250 V, followed by in vitro digestion according to the standardized INFOGEST protocol. Total phenolic content, total anthocyanins, and antioxidant capacity (FRAP) were determined before and after digestion, allowing the calculation of bioaccessibility. In addition, samples were evaluated in cellular assays for viability (MTT) and reactive oxygen species (ROS) production using Caco-2 cells. Ohmic treatments increased total phenolic content in the samples before digestion, particularly at 150 V (240.1 ± 11.8 mg GAE/100 g) and 250 V (238.5 ± 26.4 mg GAE/100 g), compared to the control (199.0 ± 2.0 mg GAE/100 g). After digestion, 250 V showed the highest phenolic content (142.0 ± 6.3 mg GAE/100 g) and bioaccessibility (59.5%), exceeding the control (42.8%). For anthocyanins, greater retention after digestion was observed at 250 V (7.6 ± 1.1 mg C3G eq/100 g) compared to the control (2.5 ± 0.1 mg C3G eq/100 g). Antioxidant activity (FRAP) was higher in ohmic-treated samples before digestion, reaching 300.3 ± 9.5 (50 V), 305.3 ± 3.6 (150 V), and 352.0 ± 10.3 mg AAE/100 g (250 V), compared to the control (217.4 ± 4.8 mg AAE/100 g). Although antioxidant values decreased after digestion, treated samples maintained higher levels than the digested control (133.2 ± 3.1 mg AAE/100 g), particularly at 250 V (191.2 ± 1.9 mg AAE/100 g). In cellular assays, samples showed no cytotoxicity in the MTT test and no significant differences in ROS production among treatments. Overall, ohmic heating, especially at 250 V, enhanced the release of bioactive compounds from the food matrix, increasing bioaccessibility and in vitro antioxidant activity without compromising cellular safety, highlighting its potential to optimize the functional properties of açaí.

Keywords

Anthocyanins, In vitro digestion,Electric field processing, Cellular bioactivity

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

MICROENCAPSULATION OF AÇAI WITH PEA PROTEIN: A STRATEGY TO INCREASE THE BIOACCESSIBILITY OF ANTHOCYANINS

Amanda Lais Alves Almeida Nascimento [1 2] , Larissa Lorrane Rodrigues Borges [3] , Janaina Gonçalves Fernandes [3] , Nathália Alves Bento [3] , Luciana Azevedo [3] , Daniel Granato [4] , Fabiana Andrea Hoffmann Sarda [2 5 6 7] , Evandro Martins [1] , Pedro Henrique Campelo [1] , Paulo César Stringheta [1]

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Abstract

Phenolic compounds, particularly anthocyanins, are recognized for their high antioxidant capacity and health-promoting effects, especially in preventing diseases associated with oxidative stress. Berries such as açaí are rich in these compounds and show functional potential in reducing the risk of chronic diseases, including diabetes, neurodegenerative disorders, and cardiovascular diseases. However, the bioaccessibility of these substances is limited and may be as low as 5% after gastrointestinal digestion. In this context, microencapsulation was investigated as a strategy to protect and stabilize phenolic compounds during digestion. The study evaluated crude extract (E) and purified anthocyanins (A), as well as their microencapsulated formulations with pea protein: EM (microencapsulated crude extract) and AM (microencapsulated purified anthocyanins). The microcapsules were characterized by microscopy, zeta potential, and Fourier-transform infrared spectroscopy (FTIR). Antioxidant capacity (DPPH and ABTS) and phenolic profile by HPLC-MS were analyzed before and after simulated gastrointestinal digestion using the INFOGEST protocol. In addition, cell viability assays were performed in HCT-8 (tumor) and HUVEC (normal) cells. Results indicated greater stability of the microencapsulated formulations, as evidenced by more negative zeta potential values for EM (−21.4 mV) and AM (−17.5 mV) compared to A (−8.3 mV) and E (−11.4 mV), suggesting reduced aggregation tendency. Samples containing crude extract showed a larger particle size (1351.0 nm) than those containing purified anthocyanins (944.1 nm). FTIR analysis revealed changes in bands around 1000 cm^{-1} and in Amide I and II bands in the microencapsulated formulations, suggesting intermolecular interactions between phenolic compounds and the protein matrix. Sample A presented the highest initial anthocyanin content (720.4 $mg \cdot g^{-1}$), followed by AM (216.1 $mg \cdot g^{-1}$) and E (46.8 $mg \cdot g^{-1}$). During the gastric phase, an apparent increase was observed in some samples, whereas in the intestinal phase, a marked reduction occurred, with losses exceeding 80% in certain cases. Only EM (128.7%) and AM (143.4%) showed bioaccessibility above 100%, indicating a protective effect of microencapsulation. Overall, encapsulation with pea protein significantly increased phenolic bioaccessibility compared to the non-encapsulated extract. Cell viability assays demonstrated higher cytotoxicity after simulated digestion, indicated by reduced IC_{50} values. The microencapsulated formulations exhibited higher selectivity indices (SIs), particularly for digested AM (SI > 3), suggesting greater activity toward tumor cells. Altogether, these findings indicate that microencapsulation with pea protein enhances the stability, bioaccessibility, and biological activity of açaí anthocyanins, supporting their application in the development of functional ingredients.

Keywords

Plant-based protein matrix, Functional ingredients, Gastrointestinal stability

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

BIOACCESSIBILITY OF LIPOPHILIC COMPOUNDS FROM A SUPERCRITICAL CO2 MUSHROOM EXTRACT USING THE INFOGEST IN VITRO DIGESTION MODEL

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Abstract

Hericium erinaceus (lion's mane) is an edible and medicinal mushroom with a long-standing history of use in traditional Chinese medicine. Its composition is marked by high protein levels and the presence of non-digestible polysaccharides such as β-glucans. Although its lipid content is relatively low, this fraction is of particular interest because it contains bioactive compounds, mainly fatty acids and sterols, that have been associated with anti-inflammatory and antioxidant activities. The potential neuroprotective relevance of these lipophilic constituents reported in the literature is likely to depend on their stability during gastrointestinal digestion and their subsequent bioaccessibility.

The present work aimed to analyze the effect of the digestion process on the composition of an extract of H. erinaceus obtained by supercritical CO_2. Supercritical CO_2 extraction (300 bar, 40 °C, 3 h and no co-solvent) yielded non-polar fractions enriched in sterols and fatty acids. GC-MS profiling of the extract identified fatty acids and sterols as the dominant chemical classes, and ergosterol as the predominant sterol. Twenty-two compounds were detected in the supercritical extract, including 13 free fatty acids, 3 fatty acid ethyl esters, and 6 sterols. Linoleic, oleic and palmitic acids, and to a lesser extent stearic acid, together with ergosterol, were the predominant detected compounds. The extract was then subjected to in vitro digestion using the standardized INFOGEST protocol. Subsequently, the fatty acid and sterol composition was analyzed by GC-MS after sample derivatization. Following digestion, an overall decrease (approximately 5%) was observed, mainly involving the major compounds. Additionally, dehydroergosterol levels increased, likely due to the conversion of ergosterol.

Overall, linoleic, oleic and palmitic acids, together with ergosterol, constituted the main compounds of the lipid fraction of the supercritical extract of H. erinaceus. Simulated gastrointestinal digestion resulted in a moderate loss of the major fatty acids and in qualitative changes in the sterol profile, indicating that digestive conditions may partially alter the composition of lipophilic mushroom extracts. These findings highlight the importance of considering digestive stability when evaluating the potential functional properties of supercritical mushroom extracts intended for food or nutraceutical applications.

Keywords

Hericium erinaceus, supercritical CO2 extraction, fatty acids, ergosterol, in vitro digestion

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

SUSTAINABLE FABA BEAN FAT REPLACERS FROM SIDE STREAMS: APPLICATION IN MUFFINS AND NUTRITIONAL AND DIGESTIVE INSIGHTS

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Abstract

With the increasing extraction of plant-based proteins, valuable by-products such as polysaccharides are generated. To support a sustainable circular bioeconomy, it is crucial to valorize these by-products through a cascading approach, thereby reducing waste and enhancing the value of plant biomass. This study aimed to valorize polysaccharide-rich residual fractions obtained as side streams from faba bean protein extraction processes.

Two extraction scales were evaluated: laboratory scale (LS) and pilot plant (PP), in order to determine how extraction scale influences the properties of the residual fractions. First, a response surface design was developed to assess different oil and residue powder concentrations for the production of emulsion gels with varying mechanical properties. Particle size and gel strength were measured as response variables. After establishing the experimental design, an optimization was performed to obtain formulations from each residue type with a gel strength comparable to that of commercial margarine. The selected emulsion gel formulations were then evaluated for their ability to replace fats in muffin formulations.

The viscoelastic properties of the batters were analyzed, and the muffins were evaluated in terms of composition, physical and mechanical properties, and in vitro macronutrient digestibility. Muffins prepared with margarine and with non-structured oil were used as controls.

The results revealed significant differences in batter viscoelasticity. The margarine-based batter exhibited a higher elastic modulus at room temperature; however, this elasticity decreased during heating. In contrast, batters containing emulsion gels became more elastic upon heating. Muffins formulated with emulsion gels contained approximately 30% less fat than the control, resulting in reduced caloric content. In addition, the lipid profile was improved, with an increase in unsaturated fatty acids and a reduction in saturated fatty acids compared to the control formulation. A slight color variation was observed, with lower L* and b* values, indicating a darker crust and crumb. In terms of texture, the higher starch content and greater gel strength of the LS formulation appeared to influence the final product, as these muffins exhibited the highest hardness. Regarding macronutrient digestibility, a lower degree of lipid hydrolysis was observed in samples containing emulsion gels, while starch and protein digestibility were not affected by their incorporation.

Overall, this study demonstrates that emulsion gels derived from faba bean side streams can effectively replace fats in bakery products, providing a more sustainable and healthier alternative with reduced fat content and an improved lipid profile characterized by higher levels of unsaturated fatty acids. The extraction scale influenced the texture and functional properties of the final product, highlighting the importance of process optimization for industrial applications.

Keywords

Digestibility, Lipid digestion, Emulsion gels, Bakery products

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

BIOACCESSIBILITY OF SELECTED MINERALS AND VITAMINS IN DAIRY AND PLANT-BASED PRODUCTS USING THE IN VITRO DIGESTION MODEL INFOGEST 2.0

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Abstract

With the growing shift toward plant-based diets, the nutritional equivalence of dairy products and their plant-based alternatives remains a critical public health question. As nutrient content does not necessarily reflect physiological availability, this study investigated matrix-dependent micronutrient bioaccessibility using the standardized in vitro digestion model INFOGEST 2.0 and a modified protocol for vitamin K analysis. Six micronutrients of public health relevance—riboflavin, vitamin K (phylloquinone and menaquinones), calcium, phosphorus, zinc, and iodine—were analyzed in dairy products and corresponding plant-based alternatives (n = 3). Post-digestion quantification was performed using ICP-OES (minerals), HPLC-MS/MS (vitamin K), and HPLC-FLD (riboflavin). Riboflavin bioaccessibility was high in dairy matrices (70–100%) and similarly high in a fortified plant-based beverage (~100%). Vitamin K bioaccessibility was moderate in liquid dairy products (~40%) but lower in solid dairy matrices (~15–20%), while several plant-based alternatives showed comparable or higher values (~20–40%). Among the minerals, calcium exhibited moderate bioaccessibility in dairy products (25–40%) but higher values in several plant-based alternatives (50–60%). In contrast, phosphorus bioaccessibility was consistently high in dairy matrices (~90%) and lower in plant-based products (~60%). Zinc and iodine showed high bioaccessibility in dairy products (70–90%) but were not detectable in the selected plant-based alternatives, limiting direct comparison. Overall, micronutrient bioaccessibility was strongly analyte- and matrix-dependent, highlighting the critical role of food matrix effects in evaluating the nutritional value of dairy and plant-based products. As the findings are based on a static in vitro digestion model, actual in vivo bioavailability may differ, and further studies are required to confirm these results.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

GERMINATION AS A STRATEGY TO MODULATE LEGUME PROTEIN DIGESTION: COMPARATIVE ANALYSIS OF PEA AND FABA BEAN VARIETIES USING THE INFOGEST MODEL

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Abstract

Legumes such as peas (Pisum sativum L.) and faba beans (Vicia faba L.) are promising protein sources for plant-based foods due to their high protein content and favourable amino acid profiles. However, their nutritional quality can be constrained by structural barriers, protein matrix interactions, and antinutritional compounds that limit protein digestibility and amino acid bioaccessibility. Germination represents a mild and sustainable processing strategy capable of inducing biochemical and structural modifications, including endogenous enzyme activation, partial protein hydrolysis, and reduction of antinutritional factors, which may influence gastrointestinal digestion behaviour. Despite this potential, systematic comparative studies across legume species and varieties remain limited.

This study investigates the impact of germination on protein digestion in three yellow pea varieties and two faba bean varieties using the standardized INFOGEST static in vitro digestion model. Germinated and ungerminated samples were subjected to simulated gastrointestinal digestion, including both complete oral–gastric–intestinal digestion and stage-specific termination after the gastric phase to assess digestion kinetics and intermediate structural changes. Protein hydrolysis was quantified using the o-phthaldialdehyde (OPA) assay, protein degradation patterns were characterised by SDS–PAGE, and total and soluble protein content was determined by Kjeldahl nitrogen analysis. The comparative experimental design enables evaluation of the effects of legume type, genetic variety, germination duration, and digestion stage, providing insight into structure–digestibility relationships and enzyme accessibility.

Preliminary observations indicate that germination influences protein hydrolysis behaviour during intestinal digestion, suggesting modifications in protein structure and susceptibility to enzymatic degradation. Ongoing analyses are expanding the dataset across additional germination conditions and legume varieties to identify consistent trends and potential mechanistic drivers underlying digestion responses.

This research contributes to improved understanding of germination as a strategy to modulate legume protein digestibility and functionality. Insights from this work may support the optimisation of plant protein ingredients for food applications and inform the development of nutritionally enhanced plant-based products within sustainable food systems.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

INFLUENCE OF CEREAL STRUCTURAL CHARACTERISTICS ON GASTROINTESTINAL PROTEIN DIGESTION IN WEANED PIGLETS

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Abstract

The present study investigated how the structural characteristics of different cereals influence protein digestibility in newly weaned piglets, a critical developmental stage marked by digestive constraints associated with recent weaning. Diets based on oat, extruded oat, wheat, corn, rice, and a starch–casein control were formulated using the cereal as the sole protein source. Digestive responses were assessed in vivo, complemented by in vitro protein digestibility measurements and small-angle X-ray scattering (SAXS) to investigate starch supramolecular organization.

Distinct digestive patterns were observed among cereals. Wheat-, corn-, and rice-based diets resulted in higher residual protein levels in the jejunum four hours after feeding, whereas protein concentrations in piglets fed oat-based and starch–casein diets were comparable to endogenous losses. Extrusion processing of oats reduced jejunal protein content, suggesting enhanced protein digestion. However, the in vitro protocol did not reveal a significant improvement in protein digestibility after oat extrusion, suggesting that the high fiber content of whole oat may continue to impede digestibility regardless of processing. Significant interactions between starch and cereal proteins were observed throughout gastrointestinal digestion. SAXS analysis revealed that starch supramolecular order was largely disrupted during intestinal digestion; however, corn starch partially retained its lamellar structure, in contrast to other cereals.

Overall, these findings demonstrate that cereal-specific structural characteristics and processing methods modulate protein digestion in weaned piglets. Understanding these interactions is essential for optimizing cereal selection and improving protein utilization in early-life nutrition.

Keywords

Cereal grains, protein digestion, starch structure, SAXS, weaned piglets.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

PROCESSING-DEPENDENT RELEASE KINETICS OF PHENOLIC COMPOUNDS FROM STARCH-PHENOLIC COMPLEXES

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Abstract

Dietary phenolic compounds could be released from the food matrix in the gastrointestinal tract to exert their bioactive functions. However, food processing-induced starch-phenolic complexation significantly interferes with phenolic release patterns. This study investigates how extrusion temperature modulates the formation of starch-phenolic complexes and subsequently controls the release of phenolics (both unbound and bound fractions) and starch digestion during simulated in vitro gastrointestinal digestion.

Cold-extruded (40°C) and hot-extruded (90°C) noodles were prepared using buckwheat starch supplemented with phenolic extracts (2.0%, w/w, starch basis) or pure rutin. The intensity and structure of starch-phenolic complexes were characterized by phenolic quantification, X-ray diffraction (XRD) analysis and size exclusion chromatography. A standardized static in vitro digestion model was employed to track phenolic release kinetics across distinct gastrointestinal phases. Unbound and bound phenolics were sequentially extracted and quantified at gastric and small intestinal stages to reveal processing-dependent release behaviors.

Hot extrusion induced the formation of more intensive starch-phenolic complexes, particularly V-type inclusion complexes, evidenced by higher proportions of bound phenolics compared to cold extrusion. This structural difference resulted in distinctly controlled release profiles: during simulated small intestinal digestion, hot-extruded noodles released significantly less unbound phenolics (59.4%) than cold-extruded noodles (68.2%). Similarly, bound phenolics exhibited more sustained release from hot-extruded noodles (41.9%) compared to cold-extruded counterparts (56.5%). For rutin-fortified systems, hot extrusion reduced small intestinal release of unbound rutin from 79.0% to 63.6%, and bound rutin release from 89.7% to 55.8%. Notably, the majority of total phenolics (85.6–94.8%) were released by the end of the gastric phase regardless of processing conditions, indicating stomach-dominated release with subsequent intestinal modulation governed by complex stability. The differential release of unbound versus bound phenolic fractions demonstrates that processing conditions can be strategically tuned to achieve targeted phenolic delivery—facilitating rapid gastric release for local antioxidant effects or sustained intestinal release for systemic bioavailability. These findings provide critical insights into food structure design for optimized phenolic bioaccessibility.

Significance: This study elucidates the pivotal role of processing-induced complexation in governing phenolic release dynamics across gastrointestinal compartments. By manipulating extrusion parameters, food manufacturers can engineer starchy matrices with tailored phenolic release profiles, supporting the development of functional foods with enhanced bioactive compound delivery for precision nutrition applications.

Keywords

starch-phenolic complexation, extrusion, gastrointestinal digestion, controlled release, food matrix

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EVALUATION OF THE BIOACCESSIBLE POTENTIAL OF MICROBIAL METABOLITES FROM BRAZILIAN RED PROPOLIS IN A CACO-2/MACROPHAGE CO-CULTURE MODEL.

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Abstract

Propolis is a resinous mixture produced by honeybees (Apis mellifera) from plant resins and flower buds. Among the different types of Brazilian propolis, red propolis stands out, with its primary botanical source being the resin of Dalbergia ecastophyllum. This variety is notable for containing more than 200 identified compounds, including flavonoids such as retusapurpurin A and B, responsible for its red color, as well as isoflavonoids such as formononetin, vestitol, neovestitol, and daidzein, among others. These compounds are widely recognized for their anti-inflammatory and antimicrobial properties (Bueno-Silva et al., 2017). However, there is still limited information regarding the influence of the microbiota associated in própolis and its botanical source, as well as whether microorganisms are capable of metabolizing to create novel metabolites with anti-inflammatory potential. The present study investigated the microbiota of Brazilian red propolis and evaluated the anti-inflammatory activity of its metabolites using an epithelial transport co-culture model with Caco-2 cells and macrophages (RAW-Luc 264.7). Samples were aseptically collected from beehives located in Alagoas (AL, Brazil). After cultivation, isolation, and identification by PCR, the microorganisms were screened for anti-inflammatory potential, and Pantoea sp. was selected as the most promising strain. The bacterium was cultured in TSB broth, followed by liquid–liquid extraction, yielding a metabolite extract (ME). Both the ME and the crude Brazilian red propolis extract (CBRP) were evaluated for anti-inflammatory activity by measuring the reduction of nuclear factor kappa B (NF-κB) activation in Caco-2/RAW-Luc 264.7) co-culture model, as well as tumor necrosis factor-alpha (TNF-α) levels. All samples were subjected to metabolic profiling by UHPLC-ESI-QTOF-MS/MS. The resulting data were analyzed using molecular networking on the GNPS platform, enabling the annotation and comparison of metabolites among the isolates. The CBRP and ME reduced NF-κB activation by 89% and 64%, and TNF-α levels by 81% and 28%, respectively (p 0.05). In the Caco-2 co-culture model, the ME significantly reduced NF-κB activation by 28% and TNF-α levels by 20%. Metabolomic analysis revealed a diverse metabolite profile under different experimental conditions among the analyzed samples. In conclusion, Pantoea sp., isolated from Brazilian red propolis, produces metabolites with anti-inflammatory activity in a co-culture model, suggesting the presence of compounds with potential bioaccessibility in the human organism.

Bueno-Silva B, Marsola A, Ikegaki M, Alencar SM, Rosalen PL. The effect of seasons on Brazilian red propolis and its botanical source: chemical composition and antibacterial activity. Nat Prod Res. 2017 Jun;31(11):1318-1324. doi: 10.1080/14786419.2016.1239088. Epub 2016 Oct 4. PMID: 27701899.

Keywords

Red propolis, Bioaccessible, Microbiota

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

COMPARISON OF MATRIX BEADS AND CORE–SHELL CAPSULES PRODUCED BY NOZZLE IONOTROPIC TECHNOLOGY FOR THE DELIVERY OF PHENOLIC COMPOUNDS FROM APPLE PEEL EXTRAC

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Abstract

Encapsulation technologies are widely applied in pharmaceuticals, the food industry etc. to improve the stability and controlled release of bioactive compounds. The present study compares two encapsulation strategies available in the Encapsulator B-390 system (Buchi®): matrix beads (MB) and core–shell capsules (CSC).

An apple peel extract (Malus domestica var. Bramley provided by The Apple Farm Ireland) rich in phenolic compounds was incorporated into a polymeric mixture containing 1% (w/v) carboxymethyl cellulose, 1% (w/v) sodium alginate and 10% (w/v) solid extract. This formulation was used to produce MB, using the regular nozzle with diameter 1mm. To produce CSC using the concentric nozzle system (external nozzles diameter 0.9 mm and 0.75 mm internal), the same mixture was used as the core phase, while the shell consisted of the same polymeric solution without the extract. Both capsule types were evaluated for encapsulation efficiency (EE%) and release under simulated gastrointestinal conditions using the standardized INFOGEST in vitro digestion method. Total phenolic content (TPC) was determined using the Folin–Ciocalteu method to further assess phenolic recovery and stability. Results were expressed as mean ± standard deviation, and statistical differences between groups were determined using Tukey's test (p 0.05).

MB showed an EE% of 50.1 ± 4.1%, indicating moderate phenolic retention. CSC achieved a significantly higher EE% of 70.0 ± 4.5% (p 0.05), reflecting improved retention due to core–shell separation. In vitro digestion enabled phase-specific release assessment. Considering only the released fraction, MB delivered 41 ± 1.6% during the gastric phase and 59.5 ± 2.6% during the intestinal phase. CSC showed gastric release to 22.8 ± 0.7%, with 78.1 ± 2.5% delivered under intestinal conditions. Overall, in addition to presenting approximately 20% higher encapsulation efficiency, the CSC demonstrated a more desirable release profile, characterized by reduced gastric release and enhanced intestinal delivery. This behaviour is particularly advantageous for compounds intended to exert their bioactivity in the intestinal environment. However, it should be considered that part of the core–shell capsule structure consists of an external polymeric layer without extract, which may require a larger consumption of total capsule mass to deliver an equivalent amount of bioactive compounds compared to matrix beads

These findings demonstrate the impact of capsule architecture on phenolic stability and targeted delivery. Moreover, considering that the phenolic extract was obtained from apple processing by-products, this approach also contributes to sustainability by promoting the valorisation of agro-industrial residues and supporting circular economy strategies.

References

Sıçramaz, Hatice, et al. "Microstructure and Release Behavior of Alginate–Natural Hydrocolloid Composites: A Comparative Study." Polymers 17.4 (2025): 531.

Keywords

Encapsulation efficiency, Core–shell capsules, Gastrointestinal release, Sustainability, Agri-food by-products

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

DIGESTING COMBINATIONS OF CLIMATE SMART AND MINOR CEREALS AND LEGUMES TO OPTIMIZE PROTEIN INTAKE BUT NOT ONLY…

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Abstract

International recommendations (FAO, WHO, EAT-Lancet…) emphasize the urgent need to shift toward more sustainable diets by rebalancing protein intake and promoting high-quality plant sources. Globally, cereals are the primary source of plant protein and essential amino acids, accounting for about 50% of total intake. However, diversification of plant protein sources is essential "from field to plate," notably by increasing the role of legumes—key to the agroecological transition—and integrating minor or climate-smart crops such as spelt, millet, sorghum, and cowpea.

Protein content varies considerably among sources: cereals and pseudocereals contain 7–19% protein (d.m. basis), while legumes contain roughly double that amount (14–40%). Beyond quantity, protein quality is crucial. Protein quality is influenced by various levels of organization of plant protein, antinutritional factors and the so-called matrix effect. Protein quality highly depends on the essential amino acid profile of the source: cereals, particularly wheat, are deficient in lysine, whereas legumes are often limited in sulfur-containing amino acids. Nevertheless, some sources achieve high chemical scores (soybean, cowpea, Bambara pea, etc.). Combining cereals and legumes within a single meal provides complementary and balanced amino acid profiles, a practice already widespread in many traditional cuisines.

Our research has built on this complementarity by developing staple foods combining cereals, legumes, and possibly including leafy vegetables rich in fibers and antioxidants. Technological processes were also applied to reduce antinutritional factors. Mixed pastas (wheat/pea, wheat/lentil, wheat/faba bean) were formulated with up to 22% protein (vs 13% in conventional pasta), 4 times more fibers, and improved amino acid balance. Tested in aged rats, they showed fair digestibility (80–90%), although lower than that of dairy proteins (Berrazaga et al., PhD thesis, 2018).

Within the INNOFOOD Africa project, bi- or tripartite formulations combining cereals, legumes, and leafy vegetables adapted to climate conditions enabled the development of pasta meeting requirements for protein, fiber, iron, zinc, and vitamin B9, with digestibility twice as high as wheat-only pasta (based on INFOGEST i-PDCAAS; Pinel et al., LWT, 2024a,b; Pinel et al., FRI, 2025). Similar combinations proved relevant for fortified infant flours, capable of delivering proteins, lipids, and micronutrients (M. Cancalon, PhD thesis, 2023).

In conclusion, while cereals remain a fundamental component of human diets, their nutritional value can be significantly enhanced through combinations with legumes and leafy vegetables. These approaches improve protein quality, support agricultural diversification, and contribute to climate adaptation. However, careful attention must be paid to the potential allergenic risk of certain new plant protein sources (Pinel et al., Food Chem., 2025).

Keywords

in vitro digestion; combination of cereal/legumes; diversification of diet; climate smart crops; minor crops;

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

FERMENTATION TIME MODULATES THE INTESTINAL FATE OF PHENOLIC COMPOUNDS AND CAFFEINE IN COFFEE-DERIVED KOMBUCHAS

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Abstract

Background: Kombucha is a functional beverage, traditionally fermented from Camellia sinensis tea. Coffee by-products, such as roasted coffee beans, cascara, and leaves, are rich in phenolic compounds and serve as innovative substrates for kombucha fermentation [1,2]. However, a significant knowledge gap persists regarding the fate of these phytochemicals during prolonged microbial fermentation and their subsequent bioaccessibility and absorption in gut.

Objective: Evaluate the effect of fermentation time on the stability, bioaccessibility and intestinal permeability of phenolic compounds and caffeine in coffee-derived kombuchas.

Methods: Coffee-derived kombuchas (from roasted bean, leaf, and cascara infusions) were prepared as previously described [1,2] and monitored at days 0, 6 and 9. Bioaccessibility was evaluated using the standardized static INFOGEST 2.0 protocol, and intestinal permeability was assessed in a Caco-2/HT-29 co-culture cell intestinal monolayer model following 180 min exposure. Fifteen phenolic compounds and caffeine were searched by LC-MS and quantified by HPLC-DAD at T0, T6 and T9 fermentation days, after simulated gastrointestinal digestion (T0, T6 and T9), and in both apical and basolateral compartments following intestinal transport (T0 and T9).

Results: Chromatographic monitoring of the kombuchas pointed T6 as the optimal point to maximize the preservation of bioactive compounds. Extending fermentation to T9 induced a marked compound degradation across matrices. Specifically, in coffee bean kombucha, prolonging fermentation from T6 to T9 drastically reduced gallic acid (12.35 to 3.88 mg/L), 5-caffeoylquinic acid (5-CQA; 11.66 to 8.54 mg/L), ferulic acid (2.32 to 1.13 mg/L) and caffeine (101.21 to 45.04 mg/L). Similar matrix-wide degradation was observed in leaf and cascara kombuchas, with pronounced reductions in representative compounds such as 5-CQA, mangiferin, gallic acid and caffeine. Following simulated digestion, T6 samples exhibited higher bioaccessible concentrations of phenolics than T9, with matrix- and compound-dependent reductions, particularly pronounced in leaf kombucha (up to ~60%). In the Caco-2/HT-29 model, native phenolic compounds exhibited poor intestinal permeability, only trace amounts of smaller phenolic metabolites, such as 3,4-dihydroxybenzoic and ferulic acids were detecte, whereas caffeine demonstrated higher permeability.

Conclusion: Extending fermentation to T9 compromises phytochemical stability and, in a matrix- and compound-dependent manner, reduces bioaccessible levels. Intact phenolic compounds exhibited limited basolateral transport in the Caco-2/HT-29 model. These findings suggest that the intestinal availability of native phenolics is restricted and that their potential physiological relevance may rely more on subsequent microbial biotransformation than on direct small-intestinal absorption.

[1] 10.3390/foods12091905

[1] doi.org/10.3390/foods12142710

Keywords

coffee-derived kombucha; phenolics; caffein, in vitro digestion; absorption

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IMPACT OF NANOEMULSION COMPOSITION ON THE IN VITRO DIGESTION OF ALGAL OMEGA-3 FATTY ACIDS

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Abstract

Algal lipids are a rich source of omega-3 fatty acids, yet their incorporation into functional foods is limited by poor water solubility and low bioaccessibility. Nanoemulsions offer a strategy to improve lipid delivery by modulating droplet size, interfacial properties, and digestion kinetics, potentially enhancing the release and micellarization of bioactive lipids. This study aimed to evaluate how nanoemulsion composition influences the in vitro digestibility of algal lipids.

Algal lipid extract was obtained from a commercial algae blend using a food-grade ultrasound-assisted extraction with ethyl acetate and then was mixed with sunflower (SUN) or medium-chain triglycerides (MCT) oil. Nanoemulsions were prepared via a two-step process involving ultra-turrax homogenization followed by probe ultrasonication. Rhamnolipids were used as biosurfactants, and two oil phase concentrations were tested, including 1% and 5% (w/w). Nanoemulsions were characterized for droplet size, polydispersity index (PDI), and zeta potential, and their stability was assessed over a pH range of 2.5–9. In vitro digestion was performed following the standardized INFOGEST protocol, and the digestibility of total omega-3 and α-linolenic acid (ALA) from algal extract was analyzed by GC–MS.

Droplet size increased with oil phase concentration and was consistently larger in SUN-based nanoemulsions (E–SUN1: 163.8±2.9 nm; E–SUN5: 208.3±6.7 nm) than MCT-based nanosystems (E–MCT1: 137.4±2.2 nm; E–MCT5: 187.6±1.2 nm). All formulations exhibited narrow size distributions (0.21±0.03 – 0.25±0.01), and highly negative zeta potential values (−68.5±2.6 to −70.9±3.2 mV). pH stability tests revealed that nanoemulsions remained stable under acidic and basic conditions, though a slight reduction in negative zeta potential at low pH suggested partial interface destabilization.

In vitro digestion showed that SUN-based nanoemulsions achieved higher total omega-3 release (E–SUN1: 76.2±5.2%; E–SUN5: 79.9±3.5%) than MCT-based nanosystems (E–MCT1: 58.5±2.6%; E–MCT5: 61.7±1.6%), likely due to the rapid hydrolysis of medium-chain triglycerides, which limits enzymatic access to longer-chain omega-3 fatty acids from the algal extract. ALA followed the same trend (E–SUN1:78.9 ± 6.0%; E–SUN5: 85.3±2.2%; E–MCT1: 73.2±1.5%; E–MCT5: 77.2±1.1%). Both oil types demonstrated a concentration-dependent effect, with higher digestibility at 5% oil phase. Moreover, nanoemulsions outperformed the corresponding oil–extract mixture.

These results demonstrate that both carrier oil and lipid loading critically modulate enzymatic access and micelle formation, highlighting the advantage of nanoemulsion encapsulation in enhancing omega-3 bioaccessibility. Tailoring formulation parameters offers a promising strategy to optimize lipid digestibility and bioaccessibility in functional food applications.

Keywords

algal lipids, nanoemulsions, in vitro digestion

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

IRON BIOACCUMULATION AND BIOACCESSIBILITY IN IRON-FORTIFIED MYCELIA OF EDIBLE MUSHROOMS

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Abstract

Edible mycelium can be a suitable vehicle for iron biofortification. Iron biofortification is the method to add iron to suitable foods intending to address iron deficiency anemia, a significant public health issue worldwide. Many strategies exist to combat iron deficiency, and among them, iron-fortified foods can improve health in a cost-effective manner without any dependence on pharmaceutical supplements. It increases population iron levels more safely. The objective of the present study was to evaluate total iron content, and iron bioaccessibility of mycelia from seven edible wood decay fungi, two edible saprotrophic fungi, and one medicinal parasitic fungus at 50, 100, 200, and 500 ppm iron concentrations.

Flammulina velutipes, Grifola frondosa, Hypsizygus tessellatus, Laetiporus sulphureus, Lentinula edodes, Pleurotus eryngii, Pleurotus ostreatus, Lepista sordida, Morchella angusticeps and Cordyceps militaris were cultivated on Potato Extract Agar where iron concentrations were 0 ppm (control), 50, 100, 200 and 500 ppm at 24-25°C for 60 days. Mycelial biomass was then harvested, washed, dried, ground and acid digested (HCl/HNO_3) for iron determination by Atomic Emission Spectroscopy. Iron solubility was evaluated using the standardized static INFOGEST in vitro gastrointestinal digestion method and expressed as the proportion of soluble iron after digestion relative to the total iron content of the samples. Dried mycelia (1 g) of Pleurotus ostreatus, Lepista sordida, and Morchella angusticeps, grown at 200 ppm Fe and selected based on their high iron bioaccumulation capacity, were subjected to sequential oral, gastric, and intestinal digestion phases at 37°C under gentle agitation. Enzyme activities and bile concentrations were adjusted according to the harmonized INFOGEST protocol.

Iron bioaccumulation in mycelia was markedly species dependent, with most fungi exhibiting their highest iron concentration at 200 ppm supplementation. At this level, iron contents were 3,864.73 ± 810.88 mg/kg db in Flammulina velutipes, 421.65 ± 177.75 mg/kg db in Grifola frondosa, 6,566.01 ± 176.14 mg/kg db in Hypsizygus tessellatus, 1,215.22 ± 32.38 mg/kg db in Laetiporus sulphureus, 4,064.09 ± 1,015.23 mg/kg db in Lentinula edodes, 5,024.15 ± 231.94 mg/kg db in Pleurotus eryngii, 9,296.66 ± 640.87 mg/kg db in Pleurotus ostreatus, 9,808.71 ± 1,064.34 mg/kg db in Lepista sordida, 13,003.49 ± 178.99 mg/kg db in Morchella angusticeps, and 32,163.26 ± 721.83 mg/kg db in Cordyceps militaris. The soluble fraction obtained after in vitro simulated gastrointestinal digestion of iron-enriched mycelia showed that Pleurotus ostreatus, Lepista sordida, and Morchella angusticeps released 35.20 ± 3.52, 43.49 ± 2.08, and 36.46 ± 8.75 mg/kg db of iron, respectively indicating that only limited amounts of bioaccumulated iron became soluble during digestion, suggesting relatively low potential iron bioaccessibility in the tested fungal mycelia.

Keywords

Mycelia, edible fungi, iron, solubility, bioaccessibility, bioaccumulation

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

ASSESSMENT OF IRON BIOACCUMULATION AND BIOACCESSIBILITY IN IRON-FORTIFIED MYCELIA OF EDIBLE MUSHROOMS

Gozde Gulseren [1] , Juan E. Andrade Laborde [1] , Matthew Smith [2]

[1] Food Science and Human Nutrition Department, University of Florida, [2] Plant Pathology Department, University of Florida

Abstract

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Keywords

mycelia, iron, bioaccumulation, bioaccessibilty, edible fungi

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

COMPARATIVE ANALYSIS OF EXTRACELLULAR VESICLES FROM HUMAN AND ANIMAL MILK AND INFANT FORMULA

Izabela Koss-Mikołajczyk [1] , Klaudia Tiszbein [1] , Natalia Maciejewska [1] , Magdalena Tiuchtij [1]

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Abstract

Milk is a biologically complex fluid that provides not only nutrients but also bioactive components that may influence infant development. Among them, extracellular vesicles (EVs) are nanoscale membrane-bound particles released by mammary and immune cells. Milk-derived EVs are relatively resistant to gastrointestinal digestion and are considered potential mediators of molecular communication between mother and infant, capable of interacting with intestinal and immune cells.

Because some infants cannot be breastfed and must rely on animal-milk–based infant formula, it is important to determine whether EVs are present across different milk sources and whether their biological activity is preserved. The aim of this study was therefore to compare EVs isolated from human, cow, goat, and sheep milk and to assess their presence in commercial infant formulas, including powdered and ready-to-use products.

EVs were isolated using PEG-precipitation method, following removal of cells, fat, and casein fractions. Vesicles were then characterized in terms of particle size, concentration, and abundance. Their identity was confirmed by detection of canonical EV protein markers using western blotting and by visualization with confocal microscopy. Functional activity was evaluated in vitro using colon-derived cell models to assess the effects of EVs on intestinal cell responses.

EVs were detected in milk from all studied species as well as in infant formula products. Although vesicles shared common structural characteristics, differences in abundance and biological activity were observed between sources. In vitro assays indicated that milk-derived EVs can modulate intestinal cell behavior, supporting their potential role as bioactive carriers of molecular signals.

Keywords

extracellular vesicles, exosomes, human milk, milk

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

ALCOHOLIC BEVERAGES MODULATE LIPID OXIDATION AND FATTY ACID ESTERIFICATION DURING IN VITRO GASTROINTESTINAL DIGESTION OF HEATED MEAT

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Abstract

Research question

Alcoholic beverages are frequently consumed alongside meat, used for culinary meat marination, or served as part of the meal. While the antioxidant potential of red wine and beer has been well-studied, recent studies indicate the formation of potentially harmful fatty acid ethyl esters (FAEE) during digestion of meat, resulting from lipase-catalysed esterification of fatty acids with ethanol. The occurrence and potential implications of FAEE formation under gastrointestinal conditions warrant further investigation.

Material and methods

A formulated pork mince (82% minced shoulder, 12.3% lard, 1.6% salt, 4.1% corn starch) was prepared and marinated with dark beer (10% ethanol), red wine (14% ethanol), or water (control) at a meat-to-beverage ratio of 1:0.67 (g:ml). Marinated meats were oven-heated under short-term high-temperature (180°C until core temperature reached 70 °C), or prolonged low-temperature (110 °C until core temperature achieved 100 °C) conditions. Heated meats with equal dry matter content were subjected to in vitro gastrointestinal digestion. Non-marinated meat was co-digested with these alcoholic beverages, and marinated meats with water, using the same volume as applied for marination. Heated and digested samples were characterized for their levels of lipid oxidation products (4-hydroxy-2-nonenal and hexanal) using HPLC-FLD, and total fatty acids and FAEE using GC-FID.

Main findings

Substantial formation of lipid oxidation products was observed during simulated gastrointestinal digestion, which was markedly reduced via marination (≤ 92%) or co-digestion (≤ 80%) of meat with alcoholic beverages. Such mitigation effects depended on both the type of alcoholic beverage and meat heating conditions. As marinades, both beverages effectively inhibited lipid oxidation during digestion of moderately heated meats (72-92%). Red wine remained highly effective (86%) compared to dark beer (51%) during digestion of intensively heated meats. When beverages were added as part of the meal, red wine consistently demonstrated a higher antioxidant capacity (59-80%) than dark beer (11-40%), irrespective of heating conditions.

Several FAEEs were detected in digests, derived from C16:0, C18:0, C16:1 Δ9, C18:1 Δ9, and C18:2 n-6. Overall, beverages added as part of the meal led to distinct FAEE formation (2-35-fold higher) following digestion, compared with their use as marinades. Red wine resulted in up to 5-fold higher levels of FAEE production compared to dark beer, when added as part of a meal, while such difference disappeared in the context of marination. Notably, co-digestion of red wine with intensively heated meat resulted in 2-fold higher levels of FAEE than with moderately heated meat, an effect not observed for other treatments. Across all conditions, the conversion of fatty acids to FAEE followed PUFA > MUFA > SFA, reaching up to 2.8% for C18:2 n-6, 1.9% for C16:1 Δ9 and C18:1 Δ9, and 0.2% for C16:0 and C18:0.

Keywords

4-hydroxy-2-nonenal, antioxidant capacity, lipase, linoleic acid, oleic acid, palmitoleic acid

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EXPLORING VITAMIN B12 ACCESSIBILITY IN THREE SPECIES OF MARINE MACROALGAE

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Abstract

Certain species of seaweed have been shown to contain vitamin B12, which makes them one of few vegan sources of this important vitamin. The green seaweed Ulva fenestrata is especially rich in B12 (Trigo et al., 2025), while the red seaweed Palmaria palmata has a more modest content. B12 content in the red filamentous Colaconema sp. has to the best of our knowledge not been evaluated yet. Overall, few studies have examined vitamin B12 in macroalgae, and information on its in vitro accessibility remains limited.

Since vitamin B12 in seaweed originates from symbiosis with cobalamin producing bacteria living on the surface of the seaweed fronds (Smith et al., 2007), we hypothesized that morphological traits that increase surface area may enhance B12 accumulation. This is of particular interest in P. palmata, where two morphologies were observed during tank cultivation: the regular flat fronds and a ruffled form characterized by thinner, smaller fronds with higher surface-area-to-volume ratio.

The focus of this study is to evaluate the in vitro accessibility of vitamin B12 in the three seaweed species U. fenestrata, Colaconema sp., and the two morphologies of P. palmata using the standardized Infogest 2.0 protocol. Vitamin B12 content will be quantified by UPLC–UV in both undigested samples and the bioaccessible fraction obtained after in vitro digestion.

Keywords

Vitamin B12, cobalamin, seaweed, in vitro digestion, Infogest 2.0, accessibility

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

DESORPTION OF ADSORBED COMPOUNDS FROM MICROPLASTICS DURING DIGESTION PROCESSES

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Abstract

One of the major problems facing our society is the high demand for plastics and their production. Food products and packaging can be a constant source of exposure to micro- and nanoplastics. It has been shown that microplastics can adsorb contaminants and then potentially transfer them to the body. In our research, we examined how gastrointestinal conditions in an in vitro model affect the desorption of compounds, using copper as an example. Desorption was highest under gastric conditions and varied depending on the type and size of polymer. We also tested microplastic impact on the gut microbiota and DNA damage (using comet assay). Our aim was to gain a better understanding of the potential impact of ingested microplastics.

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

DESIGN AND IN VITRO EVALUATION OF NUTRIENT-OPTIMIZED PLANT-BASED STAPLE FOODS FOR ADULTS AND YOUNG CHILDREN

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Abstract

Malnutrition continues to increase worldwide, affecting both adults and children. In 2022, 2.5 billion adults were overweight, while 9.2% of the global population experienced chronic hunger. Among children under five, 22% suffered from stunting and 5.6% from wasting. Addressing this double burden requires strategies that improve both diet quality and sustainability. Optimizing staple foods with locally produced, climate-smart ingredients is a promising solution.

This study developed tripartite combinations of cereals, legumes, and leafy vegetables selected for their complementary essential amino acids, favorable omega-3 content, and manageable anti-nutritional factors. Leafy vegetables were included to enhance iron, omega-3, and antioxidant levels. Linear programming was used to design formulations meeting the nutritional needs of two groups: adults (especially women) and children aged 6–24 months.

For adults, optimized blends were processed into pasta formulated to meet recommendations for protein, fiber, iron, zinc, and vitamin B9. For young children, complementary infant flours were produced and fortified with long-chain polyunsaturated fatty acids (PUFAs) and vitamins A and E. After processing and cooking, products were evaluated for culinary or oxidative properties and subjected to in vitro digestion using the INFOGEST protocol adapted to adults or infants. Nutrient bioaccessibility was then assessed.

Four optimized pasta formulations based on cowpea flour, with or without teff and amaranth leaves, were obtained. All achieved a chemical score above 100 and an omega-6/omega-3 ratio below 5. A 100 g portion met FAO recommendations for key nutrients in adults, particularly women, and two formulations also covered beta-carotene needs. Compared with conventional wheat pasta, the optimized pasta contained much more fiber (17% vs. 4%), similar or lower rapidly digestible starch, balanced essential amino acids, and comparable protein digestibility despite higher anti-nutritional factors.

The optimized infant flour, based on a teff–wheat–soy blend, was fortified with docosahexaenoic and arachidonic acids and linolenic acid. It met PUFA requirements (omega-6/omega-3 ratio of 3.7) and covered vitamins E and A as well as carotenoids. Long-chain PUFA bioaccessibility was good, although protein and vitamin E bioaccessibility were more limited.

Overall, optimized cereal/legume/leafy vegetable combinations represent a promising strategy to promote sustainable, plant-based diets tailored to adults and young children. Their success depends on proper raw material characterization, controlled processing, and careful evaluation of nutrient bioaccessibility.

Keywords

linear programing ; adult and infant in vitro digestion models ; climate smart crops ; tripartite combination ;

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECT OF IN VITRO DIGESTION ON THE BIOACCESSIBILITY AND BIOACTIVE PROPERTIES OF ENCAPSULATED OLIVE LEAF SUPERCRITICAL FLUID EXTRACT

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Abstract

Olive leaves, a by-product of the olive industry and a rich source of bioactive compounds, can be effectively extracted for polyphenolic compounds using supercritical fluid extraction, a green extraction technique. The objective of this research is to encapsulate supercritical fluid extracts of olive leaves (OLESFE) by the electrospray method, using different cyclodextrins—hydroxypropyl-β-cyclodextrin (HPβCD), hydroxypropyl-γ-cyclodextrin (HPγCD), and methylated-β-cyclodextrin (MPβCD)—and to investigate the stability and bioaccessibility of the resulting high-polyphenol encapsulated powders under simulated gastrointestinal digestion. The prepared powder particles were examined using scanning electron microscopy (SEM) to assess morphology and thermogravimetric analysis (TGA) to evaluate their thermal stability. The antioxidant activity and total phenolic content (TPC) of the powder particles were determined by the ABTS radical-scavenging assay and the Folin–Ciocalteu method, respectively. UHPLC–Orbitrap® HRMS was employed to quantify the principal individual phenolic and flavonoid compounds in encapsulated and digested samples. Both digested and undigested particles exhibited antioxidant activity and contained phenolics including oleuropein, 2-(4-hydroxyphenyl)ethanol (tyrosol), 4-hydroxybenzoic acid, salicylic acid, vanillic acid, trans-cinnamic acid, coumaric acid (trans-3-hydroxycinnamic acid), caffeic acid, caffeic acid phenyl ester (CAPE), ferulic acid, and flavonoids such as chrysin, apigenin, acacetin, genkwanin, and pinocembrin. The results of this study provide new insights into the bioaccessibility and transformation of encapsulated OLESFE, and may inform the design of novel food ingredients with enhanced functional properties and potential health-promoting effects.

Keywords

olive leaf, supercritical fluid extract, electrospray, in vitro digestion,, encapsulation, polyphenolic compounds

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CHANGES IN PHENOLIC CONTENT OF PLANT-BASED MEAT ANALOGUES ENRICHED WITH SELECTED SUPERCRITICAL FRUIT EXTRACTS DURING IN-VITRO DIGESTION

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Abstract

Plant-derived phenolic compounds are increasingly incorporated into plant-based foods to enhance their functional value; however, their stability during digestion in complex food matrices remains insufficiently understood. In this study, plant-based meat analogue formulations enriched with selected fruit extracts obtained by supercritical CO_2 extraction (beetroot (Beta vulgaris), pink barberry (Berberis spp.), and rosehip (Rosa canina)) were prepared to evaluate changes in phenolic content and antioxidant capacity during in-vitro digestion.

Plant-based patties were formulated using selected plant protein matrices combined with liquid supercritical fruit extracts. Total phenolic content (Folin–Ciocalteu method) and antioxidant activity (DPPH assay) were determined to assess antioxidant potential prior to digestion. Simulated gastrointestinal digestion based on the INFOGEST protocol is currently being performed, and changes in total phenolic content and antioxidant capacity across digestion stages are being evaluated.

This study integrates supercritical extraction, plant-based food formulation, and in-vitro digestion modelling to better understand how food matrix composition influences phenolic stability and potential bioaccessibility in plant-based meat analogues.

Keywords

plant-based meat analogues; phenolics; antioxidant capacity; supercritical CO_2 extraction; INFOGEST protocol

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

EFFECT OF 3D FOOD PRINTING PARAMETERS ON TECHNOLOGICAL AND NUTRITIONAL PROPERTIES OF PROTEIN-BASED FOOD GELS ADDED WITH ALPHITOBIUS DIAPERINUS INSECT FLOUR

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Abstract

The world population growth is consequently causing an increasing demand for meat consumption, putting immense pressure on food industry. Research on alternative protein sources is required, willing to reduce environmental impact of conventional food systems. Among meat-analogues, plant-based meat, cultured meat and edible insects currently attract the most consumer attention. Edible insects are particularly notable for their high feed utilization rate, high feed efficiency, rapid life cycles, extended shelf life, and low disposal rates compared to conventional meat sources. Beyond these environmental benefits, edible insects also possess high nutritional value and are increasingly viewed as a key candidate to meet the growing global demand for meat. Despite these advantages, challenges persist regarding consumer acceptance, particularly the unappealing appearance of insects and perceptions that their consumption is primitive. 3D food printing (3DFP) emerges as a promising approach to enhance the acceptance of insect-based foods. By allowing precise control over shape, texture, and presentation, it can transform insect ingredients into visually appealing and familiar products that improve consumer perception and willingness to try them.

This study investigates the relationship between process parameters, protein denaturation, and digestion kinetics in 3D-printed protein-based food systems added with Alphitobius diaperinus insect flour. The research aimed to elucidate how temperature and shear during extrusion influence the structural, functional, and nutritional properties of printable protein matrices, composed of soy protein isolate (SPI), Alphitobius diaperinus flour, potato starch, gluten, cellulose and water. Three main formulations were designed: a control system containing only SPI as novel food and two partially substituted with insect flour at increasing concentrations (2.5% and 5%). Thermal, rheological, and morphological analyses (DSC, oscillatory rheology, and SEM) revealed that printing temperature strongly affected protein denaturation and the resulting microstructure. In vitro digestion assays demonstrated that samples printed at denaturation temperature displayed enhanced proteolytic degradation compared with those printed cold, indicating improved enzyme accessibility due to controlled denaturation.

References

1) Tang et al. Edible insects as a food source: a review. Food Prod Process and Nutr. (2019).

2) Mantihal et al. 3D food printing of as the new way of preparing food: A review, International Journal of Gastronomy and Food Science (2020)

Keywords

3D Food Printing, Alphitobius Diaperinus, Alternative Proteins, In Vitro digestion, Novel Food, Rheology

Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

BIOACCESSIBILITY OF AMINO ACIDS FROM EDIBLE INSECTS AS NOVEL FOOD AFTER IN VITRO DIGESTION

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Abstract

Over the last decade, the scientific interest in edible insects as an alternative food source for the human diet has increased drastically. However, insects are not a new phenomenon in human nutrition. In tropic regions, parts of Africa, Asia, and Latin America edible insects are a part of traditional diets, but are also consumed nowadays. In the European Union four species of edible insects were approved as Novel Food until 2026: House cricket (Acheta domesticus), Migratory locust (Locusta migratoria), Lesser Mealworm (Alphitobius diaperinus) and Yellow mealworm (Tenebrio molitor).
Independent of species, edible insects have a high protein content with a beneficial amino acid profile, are rich in mono- and polyunsaturated fatty acids and contain minerals and vitamins. Insects contain chitin, that acts as a dietary fibre, which could influence digestibility. Additionally, in terms of environmental sustainability insects are a promising alternative protein source to conventional livestock – particularly in the context of a projected world population increase to ten billion people by the year 2050. However, due to their novelty in Western diets, there are challenges of consumer acceptance, legislation and food safety (microbial contamination or allergic reactions).

Huge effort was put into characterising the nutrients content and food safety aspects of edible insects. However, the digestibility and even more the bioavailability of nutrients from edible insects has not received the same attention yet.
Using a static in vitro digestion model (INFOGEST 2.0), the digestibility of samples derived from edible insects was investigated, with a focus on the bioaccessibility of amino acids. This method simulates the digestion in the upper gastrointestinal tract until the small intestine in vitro under static physiological conditions. A commercial whey protein powder served as a control, as it had a digestibility of almost 100%. The success of the in vitro digestion, was confirmed by polyacrylamide gel electrophoresis.
The individual amino acids of the digested samples were measured using an UPLC-MS/MS approach. Additionally, the amino acids in the undigestible fraction of the samples were analysed after a subsequent HCl hydrolysis.

In the future, the determined in vitro protein and amino acid digestibility scores will be verified in an in vivo human study.

Reference:
Nachtigall, L.; Grune, T.; Weber, D. Proteins and Amino Acids from Edible Insects for the Human Diet—A Narrative Review Considering Environmental Sustainability and Regulatory Challenges. Nutrients 2025, 17, 1245, doi:10.3390/nu17071245

Keywords

edible insects, alternative protein sources, in vitro digestion, bioaccessibility, amino acids, UPLC-MS/MS

Acknowledgements

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Topic: Food Digestion and Its Effect on the Bioaccessibility and Bioavailability of Nutrients and Bioactives

CHANGES IN HUMAN MILK STRUCTURE DURING LACTATION: EFFECT OF GESTATION

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Abstract

Human milk (HM) is a natural oil-in-water emulsion, consisting of lipids, proteins, carbohydrates, minerals, and bioactive components. Its colloidal organization plays a significant role in nutrient delivery, digestion, and bioavailability for the developing infant. The (bio)chemical composition of HM can vary significantly throughout the lactation period, and differs between milk produced after full-term and preterm delivery. This study investigates the influence of lactation stage and gestational age on the colloidal structure of HM. Milk samples were collected at two lactation stages (days): colostrum (1-7 days) and mature milk (30-60 days) with each participating donor providing a pair of samples. Samples were obtained from mothers of full-term and preterm infants, resulting in four experimental groups of milk samples. The colloidal structure of HM was characterized by particle size measurements using laser diffraction. Mid-infrared spectroscopy (MIRIS) was applied to determine the macronutrient composition of each sample. Statistical analyses were then conducted to evaluate whether variations in particle size distribution were systematically associated with lactation stage, gestational age, and milk composition. Our findings indicate differences in the colloidal properties of HM between different groups analysed. Variations in particle size distribution were detected, suggesting structural changes that may occur during lactation. Associations between compositional parameters and particle size were also observed, pointing to a potential relationship between milk composition and colloidal organization, which in turn can potentially affect milk digestibility. These insights may contribute to the development of infant formulas that better mimic the structural and compositional characteristics of human milk at different stages of lactation and dedicated to either full-term or preterm infants.

Keywords

human milk, colloidal structure, particle size measurements, mid-infrared spectroscopy, statistical analysis

Acknowledgements

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SESSION

3 Advances in Digestion and Absorption Models

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Engineer in Food Sciences, Stéphanie Blanquet-Diot is full professor in Microbiology and Biotechnology at University Clermont Auvergne (Clermont-Ferrand, France). She is currently deputy Director of the UCA/INRAE 0454 MEDIS research group (Microbiology, Digestive Environment and Health).
She leads the HOMIGUT (Host Microbes Interactions in the Human Gut) international associated lab with Ghent University in Belgium (Prof Tom Van de Wiele) working on human small intestine and associated microbiome. She is also managing an in vitro gut simulation platform combining gastric, small intestine and colon models (including TIM, ESIN, ARILE, ARCOL and SHIME models) and is since 2024 the vice-chair of the INFOGUT CA23110 COST action on in vitro colon models simulating gut microbiota.
She has strong expertise in digestive physiology, intestinal microbiology and in vitro gut modelling of human and animal gastrointestinal tract, under both physiological and pathological situations (digestive diseases, obesity, antibiotherapy).
She co-authored almost 100 publications in this field of research.

ABSTRACT

New *in vitro* models of the small intestine to study microbiota: Bridging the gap between the bench and the human gut

Auriane Bron, Karen Delbaere, Charlotte Deschamps, Maurice Tandefe, Sylvain Denis, Jana de Bodt, Lucie Etienne-Mesmin, Ludovica Marinelli, Tom Van de Wiele, Stéphanie Blanquet-Diot

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The small intestine is the primary site of food digestion and nutrient absorption in humans. Its associated microbial communities are widely shaped by continuous shifs in nutritional and physicochemical conditions along this compartment. Despite its crucial role in nutrient metabolism, bile acid homeostasis and immune function, small intestinal microbiota remains poorly studied due to sampling challenges and ethical constraints. Consequently, most human research is still relying on fecal samples or, to a lesser extent, colonic microbes. In line with the European 3Rs rules, one alternative to *in vivo* assays is the development of *in vitro* models that simulate the human digestive tract. However, to date, only a limited number of artificial digestive systems integrates resident microbes in small intestine compartments. This presentation will first highlight the key role of small intestine and its associated microbiota in human health and diseases, followed by a state-of-the art overview of available *in vitro* models integrating small intestinal microbes. Then, the talk will focus on two dynamic models that have been recently developed in the frame of the HOMIGUT International Associated Laboratory, the mono-compartmental Mucosal Artificial Ileum (M-ARILE) and the multi-compartmental Small Intestine Mucosal Simulator of Human Intestinal Microbial Ecosystem (SI-M-SHIME). Finally, potential applications in nutritional and biomedical research will be discussed.

ORAL PRESENTATIONS

Topic: Advances in Digestion and Absorption Models

ISO DIS 24223 | IDF 253 - IN VITRO DIGESTION PROTOCOL FOR THE DETERMINATION OF PROTEIN DIGESTIBILITY AND IN VITRO DIGESTIBLE INDISPENSABLE AMINO ACID SCORE

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Abstract

Protein quality assessment is increasingly critical for the food sector, with the FAO recommended Digestible Indispensable Amino Acid Score (DIAAS) as the preferred metric. While in vivo, human and porcine models remain the gold standard, the demand for cost-effective and ethical alternatives has led to the development of robust in vitro methodologies.

Here we present the results of an international standardization process launched within the International Dairy Federation (IDF) and the International Organization for Standardization (ISO) leading to ISO DIS 24223 | IDF 253. The initiative standardized an advanced in vitro protein digestibility protocol (based on the INFOGEST 2.0 protocol) that incorporates a specific analytical workflow for determining both total protein digestibility and individual amino acid digestibility, which demonstrated high correlation with in vivo data across seven diverse food sources.

Validation was performed in two international collaborative studies involving >25 laboratories within the INFOGEST network and including seven protein sources (five dairy and two plant-based). Statistical analysis established method repeatability and reproducibility at 10% and 15%, respectively, for total protein digestibility, using OPA (primary amines) and total nitrogen (TN by Kjeldahl/Dumas). Notably, digestibility results obtained via OPA, TN, or total amino acid analysis (TAA by UPLC) were not statistically different, offering laboratories significant analytical flexibility.

While validation for TAA analysis in digesta remains ongoing, the method's applicability to various food matrices has been successfully demonstrated. This new IDF/ISO standard method marks a milestone in international harmonization, providing a validated, accessible framework for comparing protein quality under standardized in vitro conditions.

Keywords

Protein digestibility, DIAAS, Protein quality, ISO/IDF

Acknowledgements

The successful development of the ISO/IDF standard method for in vitro protein digestibility is a direct result of the dedicated participation of INFOGEST network members in two collaborative validation studies. We thank them for their critical contributions to this international milestone.

Topic: Advances in Digestion and Absorption Models

POPULATION-SPECIFIC IN VITRO DIGESTION: HOW ALTERED DIGESTION CONDITIONS RELEVANT FOR PEOPLE WITH OBESITY AND AFTER BARIATRIC SURGERY IMPACT PROTEOLYSIS

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Abstract

Both obesity and bariatric procedures, such as gastric sleeve and gastric bypass, are associated with alterations in both gastrointestinal (GI) anatomy and physiology, including changes in pH, transit time, and bile salt concentration (Steenackers et al., 2021, 2023). These changes may substantially affect nutrient digestion, particularly protein, which is a key nutrient in the dietary management of both populations. Food digestion is typically studied using a standardised static in vitro digestion model (Brodkorb et al., 2019) that represents the GI conditions of healthy adults. However, this model does not reflect the altered physiology of people with obesity and people after bariatric surgery. To effectively evaluate and develop food products tailored to their specific nutritional needs, population-specific in vitro digestion models adapted to their physiological conditions are crucial.

First, the impact of altered gastric and small intestinal pH and transit time in people with obesity and after bariatric surgery (gastric sleeve and gastric bypass) was investigated by adapting the standardized static in vitro digestion model of the INFOGEST network (Brodkorb et al., 2019). The impact of altered digestion conditions was determined for whey and casein (fast- and slow-digesting protein, respectively). Compared to healthy adult conditions, application of a lower gastric pH (relevant for people with obesity) resulted in a significantly greater extent of gastric whey digestion. Although a clear impact was observed for whey, this was not the case for casein, underscoring the importance of considering food-specific properties when assessing the effects of specific digestion conditions.

Second, we hypothesized that enzyme activity could also play a major role. However, in vivo data on enzyme activity in the gastrointestinal tract of people with obesity and after bariatric surgery are currently lacking and challenging to collect. To assess the potential impact of altered enzyme activity, a range of digestive enzyme activities was tested. A significant effect of applied enzyme activity on protein digestion kinetics was observed. For example, under conditions relevant to people with a gastric sleeve, a lower enzyme activity led to a significant decrease in the extent and rate of both gastric and small intestinal casein digestion.

To conclude, since digestion conditions relevant to people with obesity and after bariatric surgery clearly affect protein digestion kinetics, there is an urgent need for digestion models adapted to these population groups. Our results highlight that developing these models requires accurate in vivo enzyme activity data from people with obesity and after bariatric surgery.

Keywords

adapted digestion models, physiological changes, whey versus casein, gastric sleeve, gastric bypass

Topic: Advances in Digestion and Absorption Models

MODELING HUMAN DIGESTION IN VITRO: TIM UPPER GI FOR NUTRITION APPLICATIONS

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Abstract

Digestion in the human gastrointestinal (GI) tract is a complex and dynamic process. Studying digestion processes is challenging, due to in vivo studies being invasive and expensive (1). Additionally, in vivo studies do not always provide information on the processes inside the gastrointestinal lumen. In vitro methods can be instrumental in facing some of these challenges, as they focus on the luminal digestion process itself. However, most in vitro digestion methods are static, which can help in screening and providing a first insight, but lack the complexity that is required to faithfully replicate the digestive system. The TIM® systems are computer-controlled, dynamic in vitro models which closely mimic the physiology of the human gastrointestinal tract. TIM Upper GI models consist of an advanced gastric compartment (AGC), with realistic simulation of gastric morphology and motility (2), and one or multiple small intestinal (SI) compartments. These systems include continuous secretion of enzymes, control of pH and the removal of digestion products, which maintains physiological conditions and allows for time-dependent assessment of bioaccessibility. This can provide insight into differences in digestion kinetics, even if the total digestibility between products is similar (3).
Pressure sensors installed in the AGC can register relative differences in (digestion-induced) viscosity changes between test products, providing valuable information on differences in product behavior during gastric digestion (4). Luminal samples can also visualize the digestion process, such as coagulation during gastric residence. Additionally, they can provide insight into the composition of the GI contents, at specific timepoints, and thereby intermediate steps in the digestion process.
TIM® systems can be programmed to mimic specific populations, such as pediatric subjects or patients with certain gastrointestinal diseases. In this way, TIM Upper GI can help study the effects of diseases such as pancreatic insufficiency on (lipid) digestion (5). Concluding, the TIM® platform offers comprehensive, physiologically relevant insights in digestion, making it a useful tool in scientific research or product development.

References

1. Bohn, T. et al. Crit. Rev. Food Sci. Nutr. 58, 2239–2261 (2017).
2. Bellmann, S. et al. Food Research International 88, 191–198 (2016).
3. Maathuis, A. et al. J. Pediatr. Gastroenterol. Nutr. 65, 661–666 (2017).
4. Bellmann, S. et al. Food Research International 122, 77–86 (2019).
5. Martins Garcia, T. et al. Heliyon 11, e43379 (2025).

Keywords

In vitro, digestion kinetics, nutrition, bioaccessibility

Topic: Advances in Digestion and Absorption Models

IN SILICO PREDICTION OF POSTPRANDIAL GASTRIC EMPTYING HALF-TIMES IN HUMANS, AS MEASURED BY MRI: INFLUENCE OF MEAL PROPERTIES

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Abstract

Gastric emptying is a major determinant of the kinetics of nutrient absorption and of the satiety response in vivo. Realistic modelling of gastric emptying is also essential for fair simulation of the in vivo situation in (semi-)dynamic in vitro research. However, gastric emptying is influenced by individual characteristics, such as age and sex, as well as the physicochemical properties of the meal, such as its caloric density and viscosity. Therefore, a better understanding of the factors governing the rate at which foods leave the stomach is still required.

The present study comprised three parts. Firstly, it presents a novel database of mean gastric emptying half-times (t1/2) for 90 meals, alongside information on their key properties: liquid or mixed meals, volume, energy content, and macronutrient composition. This database is the result of a comprehensive meta-analysis of 37 studies in which postprandial gastric emptying was assessed using magnetic resonance imaging (MRI) in healthy volunteers ($n \geq 5$).

Secondly, a linear model analysis was performed to evaluate the dependency of t1/2 on the properties of the meal. Following a variable selection process (AIC criterion), two models were selected: one without interaction (coefficient of determination, R^2, of 0.79), and one with interactions ($R^2 = 0.85$). Both models highlighted the key role of the energy content and the meal type (liquid vs. mixed meal). They also provided a much better fit than other approaches typically used for the same purpose, since the R^2 values were -0.85 (worse than pure randomness) for the model of Hunt & Stubbs (1975) and 0.57 for the rule proposed in the semi-dynamic INFOGEST protocol (Mulet-Cabero et al., 2020), respectively.

Finally, the accuracy of various in silico approaches for predicting t1/2 based on the meal properties was compared using a cross-validation workflow. In this workflow, 75% of the data was used to fit the model, and the remaining 25% was used for prediction (20 iterations). In addition to linear models, seven machine learning algorithms suited for modelling complex relationships were also considered. The linear model proved to be the most robust approach ($R^2 = 0.78$, mean absolute error = 12 min), resulting in a straightforward equation for predicting t1/2 based on the meal's primary properties.

These results highlight the relevance of data-driven models in predicting postprandial gastric emptying half-times, and pave the way for fairer programming of gastric emptying in in vitro (semi-)dynamic digestion studies. Further work remains needed to study the influence of individual characteristics.

Hunt, J. N., & Stubbs, D. F. (1975). The volume and energy content of meals as determinants of gastric emptying. The Journal of Physiology, 245(1), 209-225.
Mulet-Cabero, A. I., et al. (2020). A standardised semi-dynamic in vitro digestion method suitable for food – an international consensus. Food & Function, 11, 1702.

Keywords

Modelling, Stomach, Half-emptying time, T50

Topic: Advances in Digestion and Absorption Models

AN IN-VITRO GASTROINTESTINAL MODEL FOR WEANING INFANTS AND ITS PERFORMANCE ON PROTEIN DIGESTIBILITY UNDER LIQUID AND SOLID DAIRY FOOD MATRICES

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Abstract

Background: The digestibility of milk is well characterised for infants. However, little is known about the effect of food structure on nutrient bioaccessibility at the time of weaning.

Method: Reconstituted skim bovine milk was selected to prepare four food matrices, namely skim milk control (SM), heated skim milk (HM), rennet-induced milk gel (RG) and starch-induced milk gel (SG) with 5% (w/w) protein content. The matrices were characterised for apparent viscosity and digested by three digestion models, including a weaning infant in-vitro model (WI), an adult (A) INFOGEST in-vitro model and an infant (I) in-vitro model. The weaning infant (WI) model was specially developed based on known physiological changes that occur at weaning and used to simulate digestion for all samples. Resulted digesta from all models was analysed for protein digestibility by SDS-PAGE, degree of hydrolysis (DH) by OPA and peptidomics by LC-MS/MS. Obtained results were compared between models and matrices.

Results: Food structure influenced digestibility under simulated WI conditions, with notable differences between the two liquid milk (SM, HM) and the two solid gel (RG, SG) matrices during gastric digestion. Moreover, the food matrix was shown to influence DH under intestinal conditions with higher levels in RG compared to HM from 30 min onwards. Liquid sample have similar digestion patterns as solid samples across digestion, but the intensity and pattern of individual proteins are notably different. Samples digested under A conditions presented higher peptide abundance across all matrices compared to the I and WI conditions, where most of the caseins are digested during the A model gastric phase. Peptides sourced from β-lactoglobulin were also notably different under A conditions.

Conclusion: The food matrix affected protein digestibility for liquid and gelled bovine skim milk matrices. Moreover, protein digestibility for both liquid milk and gels digested under WI simulated gastric conditions is different to those digested under I or A conditions in the order of A > WI > I. Differences in the matrices' peptidome profile were also noted between digestion models, but differences in peptidome profile is only observed for individual proteins. Limited differences, on the other hand, were measured in digestibility between matrices under intestinal conditions regardless of the model applied, yet differences in the peptidome profile remained. This indicates that weaning infants exhibit unique digestive patterns compared to infant or adults, exhibiting differences in protein digestibility under gastric conditions and differences in the breakdown of proteins to form peptides across both gastric and intestinal digestion.

Keywords

Food matrix, Weaning infant digestion, Digestion model, Milk protein, LC-MS

Topic: Advances in Digestion and Absorption Models

BEHIND THE SCENES OF INFOGEST QUANT: INSIGHTS INTO THE STEP-BY-STEP PROTOCOL FOR PROTEIN DIGESTIBILITY AND DIAAS DETERMINATION

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Abstract

The INFOGEST 2.0 protocol is widely used to simulate human gastrointestinal digestion but was not specifically designed for the assessment of protein quality. Recently, an adapted method based on the INFOGEST static in vitro protocol was introduced, incorporating targeted modifications to the digestion conditions and a dedicated post-digestion analytical workflow for the determination of protein digestibility and digestible indispensable amino acid scores (DIAAS) (Sousa et al., 2023). This novel method, named INFOGEST Quant, has been validated with in vivo data, is undergoing international standardisation, and recently resulted in a comprehensive, step-by-step protocol published in Nature Protocols (Egger et al., 2026). Here, we present the insights into the key modifications to the original INFOGEST protocol and the critical analytical steps, supported by experimental data generated during method development.

Key aspects of the INFOGEST Quant protocol can be summarized into six. i) The enzyme blank tube to correct for enzyme autolysis must include a protein-free substrate (e.g. a protein-free cookie). ii) The amount of sample for digestion is adjusted to 40 mg of protein in order to normalize the protein-to-enzyme ratio among experiments. iii) Mastication is simulated by grinding the solid samples to a particle size of 2-3 mm. iv) Following digestion, absorbable and non-absorbable protein-derived molecules are separated by methanol precipitation. In this step, the pellets (non-absorbable fractions) must not be aliquoted for various analytical workflows; instead, the whole pellets are used for chemical analysis. v) After fractionation, both the absorbable and the non-absorbable fractions are analysed, and calculations are done based on the chemical composition of the absorbable fraction in relation to the sum of the absorbable and non-absorbable fractions. vi) The acid hydrolysis of the digested samples that is required prior to quantification of total amino acids (for DIAAS estimation) and total amino groups (alternative for proxy-DIAAS estimation) has been shortened from 24 h to 15 h to prevent degradation of thermosensitive amino acids, which may already be minor in highly digestible protein sources. Accounting for all these critical steps enables robust evaluation of the quality of dietary proteins across laboratories.

Sousa et al. (2023), Food Chem. 404, 134720 (DOI: 10.1016/j.foodchem.2022.134720); Egger et al. (2026), Nature Protocols, in press (DOI: 10.1038/s41596-025-01307-9).

Keywords

Nature Protocols, protein digestibility, DIAAS, method optimisation

Topic: Advances in Digestion and Absorption Models

TOWARDS AN INTERNATIONAL CONSENSUS INFANT IN VITRO DIGESTION MODEL FOR DIFFERENT AGES : FROM PREMATURITY TO MATURITY

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Abstract

Intro

Understanding infant digestion remains a scientific challenge due to the absence of fully consensual in vitro models covering early life stages. To address this gap, a group of over 16 experts from 7 countries within the INFOGEST network conducted a systematic review of available physiological data. This initiative aimed to define a consensual framework for infant digestion from the preterm until the mature stage.

Materials and Methods

First, the different preterm stages were defined based on gestational age at birth and infant age at the time of the study. A comprehensive review of the scientific literature was then conducted, including a large number of publications reporting gastrointestinal parameters in infants in comparison with adults. Four physiological stages were defined: preterm, 1 month, 6 months and mature. Extracted data included gastric emptying kinetics, pH values, enzyme activities, food to secretion ratios. Recent studies provided updated information on gastric emptying and pH evolution, whereas older but still relevant publications informed on enzyme concentrations.

Results

The analysis revealed substantial variability in reported physiological values, confirming the need of harmonized parameters. A few parameters were found to substantially vary during infancy. Particularly, gastric pH decreased after meal ingestion, reaching values of approximately 4 in preterm and 1-month-old infants and around 2 at 6 months of age, which is comparable to adult. Pepsin activity increased markedly from birth to 6 months, reaching approximately 71 U/mL/kg of body weight, while pepsin and pancreatic lipase seem to mature progressively until 2 years of age, when they reach a level similar to adult (2,000 U/mL for both enzymes). Bile salts concentrations increased throughout infancy, from approximately 1.6 mM in preterm infants to 4.3 mM at 6 months of age, and up to 10 mM at maturity. Trypsin is present at a mature level at birth; however, enterokinase, which activates trypsin, is immature, resulting in only 17% to 38% of effective trypsin activity from the preterm stage to 6 months of age. Conversely, other parameters—such as gastric lipase (mature at birth), gastric emptying rate, intestinal pH, duration of the intestinal phase, dilution of intestinal contents by secretions—remained relatively stable throughout infancy. However, the authors were also confronted with a lack of infant digestion data e.g. older infants or some intestinal parameters of any ages.

Conclusion

Based on the expert consensus and data consolidation, parameters for in vitro gastrointestinal digestion models are proposed for preterm, 1-month-old and 6-month-old infants in comparison with adults. These settings will be applicable for static, semi-dynamic, and dynamic in vitro digestion systems. Complementing the models developed within the INFOGEST network for adults and the elderly, this latest development will ultimately enable us to cover all stages of life.

Keywords

Digestion models, preterm, term, maturity, digestive parameters

Topic: Advances in Digestion and Absorption Models

INNOVATIVE HUMAN GASTRIC AND SMALL INTESTINAL MODEL SIMULATING DIFFERENTIAL GASTRIC EMPTYING OF REAL-SIZE FOOD PARTICLES AND ILEAL MICROBIOTA

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Abstract

The impact of food structure and gut microbiota in nutrient digestibility has been investigated in numerous studies. However, the specific role of small intestinal microbiota is largely unknown due to sampling invasiveness. In line with European 3Rs rules, a relevant alternative to in vivo assays is the use of in vitro models simulating the human digestion processes. Up to now, there is no validated multi-compartmental model of the full upper human gastrointestinal tract combining the ability to: digest real-size food particles; simulate the absorption of nutrients; and incorporate & retain biorelevant microbiota. To fill this gap, we are currently developing a new gastric and small intestinal system, called Engineered Stomach and Small Intestine (ESIN).

ESIN is a dynamic system reproducing the stomach, duodenum, jejunum and ileum, set-up with in vivo data collected from healthy adult humans. This model reproduces the main physicochemical parameters of digestion, such as body temperature, kinetics of pH in each compartment and oral, gastric, biliary and pancreatic secretions. The innovative structure of the stomach enables digestion of food particles and differential emptying of liquids and solids, with a realistic meal ingestion time. Passive absorption of nutrients, drugs and/or water is realized through dialysis fibers connected to the jejunal and the ileal compartments. Progressive reduction of oxygen along the gastrointestinal tract is achieved thanks to nitrogen flushing. Lastly, resident microbiota will be introduced into the ileal compartment using an immobilization process to avoid microbial wash-out during digestion.

First validation experiments of the ESIN model were based on a fasted protocol reproducing the ingestion of a glass of water. Dextran blue and paracetamol were used as transit marker and absorbable compound respectively. Stomach (n=8) and ileal (n=4) transit time as well as paracetamol bio-accessibility (n=4) showed good correlation with in vivo data in humans. Employing a fed-state protocol, experiments were performed using real-size food particles, showing that ESIN allows differentiated emptying of liquids and solids up to a size of 8 mm. Moreover, three different immobilization protocols of bacteria have been tested and compared to evaluate their ability to maintain and release a strain isolated from the small intestinal microbiome (Lactobacillus salivarius) into the in vitro ileal environment. From these, a procedure producing gellan/xanthan beads showed the most promising results.

This new ESIN model will help to move towards a better understanding of the role of food structure and ileal microbes in human nutrition and health, particularly in terms of food matrix-microorganism interactions as well as the impact of the intestinal microbiota on macronutrient digestibility or drug bio-accessibility and availability.

Keywords

In vitro model, ESIN, food structure, small intestinal microbiota, drug

Topic: Advances in Digestion and Absorption Models

BRUSH BORDER MEMBRANE VESICLES AS A SUPPLEMENTARY STEP PROCESS IN THE IN VITRO GASTROINTESTINAL DIGESTION MODEL INFOGEST: CONSEQUENCES ON THE PROTEOLYSIS

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Abstract

To determine peptide and amino acid profiles released during food digestion, reproducible methods such as INFOGEST have been used. However, amino acids and peptides released under in vitro conditions can still greatly differ from those generated in vivo. In vitro/in vivo differences can be attributed to the lack of epithelial enzymes that finalize digestion and hydrolyze peptides into short peptides and free amino acids. To address this, a few research groups have isolated Brush Border Membrane Vesicles (BBMv) to mimic the action of the intestinal epithelium. However, it hasn't been established which incubation time with BBMv is physiologically relevant.

BBMv were isolated by scraping the mid-jejunum of a pig and characterized by the assessment of aminopeptidase activity. In vitro gastrointestinal digestion samples were prepared according to the INFOGEST protocol and included two milk proteins collected at four time points: casein at 5 and 120 min after gastric and intestinal digestion (Cg5, Cg120, CI5, CI120), as well as whey proteins (Wg5, Wg120, WI5, WI120). All samples were incubated for 6 h with 13 µU/µg protein of BBMv, consistent with aminopeptidase activity described in humans. Total amino acid analysis, quantitative determination of α-amino groups by the OPA method, and peptidomic analysis by mass spectrometry were performed to characterize proteolysis at different time points. Statistical analysis was performed using t-tests, one-way ANOVA, and Pearson's correlation.

Our results showed that whey proteins are hydrolysed more rapidly than casein. Significant differences between casein and whey protein hydrolysis were observed in the intestinal samples after BBMv 6h incubation, compared with the gastric phase, which was steady and sustained. Proteolysis progressed towards free AA release (Pearson r=0.83-0.94, p 0.05). Proportion of FAA release after BBMv incubation in WI5 reached 53.6%, while WI120 showed 60.1%. Regarding casein, the percentage of I5 increases from 23.2% to 48%, and that of I120 increases from 33.7% to 56%. FAA release never reached a plateau even after 6h of incubation. For both matrices, supplementation with BBMv significantly increased cleavage at alanine, isoleucine, and proline residues, consistent with Aminopeptidase A, N, and DPP4 specificity. In contrast, the release of arginine, cysteine, and phenylalanine was extremely limited. The full peptidome was characterized, and specific sequences consistent with previous publications were found. In both matrices, supplementation with BBMv enhanced protein hydrolysis and reduced persistence of digestion-resistant fragments.

The peptide patterns obtained after incubating the digested samples with BBMv for different times will now be compared with those from in vivo effluents collected by naso-intestinal probes from volunteers fed either casein or WP. The incubation time that leads to the closest in vitro peptidome to the in vivo one will be selected.

Keywords

Keywords: Protein hydrolysis, BBMv, Free amino acids, In vitro digestion

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

THE MISSING LINK IN DIGESTION MODELS: A HIGH-RESOLUTION FUNCTIONAL BLUEPRINT OF THE CACO-2 HUMAN BRUSH BORDER INTERFACE

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Abstract

Terminal nutrient breakdown occurs at the enterocytes microvilli, which form the jejunal brush border membrane (BBM) (1). This specialised functional interface acts as a metabolic gateway, distinct from the duodenal digestion mediated by pancreatic juice. This process relies on a complex architecture of enzymes that are either anchored to the microvilli membrane or secreted into the immediate luminal environment, working alongside nutrient transporters and the glycocalyx matrix (2). Within the Giant Leaps project, this study aims to enhance the INFOGEST model by implementing a BBM digestion phase using BBM enzymes from Caco-2 monolayers. This provides a standardised, ethical, and reproducible alternative to traditional porcine extracts.

Caco-2 cells were grown using an optimised 21-day polarisation protocol, to ensure a full columnar phenotype. This scalable platform, on a 75 cm2 flasks was designed to provide a consistent source of membrane-anchored enzymes for the modified INFOGEST model.

The digestive capacity of Caco-2 extracts was characterised by combining semi-quantitative mass spectrometry mapping with targeted enzymatic assays. This dual-platform methodology allows for the precise identification of enzymes responsible for terminal digestion while simultaneously quantifying their functional efficiency. Our findings demonstrate that integrating proteomic mapping with kinetic data offers a robust model for characterising the enzymatic pool, providing a definitive framework for the standardisation of the enzymatic extracts.

At the proteomic level, the identification of structural and functional proteins including the Annexin family, Villin-1, Ezrin, Tight Junction Proteins 1, 2, and 3 (TJP1–3), and F-actin-capping protein subunit beta (CAPZB) suggests the development of an apical-lateral scaffold and the stabilisation of the actin cytoskeleton at the cell-cell interface, indicating the potential for barrier integrity and cellular polarization. Enzymatic activity was measured by a spectrophotometric assay for Leucine Aminopeptidase (LAP), which demonstrated consistent catalytic output across experimental cultural conditions. High-resolution proteomics further confirmed the recovery of a diverse human-specific Caco-2 BBM enzymes, including exopeptidases (DPP4, Aminopeptidase B), carboxypeptidases, disaccharidases (Lactase/phlorizin hydrolase), and phosphatases (Intestinal-type alkaline phosphatase).

Overall, this study demonstrates that Caco-2 monolayers possess BBM digestive capacity. Building on this finding, this enzyme pool can be harvested and integrated as a final digestion step within the INFOGEST static protocol. Consequently, this system offers a robust and reproducible tool for exploring intestinal brush border processing and metabolic interactions in food science.

References:

(1) Mamone G, Picariello G. Food Res Int. 2023;164:112326

(2) Holmes R. Gut. 1971;12(8):668

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Topic: Advances in Digestion and Absorption Models

A COMPARATIVE ASSESSMENT OF RAT SMALL INTESTINE EXTRACT AND PURIFIED BRUSH BORDER MEMBRANE VESICLES FROM PIG TO STUDY POLYPHENOL DIGESTION

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Abstract

Most dietary polyphenols naturally occur as glycosylated derivatives, influencing their metabolic response and biological activity. Nevertheless, the bioactivity of these compounds is fundamentally restricted by their bioaccessibility, as the attached sugar moiety must be removed before absorption can take place. Membrane-bound enzymes located in the small intestinal epithelial cells, particularly lactase-phloridzin hydrolase (LPH), play a key role in this process. Despite their relevance, these enzymes are usually disregarded in the standardized INFOGEST 2.0. protocol. The incorporation of a small intestinal extract from rat (RSIE) has been proposed to introduce mammalian glycosidases; however, its complex unrefined biological matrix may promote non-specific interactions or entrapment of polyphenols, potentially distorting their digestibility. In this work, two enzymatic sources of brush border enzymes—RSIE and purified brush border membrane vesicles from pig (BBMV)—were characterized and included into the INFOGEST protocol, using a pancreatin only-condition as a control. Whole apple phenolic compounds digestion and bioaccessibility were evaluated by coupling the upper gastrointestinal tract static INFOGEST 2.0, to the physical simulation of transepithelial absorption of polyphenols via centrifugal filtration and UHPLC-ESI-QTOF-MS/MS analysis.

The inclusion of RSIE resulted in a marked reduction in the bioaccessibility of all polyphenol subclasses, accompanied by an extensive release of aglycones such as ferulic acid, caffeic acid, ρ-coumaric acid, quercetin, and phloretin. The broad hydrolytic profile suggests excessive and non-selective deglycosylation, potentially associated with the crude nature of the extract and the presence of microbial enzymatic activities. By contrast, digestion with BBMV resulted in a more moderate decrease in polyphenol bioaccessibility, being more precise and consistent with the specific β-glycosidase activity of LPH, which preferentially targets β-linked glycosides (dihydrochalcones). The high purity of BBMV minimizes non-specific interactions and matrix entrapment, allowing an intestinal environment including LPH-derived β-glycosidase activity deprived from microbial enzyme activities. Overall, the enzymatic source critically influences the assessment of polyphenol digestion. RSIE may better approximate intestinal conditions including microbial activities, which are known to occur in the distal parts of the small intestine, whereas purified BBMV provides a more accurate model to measure the brush border mucosal small intestinal digestion.

Keywords

Brush border enzymes, deglycosylation, dihydrochalcones, aglycone, INFOGEST

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Topic: Advances in Digestion and Absorption Models

A NOVEL IN VITRO REAL-TIME DIGESTION, ABSORPTION AND HEPATIC UTILIZATION CACO-2/HEPG2 MODEL LINKS INFANT MILK LIPID STRUCTURE TO METABOLIC FATE

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Abstract

Objectives

Lipid digestion kinetics influence postprandial lipid availability and subsequent metabolic outcomes. Because lipid droplet size and interfacial coating govern these kinetics, these may determine whether lipids are preferentially stored or oxidized by liver cells. We developed a real-time lipid digestion–absorption–utilization model that links digestion kinetics to intestinal absorption, and hepatic metabolism. Using this model we examined human milk (HM) which contains large lipid droplets with a milk fat globule membrane (MFGM), a standard infant formula containing small protein coated lipid droplets (sIF) and a concept infant formula containing large MFGM coated lipid droplets (cIF; Nuturis).

Methods

A Transwell co culture of apical Caco 2 and basolateral HepG2 cells simultaneously modelled intestinal digestion, epithelial transport, and hepatic lipid handling in real time. Gastric digested HM, sIF, and cIF, at 0.8 mM triglycerides (TG), were subjected to intestinal lipolysis via addition of pancreatic lipase, phospholipase, and bile salts in the apical chamber. The model was run both with and without cells. Apical free fatty acids were quantified continuously as indicators of lipolysis, while basolateral TG secretion served as a readout of postprandial lipid delivery. After 24 h, hepatic lipid partitioning was assessed using the ratio of secreted 3 hydroxybutyrate (3HB), a marker of ketogenesis, to intracellular TG accumulation.

Results

sIF showed a higher lipolysis rate than HM and cIF from 3–5 h, although cumulative 24 h fatty acid uptake was similar across milks, reaching ~65%. The increased lipolysis rate of sIF translated into higher basolateral TG secretion rates at 3-5h, and a higher TG appearance at 24 h than HM. After 24 h, ~15% of apical triglycerides were secreted. TG secretion rose linearly from 0.2–0.8 mM but plateaued at 1.6 mM. HepG2 cells exposed to sIF accumulated more intracellular TG and displayed the lowest 3HB:TG ratio, indicating preferential lipid storage. In contrast, cIF more closely resembled HM across all endpoints: showing slower digestion kinetics, lower TG delivery, reduced hepatic TG storage, and higher 3HB:TG ratio than sIF.

Conclusions

The real time digestion–absorption–utilization model showed that rapid lipolysis of small lipid droplet sIF accelerated lipid delivery to the basolateral compartment and promoted hepatic TG storage. In contrast, the slower lipolysis of the larger, MFGM coated lipid droplets in HM and cIF supported a metabolic phenotype biased toward lipid oxidation over TG storage. These findings demonstrate that the kinetics of lipolysis can shape the kinetics of absorption, secretion, and hepatic utilization, suggesting that formula with large MFGM coated lipid droplets can shape early lipid metabolism in a manner more similar to breastfed infants. Finally, this model illustrates the value of integrating real time intestinal digestion with epithelial transport

Keywords

Lipid digestion & absorbtion, Caco-2 cells model, Hepatic metabolism

ICF Gdańsk 2026

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POSTER PRESENTATIONS

Topic: Advances in Digestion and Absorption Models

IN VITRO DIGESTION FOR ESTIMATING REAL PROTEIN DIGESTIBILITY, AN EFFECTIVE METHOD WHEN ENDOGENOUS NITROGEN IS ACCURATELY TAKEN INTO ACCOUNT

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Abstract

The in vitro estimation of protein digestibility can be biased by the presence and release of endogenous N/AAs due to the autolysis of digestive enzymes. The use of 15N-labelled dietary proteins allows to estimate the real digestibility by tracing specifically dietary N and accurately correcting for the endogenous N/AA contribution. Beforehand, a fractionation step is needed after in vitro digestion to separate bioaccessible (i.e. digestible) from non-bioaccessible materials. The present study aimed (1) to compare different methods of in vitro digesta fractionation and (2) to compare real N and AA digestibility obtained in vitro or in humans on the same 15N-labelled protein sources and in the same laboratory.

Proteins with different structure and hydrophobicity were extensively (100U/mL trypsin, 2h, INFOGEST protocol) or moderately (10U trypsin/mL, 5min) digested. Digesta were centrifuged, with or without prior precipitation with trichloroacetic acid (TCA), sulfosalicylic acid (SSA) or methanol. Supernatants were analysed by size-exclusion chromatography to estimate the size of peptides eliminated by each fractionation method. 15N-labelled proteins (milk, pea, flaxseed, rapeseed, sunflower, and wheat proteins), with real N ileal digestibility in humans from 81 to 95%, were incorporated into meals (8.5%) and digested in vitro (INFOGEST). After digesta fractionation with methanol, the N, AA content and 15N enrichment were measured in dried pellets using elemental analysis or chromatography coupled with isotope ratio mass spectrometry. The real in vitro digestibility of proteins (RIVPD) and AAs were determined and compared with values obtained in humans using Bland & Altman analysis. The contribution of endogenous N was estimated.

Methanol, TCA and SSA mainly eliminated peptides >10 kDa with an efficiency depending on the protein structure and AA composition. The maximal size of the bioaccessible peptides (11-2 kDa) was strongly influenced by the digestion extent and exceeded physiological thresholds of intestinal absorption (1-0.5 kDa), likely explained by the absence in vitro of brush border peptidases. RIVPD values closely matched human data (r=0.70, slope: 0.99). The average in vitro - in vivo bias varied from -3.9±3.5% to +4.1±3.4%. Pellet N was mainly endogenous (66 to 87%), in line with protein digestibility (r=0.70), but accounted for only 22±3% of total endogenous N, with no significant difference among dietary proteins, indicating >75% of endogenous N was bioaccessible due to autolysis, with a similar % found across dietary proteins digested under isoprotein level.

Overall, in vitro static digestion provided an accurate estimation of real N and AA digestibility when endogenous N/AA was accurately corrected for. Further research is needed to assess the impact of dietary protein level and matrix on enzyme autolysis and to identify the optimal digestion blank for correction in the absence of 15N-labelled proteins.

Keywords

Fractionation methods, Bioaccessible peptides, Protein digestibility, in vitro vs. in vivo, Endogenous nitrogen

Topic: Advances in Digestion and Absorption Models

ISOLATION OF INTESTINAL BRUSH BORDER ENZYMES: RESULTING ENZYME ACTIVITIES ARE HIGHLY DEPENDENT ON CELL HARVESTING PROCEDURE.

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Abstract

Background: The bio-accessibility of amino acids/peptides from food proteins can be determined by applying simulated in vitro digestion protocols, which in general include the oral phase (amylase), gastric phase (pepsin and gastric lipase) and intestinal phase (pancreatin and bile acids). However, simulation of brush border enzymes (BBEs) activities that contribute to the bio-accessibility and bio-availability of protein peptides and amino acids is still missing in most standard in vitro digestion protocols. BBEs are produced by small intestinal epithelial cells and can either be secreted or anchored to the cell membrane. Different methods are described in literature to harvest brush border membranes and isolate BBEs. Here, we compare the isolation efficiency of different methods applied and how enzyme unit activity might be influenced.

Methods: Brush border enzymes were isolated from the small intestine of a slaughtered pig according to Cheeseman et al. 2006 and Picariello et al. 2015. Comparisons were made between the isolation of brush border membranes by tissue scrapings versus vibrational cells de-attachment (FundaMix), between fresh and stored (-80 degrees Celsius) tissue, and between intact tubular tissue versus cut-open tissue. Quantification of the brush border enzymes aminopeptidase N, glutamate carboxypeptidase 2 and neprilysin was performed by ELISA. Unit activity of the brush border extract (BBE) was defined by checking aminopeptidase N activity using L-Leucine p-nitroaniline as a conversion substrate and quantifying color changes at OD405nm.

Results: Higher amounts of BBEs were isolated from the jejunum and ileum sections of the small intestine. Stored tissue released a higher amount of BBEs while the de-attachment of membrane cells by vibration worked best on ileum tissue. Distribution of the BBEs over the duodenum, jejunum and ileum was different for aminopeptidase N, glutamate carboxypeptidase 2 and neprilysin. Unit activity of aminopeptidase N was highest for the jejunum using fresh tissue scrapings, however the yield for this sample was low.

Conclusion: Harvesting brush border membrane cells by vibrations rather than tissue scrapings seems to preserve the activity of isolated BBEs. Most likely with tissue scrapings, proteases or inhibitors are released that decrease the enzymatic unit activity of the BBEs extract. Not much difference in unit activity was seen between using fresh tissue or stored tissue, which makes the procedure for isolation more manageable.

References
Cheeseman et al., Cur. Protoc. Cell Biol. 2006, 3, 3.21.1-10
Picariello et al., Mol. Nutr. Food Res. 2015, 59, 948–956

Keywords

Brush border membranes, Brush border enzymes

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

COMPUTER-AIDED SIMULATION OF PEPTIDE AND PROTEIN DIGESTION INVOLVING INFORMATION ABOUT MODIFIED AMINO ACIDS

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Abstract

The BIOPEP-UWM database (https://biochemia.uwm.edu.pl/biopep-uwm/) has become a standard tool in research on bioactive peptides primarily those of food origin [1]. The database is equipped with a program designed for proteolysis simulation. This application incorporates, among other features, the specificty of pepsin, trypsin and chymotrypsin, enzymes of digestive tract.

Recently, the database has been expanded to include not only proteinogenic amino acids but also products of their enzymatic or chemical modifications, such as phosphorylation of hydroxyl groups in serine, threonine, or tyrosine, glycation of lysine, and oxidation of methionine. These modification products along with other residues being components of peptides are annotated in the BIOPEP-UWM repository of amino acids and modifications.

Sequences of peptides and proteins, annotated using the BIOPEP-UWM code. Symbols written in this code are inserted into the sequences of proteins and peptides. The following sequence of peptide can be used as an example:

{K[7α-D-Frup]}RS{M[5(R)O]}

Where:

{K[7α-D-Frup]} – α-D-fructopyranosyllysine (product of lysine glycation)

{M[5(R)O]} - methionine sulfoxide with configuration Rectus around asymmetric sulfur atom (product of methionine oxidation)

Simulated proteolysis of the above peptide (using trypsin, chymotrypsin and pepsin) results in the following peptides: {K[7α-D-Frup]}R and S{M[5(R)O]} corresponding to hydrolysis of bond between arginine and serine residues by trypsin. The bioactivity or metabolic fate of these peptides may be predicted using specialized programs (e.g. SwissTargetPrediction [2] and ADMETLab 3.0 [3]), which utilize SMILES representations [4] as an input. These representations can be generated from amino acid sequences using a program associated with the BIOPEP-UWM database. Similar results concerning phosphopeptides as possible products of casein digestion (according to in silico digestion simulation) were published in our previous article [5].

The BIOPEP-UWM repository of amino acids and modifications can be enriched with new compounds as necessary.

References:

1. Iwaniak A. et al. Curr. Opin. Food Sci., 55, 2024, 101108. DOI: 10.1016/j.cofs.2023.101108
2. Daina A. et al. Nucleic Acids Res., 47, 2019, W357–W364. DOI: 10.1093/nar/gkz382
3. Fu L. et al. Nucleic Acids Res., 52, 2024, W422–W431. DOI: 10.1093/nar/gkae236
4. Weininger D. J. Chem. Inf. Comput. Sci. 28, 1988, 31–36. DOI: 10.1021/ci00057a005
5. Minkiewicz P. et al. Appl. Sci., 13, 2023, 8091. DOI: 10.3390/app13148091

Keywords

BIOPEP-UWM database, modified amino acids, peptides, proteins, proteolysis simulation

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

EFFECT OF CONSUMPTION TEMPERATURE OF WHOLE MILK ON IN VITRO GASTRIC DIGESTION USING A BIOMIMETIC DIGESTION SIMULATOR

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Abstract

It is well-established that milk coagulates during gastric digestion due to the combined action of pepsin, the gastric protease, and the increased acidity of the stomach contents caused by gastric acid secretions. However, consumption practices may also affect milk digestion. In particular, there is a lack of research concerning the effect of consumption temperature on the behaviour of milk within the gastrointestinal tract, considering that milk coagulation partly depends on temperature and that intragastric temperature is significantly influenced by the temperature of the ingested meal. This study evaluates the effect of consumption temperature on the gastric digestion behaviour of whole milk using a dynamic in vitro model (NERDTTM, computer-controlled biomimetic system). Human intragastric temperature profiles in response to cold, warm, and hot beverages were accurately reproduced to mimic the conditions encountered after consumption of milk at 4°C, 37°C and 60°C. For comparison, control experiments were performed with milk at 37°C without any pepsin addition. Results show that milk coagulated more rapidly within the stomach when consumed at 37°C or 60°C compared to 4°C and 37°C without pepsin. This result perfectly fits with an MRI study that we published in 2024 that showed a slower milk coagulation for the milk consumed at 4°C (Fitzpatrick et al. 2024). Moreover, the gastric emptying rate of milk proteins in the presence of pepsin was slower with milks at 37°C and 60°C compared with the milk at 4°C. The hydrolysis of milk proteins also appeared to be temperature-dependant, with proteolysis increasing with higher milk temperatures toward the end of digestion. Regarding the fat fraction of milk, our data suggest that both hot (60°C) and cold milk (4°C) could plausibly increase the gastric emptying rate of milk fat, compared to a 37°C milk, but further studies remain needed to verify this hypothesis. At the end of the experiments, the wet and dry masses of the residual gastric digesta was similar across the three conditions with pepsin, but significantly lower than in the absence of pepsin at 37°C. Even though the main effects of milk consumption temperature take place during the early stages of gastric digestion, this study demonstrates that they are likely to have repercussions over the whole postprandial period. It remains to be confirmed whether these effects will further affect the fate of proteins and lipids in the small intestine. Provided that in vivo studies suggest a possible impact of consumption temperature on gastric emptying, and that our current results indicate it is likely the case for the protein and lipid of milk, another interesting area for future research relates to the possible influence of meal temperature on satiety

Keywords

In vitro digestion; Milk; Coagulation; Temperature; Stomach; Gastric emptying

Topic: Advances in Digestion and Absorption Models

THE EFFECT OF DIGESTED CARBOXYMETHYLATION-MODIFIED (CML-BLG) AND FUROSINE-MODIFIED (FL-BLG) B-LACTOGLOBULIN ON SMALL INTESTINE HEALTH IN EARLY LIFE

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Abstract

Thermal treatment during infant formula processing results in the generation of various non-enzymatic protein modifications (nePTMs). In our previous study, dry heating of a casein or whey protein model system was used to investigate the formation of protein-bound nePTMs after heating from 0 to 72 h. We found that oxidation, lactosylation, carboxymethylation, 3-deoxyglucosone and hexose modification were the predominant nePTMs formed, which were found to be protein- and site-specific. After intestinal digestion, the level of carboxymethylation and furosine in the soluble phase increased, especially for 72 h glycated casein, which also showed the highest soluble nitrogen level. We hypothesized that peptides with nePTMs could promote the inflammation by activating receptors such as RAGE (receptor of advanced glycation end products) and thereby lower peptide bioavailability. Motivated by the dominant abundance of carboxymethylation and furosine in digesta, we used an intestinal organoids model derived from newborn mice ileum (n=3, C57BL/6J, 11 days) to investigate absorption and transportation mechanisms of digested carboxymethylation-modified (CML-BLG) and furosine-modified (FL-BLG) β-lactoglobulin. Results from an initial screening showed that digested CML-BLG and FL-BLG induced higher apoptosis and LDH activity than the free-form CML and FL. In the evaluation of gut barrier integrity, membrane receptors related to advanced glycation products (AGEs) and transportation via the paracellular pathway will unravel the structure-function relationship on the bioavailability of modified BLG in early life.

Keywords

intestinal Organoids, baby mice, ileum, milk protein, Maillard reaction, peptides, absorption, transportation.

Topic: Advances in Digestion and Absorption Models

DYNAMIC IN SILICO GASTROINTESTINAL DIGESTION OF CONVENTIONAL BEEF VERSUS CELL-BASED BEEF PROTOTYPES

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Abstract

Cell-based meat products are emerging as promising alternatives to conventional meat and create new opportunities to modulate digestion through formulation and matrix design. Here, a dynamic, semi-mechanistic, compartmental in silico digestion model based on mass balance was used to compare the gastrointestinal fate of cooked conventional beef (CB) with a cell-based meat and fat prototype (CMF) and a scaffold-free prototype with cultured fat (SF). The model coupled ordinary differential equations that describe (i) mass transfer between gastrointestinal compartments, (ii) time-dependent secretion of digestive fluids and enzymes, and (iii) enzymatic hydrolysis and absorption of protein- and lipid-derived pools. The gastrointestinal tract is represented by 13 interconnected compartments (diet; stomach; parietal/chief cells; pancreas; duodenum; jejunum 1–2; ileum 1–4; portal blood; and colon). Within each compartment, the model tracks masses (or molar amounts) of water (free and bound), intact proteins (myofibrillar and collagen) and their digestion products, lipid pools (saturated and unsaturated) and their hydrolysis products, dietary fibre, and selected secretions. The prototypes were more water-rich and less protein-dense than CB, resulting in lower energy density and distinct transit and hydrolysis kinetics. Simulations predicted faster gastric emptying for CMF and SF compared with CB, advancing nutrient delivery to the duodenum while reducing gastric residence time available for pepsin and gastric lipase catalyzed-hydrolysis of collagen and myofibrillar proteins, and saturated and unsaturated TAG, respectively. Consequently, protein hydrolysis and peptide and amino acid appearance differed in both timing and magnitude, with earlier but lower cumulative portal amino acid absorption for the prototypes (CMF and SF), consistent with their lower protein load and faster transit. Lipid outputs showed more unsaturated free fatty acids absorption in the jejunum and ileum compartments for CMF and SF than CB while CB showed more absorbed saturated free fatty acids. The alginate present in the scaffold of CMF reduced its caloric density compared to CB and, impacting the overall transit throughout the gastrointestinal tract . Overall, the results indicate that nutritional comparisons between conventional and cell-based meats should incorporate postprandial release kinetics alongside compositional targets. Dynamic modelling is proposed as an initial tool to redesign formulations toward targeted digestive patterns. Future work should integrate measured microstructural descriptors and validate predicted kinetic metrics using dynamic in vitro digestion systems and relevant in vivo markers.

Keywords

cultivated meat, cell-based fat, dynamic digestion model, gastric emptying, proteolysis, lipolysis, fibre

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

EVALUATION OF THE CYTOTOXIC EFFECT OF FERMENTED APPLE POMACE-BASED FOODS SUBJECTED TO STATIC DIGESTION ON THE HT29 CELL LINE

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Abstract

Fermented foods have been consumed as a way to preserve raw materials and to obtain new products with healthier properties and appreciated sensory qualities. This approach can also be applied to reuse byproducts obtained from foods processing, contributing to the circular economy. But it is necessary to check for the absence of negative effects of any new food product. The objective of this work was to in vitro evaluate the effect of apple pomace-based products fermented with different microbial consortia on the intestinal epithelium.

The cytotoxicity model upon HT29 cell line using the label-free, impedance based RCTA (real time cell analyser) xCELLingence technology was used (Valdés et al. 2015; doi/10.1016/j.mimet.2015.09.022). Dehydrated flour obtained from apple pomace generated in the production of Asturian cider was used as fermentable substrate for three microbial consortia at three temperatures for variable times. Each fermentation condition was carried out in duplicate and a total of 24 samples were collected. The static Infogest 2.0 digestion was carried out using 2.5 g of the fermented products, and final digesta were centrifuged to collect supernatants which were lyophilised. For cytotoxicity analyses, lyophiles were resuspended in complete McCoy medium (MM) at the same initial volume. Digested and undigested samples were tested at seven doses (from 1x to 0.016x). Additionally, the effect of Infogest digesta, using water instead of food, or the protease inhibitor Pefabloc, was analysed. The negative cytotoxic control was MM and the positive control 1 ng/ml of toxin TcdA (from C. difficille). Cell index (CI, i.e. impedance) was followed for 24 h and normalized-CI calculated at several points post-sample addition.

Digested fermented-foods at high doses (1x) were toxic for the cell line and doses equal or lower than 0.25x showed positive values close to “0” normalized-CI being no toxic. Undigested foods had no toxic effect at any dose. HT29 monolayers facing Infogest-water digesta showed that dose higher than 1x were toxic due to the presence of digestive enzymes. The 0.5x dose produced an increase in the normalized-CI at the initial steps, but also induced cell damage later on since the normalized-CI presented negative values after 3 h. Similar behaviour was detected for the 0.25x dose but at a later time point (after 8 h), which can be considered as no physiologically relevant since we are using a static model that lacks the flow that occurs in vivo. Pefablock treatment at concentrations 2.5 and 5 mM were also toxic for the cellular line and not able to counteract the effect of digestive enzymes.

In conclusion, under our experimental conditions, the cytotoxic effect of the digested fermented foods at the highest dose tested was due to the presence of digestive enzymes and no toxic effect was detected at dose lower or equal to 0.25x (dilution factor 1/4) for short (less or equal than 8 h) periods of exposition.

Keywords

cytotoxicity, HT29 cell line, RTCA, fermented foods, digested foods, apple pomace valorisation

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

ALLERGENIC RISK- ASSESSMENT OF CASEINS PRODUCED BY PRECISION FERMENTATION: A 96-WELL DIGESTION FORMAT

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Abstract

Background: One of the developments within the sustainable protein transition movement is replacing the production of animal proteins by livestock, for protein production via precision fermentation. This also applies to the production of milk proteins. Caseins in milk largely determine the unique structure, texture and taste of cheese, aspects that have proven difficult to imitate in vegan cheese using plant proteins. Caseins produced through precision fermentation could therefore significantly enhance the taste quality of vegan cheese. When submitting a novel food dossier, one of the components that must be provided is a description of an allergenicity risk assessment of a novel protein source. Ingested food proteins can, when incompletely digested in the stomach, potentially give an allergenicity risk due to partially digested proteins reaching the small intestine. Current in vitro digestion consensus protocols, comprising an oral, gastric and intestinal stage, are not suitable for investigating single purified allergens, due to the use of large volumes. Therefore, a recently developed 96-well plate format (1) was evaluated that is suitable for the application of small amounts of protein, to see whether this method can be applied to compare caseins of animal origin with caseins produced via precision fermentation

Method: Casein (Sigma Aldrich) was used as a general reference protein for protocol validation. Casein concentration and solubility was optimized for this protocol. A pre-titration was done with the protein in gastric condition, determining the amount of HCl used for pH 2.5 and pH 5.5. Digestion of casein was performed in a 96 well format using the INFOGEST digestion protocol (2) with adaptations on pH and pepsin activity. Two pH conditions were tested mimicking the fed (pH 5.5) and fasted (pH 2.5) stomach conditions (3) and a high and low pepsin activity, 1000 U/ml and 100 U/ml representing adult and infant pepsin conditions. For each timepoint a separate well digestion was made and stomach phase digestion was stopped by adding 0.5 M ammonium bicarbonate to raise the pH above 7 and stopping the pepsin activity. Timepoints during gastric digestion were 0-0.3-5-10-20-120 minutes.

Results: Alle timepoints of the four conditions during digestion were put on SDS PAGE. Digestion condition : 1. pH 2.5 and 100 U pepsin/ml, 2. pH 2.5 and 1000 U pepsin/ml, 3. pH 5.5 and 100 U pepsin/ml, 4. pH 5.5 and 1000 U pepsin/ml. Casein digestion was best in condition 2, almost no large protein particles were seen on SDS PAGE whereas condition 3 shows larger protein fragments between start and 20 minutes of digestion.

Conclusion: The 96-well digestion protocol appears well suited for digesting small protein volumes, such as precision fermentation test samples, and could therefore become part of a screening method toolbox for allergenicity risk assessment.

1. Wang et al., 2022, 2023a
2. Brodkorb et al., 2019
3. Spiller and Marciani., 2019

Keywords

96-well digestion,Gastric digestion,Allergenic,sustainable protein transition

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

A SEMI-DYNAMIC IN VITRO GASTRIC DIGESTION MODEL TO INVESTIGATE THE EFFECT OF PROTON PUMP INHIBITOR USE ON NUTRIENT BIOACCESSIBILITY

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Abstract

Proton pump inhibitors (PPIs) are a class of medications used by nearly 25% of adults in Western countries (Shanika et al., 2023). PPIs induce a profound and prolonged reduction of gastric acid secretion and are prescribed for several gastric acid-related disorders. Although they are generally considered safe for short-term use, emerging evidence suggests that PPIs are overprescribed and may be associated with a number of side effects that are presumably linked to gastric hypoacidity. By increasing postprandial gastric pH, PPI use may thus impair food digestion and nutrient absorption.

The first objective of this study was to propose an in vitro gastric digestion protocol dedicated to model PPI use. This protocol is an extension of the semi-dynamic INFOGEST protocol (hereinafter referred to as the "Standard" protocol), with two modifications based on a literature search on the PPI-induced changes in postprandial gastric conditions in human adults: (i) a final gastric pH of 4.2 and (ii) a 50 % reduction in simulated gastric acid fluid volume. The second objective was to compare the release kinetics of peptides, soluble carbohydrates, lipids, and minerals during simulated gastric digestion of a mixed meal (bread, cheese, and tomato) with both standard and PPI versions of the digestion protocol.

Results demonstrated that the release of peptides, arabinose, and minerals, including calcium, magnesium, and phosphorus, was significantly reduced (p 0.05) in the PPI model. These findings are in agreement with the expected effects of reduced gastric acidity on pepsin activity and mineral solubility. They are also consistent with known or presumed side effects of PPIs, such as an increased risk of hypomagnesemia, fractures, skeletal muscle loss, and vitamin B12 deficiency. However, the hydrolyses of starch and lipids, assessed through maltose release and triacylglycerol disappearance, respectively, were not significantly affected.
In conclusion, this modified INFOGEST protocol appears to serve as a valuable in vitro tool to study the side effects of PPI use on food digestion. Future research building on this protocol could further investigate the effects of PPIs on nutrient bioaccessibility and bioavailability throughout the gastrointestinal tract, thereby providing insights into the mechanisms underlying PPI side effects and informing strategies for their mitigation.

Shanika, L. G. T., Reynolds, A., Pattison, S., & Braund, R. (2023). Proton pump inhibitor use: systematic review of global trends and practices. European Journal of Clinical Pharmacology, 79(9), 1159–1172. https://doi.org/10.1007/s00228-023-03534-z

Keywords

Nutrient, Mineral, Bioaccessibility, Gastric pH, Acid suppression

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

CHARACTERIZATION OF PORCINE INTESTINAL ORGANOID CELL TYPES AND FUNCTIONAL MONITORING OF DERIVED MONOLAYERS USING RTCA

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Abstract

Intestinal organoids are three dimensional and multicellular structures that mimic the cell types, structure, and functions of the intestinal epithelium. Unfortunately, their 3D geometry, while advantageous for various studies, prevents easy access to the apical region of the epithelium. To overcome this problem, two dimensional polarized monolayers have been derived from organoids. The development of reproducible organoids monolayers relies on the optimization of experimental parameters that can influence the formation of a confluent monolayer. Therefore, the objectives of this study were to, first, characterize the cellular composition of generated porcine duodenal organoids and subsequently evaluate the effects of cell seeding density (CSD) and the timing of medium changes (TMC) on the development and formation of organoid cell-derived 2D monolayers.

Intestinal organoids were obtained from a healthy adult (5-month-old) male pig (Sus scrofa domestica, “Gochu Asturcelta” breed) and stored in liquid N2. For reactivation, organoids were seeded in Matrigel® and cultured in organoid growth medium (Stemcell™). After several passages, organoids were disrupted for single-cell suspension and seeded into three 16-well plates for RTCA (E-Plate VIEW, Agilent) using serial two-fold dilutions, with each CSD plated in duplicate per plate, and with an initial CSD of 1×10^5 cells. The three E-plates were monitored simultaneously using the RTCA-DP (real time cell analyzer) equipment for 6 days. To evaluate the effect of the TMC, in the first plate the medium was changed at 24 h (ODM, Stemcell™), in the second at 24 h and 40 h, and in the third one at 24 h and 48 h. On the other hand, three droplets of Matrigel containing organoids were used for RNA extraction. The RNA was reverse transcribed to cDNA and the presence of the cell type markers was detected by PCR. The markers used were: lgr5 (intestinal stem cells), lyz1 (Paneth cells), mucin-2 (goblet cells), chgA (enteroendocrine cells) and villin (enterocytes).

RTCA data showed that, independently of the CSD and TMC conditions, the cell index (CI) increased with time, suggesting cell proliferation, reaching a plateau phase (PP) after several days and a final decrease in CI which may reflect growth arrest or viability reduction. Notably, the higher the CSD, the sooner the maximum CI and PP were reached, but the highest CSD tested also showed the greatest instability in the CI signal (impedance) on all plates. Based on these observations, 5×10^4 cells is proposed as the most suitable CSD for the generation of organoid monolayers, as it showed greater stability than the highest density and required less time (approximately 57 h) than the next lower density (2.5×10^4 cells) to reach the PP (approximately 70 h). Regarding the TMC, no major differences in CI dynamics were observed among the three conditions tested. Lastly, PCR analysis confirmed the presence of all the cell markers evaluated.

Keywords

monolayer, duodenal pig organoids, RTCA, PCR, Lgr5, Mucin-2, LYZ1, Villin

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

INSIGHT INTO THE ACTION OF THE DIGESTIVE PROTEASES

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Abstract

Within the ICFD network, many researchers use Infogest protocols to simulate food digestion in vitro. Often, foods with novel ingredients, processing techniques, or varying structures are evaluated for their digestibility. However, limited studies aim to fundamentally understand the digestive process itself. In our laboratory, we studied the action of proteases (pepsin, trypsin, and chymotrypsin), the main players in protein digestion, to better understand how proteins are broken down and peptides are released. Over the last ten years, we developed a unique method to identify and quantify which peptides are released at which rate, during in vitro enzymatic protein hydrolysis. Using this approach, we were able to characterize which bonds in a protein can be cleaved. We aim to eventually rationalize and predict how proteins will be digested into peptides and amino acids. Moreover, the influence of hydrolysis conditions (pH, substrate concentration) and food processing-induced modifications (glycation) was evaluated with respect to peptide release. The developed approach uses UHPLC-PDA-ESI-MS for robust peptide mapping, according to data-processing guidelines established using a manual reference analysis of simple tryptic hydrolysates. The method uses UV absorbance at 214 nm and sequence-dependent extinction coefficients to calculate absolute concentrations of individual peptides. The major advantage of this technique is that it avoids (isotopic) labelling treatments and does not rely entirely on MS intensity. Furthermore, the method is highly reproducible (≤ 10% RSD of individual peptide concentrations). By analyzing the peptide composition at different time points during in vitro hydrolysis, unprecedented insights were obtained into the digestive enzymes trypsin, chymotrypsin and pepsin. For trypsin, we observed differences in (secondary) specificity between bovine, porcine and human origin. The trypsin obtained from cows had considerably more difficulty in hydrolyzing cleavage sites that were surrounded by charged amino acids. For chymotrypsin, the specificity and preference were revisited by correlating hydrolysis rates of individual cleavage sites with the amino acids in P4-P4’. We observed clear hindrance, leading to missed cleavages, when proline occupied specific binding site positions (P3, P1’ & P2’). For pepsin, we studied whether pH affected the type of bonds hydrolyzed, since the pH in the stomach is naturally dynamic. pH influenced the rate at which peptides were released, but not necessarily the preference of pepsin for certain amino acid residues. Through the detailed characterization of these digestive proteases, we have taken a step forward in understanding their action, predicting missed cleavages and understanding what impacts peptide release.

Keywords

Protein digestion, Proteases, Peptide release kinetics, Pepsin, Trypsin, Chymotrypsin, LC-MS, missed-cleavages

Topic: Advances in Digestion and Absorption Models

REFINEMENT OF THE INFOGEST PROTOCOL FOR ACCURATE AMINO ACID DIGESTIBILITY ASSESSMENT ACROSS DIVERSE INGREDIENTS AND PRODUCTS.

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Abstract

Background

The static INFOGEST digestion protocol (1) is widely used to assess protein and amino acid (AA) digestibility. However, its high pancreatic enzyme load can hinder accurate digestibility determination, especially for foods with low protein content or low digestibility. To address this challenge, we refined the protocol to improve accuracy, reproducibility and sensitivity across a broad range of dairy and plant proteins, products with varying digestibility (low to high) and protein concentration. The refined method was then compared with the standardized method.

Methods

Optimization focused on decreasing enzyme/substrate (E/S) ratios by reducing pepsin and pancreatin activities relative to substrate protein content. Additional modifications included lowering bile salt concentration, extending the intestinal phase, and replacing the protein-free cookie with a water blank. AA digestibility was calculated using initial AA input. A representative set of dairy and plant proteins, including liquid and solid products, was tested. After 80% methanol precipitation, AA were quantified by standard techniques. Method performance was assessed through repeatability testing and inter-laboratory reproducibility, with INFOGEST method run in parallel. Three E/S ratios (12.5%, 22.2%, 41.6%) corresponding to pepsin activity of 1000 U/mL and trypsin activity of 2.5, 5 and 10 U/mL, were pre-selected and compared with INFOGEST using whey protein isolate (WPI), soy concentrate and raw chickpeas.

Results

Across all ratios, mean digestibility values for WPI and soy concentrate remained high (92.7 □ 0.5% and 90.3 □ 1%) and aligned with published in vivo data. For raw chickpea, digestibility values were consistent with literature but decreased at the 12.5% and 22.2% ratios. In contrast, the INFOGEST protocol overestimated digestibility for all matrices, with deviation up to 25% for low-digestible protein, likely due to biased estimation of endogenous N level, present in high proportion in such model. Among tested conditions, only the 41.6% ratio provided consistent digestibility across all proteins. This ratio was therefore selected for an inter-laboratory comparison using three products varying in protein concentration and digestibility. The optimized model produced consistent AA digestibility across laboratories and showed closer alignment with expected in vivo data than INFOGEST protocol, particularly for low-digestible product.

Conclusion

The optimized digestion model offers a reliable predictor of in vitro AA digestibility, closely matching in vivo data by reducing digestive enzyme background. The method shows strong repeatability and inter-laboratory reproducibility. These optimized conditions are particularly suitable for ingredients/products with low protein content or low digestibility, offering an accurate adaptation to the INFOGEST protocol.

1. Sousa R., Recio I., Heimo D., Dubois S., Moughan P. J., Hodgkinson S. M., Portmann R., Egger L. (2023)

Keywords

Refinement, digestion protocol, reduced enzyme/substrate ratio, low digestibility, low protein concentration

Topic: Advances in Digestion and Absorption Models

INTRINSIC STABLE ISOTOPE LABELLING REMOVES THE NEED FOR COOKIE BLANKS IN INFOGEST QUANT: DEVELOPMENT, VALIDATION, AND CROSS-CHECK OF THE ESTABLISHED PROTOC

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Abstract

Determining true ileal indispensable amino acid digestibility and the resulting protein quality (DIAAS) in vivo is labor-intensive and constrained by ethical and practical limitations. Conversely, in vitro approaches are often affected by the analytical contribution of digestive enzymes (background).

In vivo, the established dual stable isotope tracer method overcomes the challenge of differentiating background from input protein by directly analyzing the labelled amino acids of the food. We applied a similar intrinsic labelling strategy to the in vitro INFOGEST Quant method using intrinsically ^{2}H-labelled T. molitor, as well as maize and yeast, which were produced to rear T. molitor.

For digestibility assessment, we developed a new selective LC-MS detection of ^{2}H-labelled amino acids. This allows for the direct exclusion of the unlabelled enzyme background without the need for a cookie blank. Digestibilities obtained with this new selective detection closely matched those from the standard INFOGEST Quant protocol.

In conclusion, the study (i) validates that tracer-based in vitro digestibilities align with results from the in vivo dual stable isotope method; (ii) establishes strong comparability with the conventional INFOGEST Quant method; and (iii) confirms that the cookie-blank correction in the established INFOGEST Quant method accurately accounts for enzyme-derived background protein.

Keywords

intrinsic stable isotope labelling, in vitro protein digestibility, INFOGEST Quant, enzyme background correction

Topic: Advances in Digestion and Absorption Models

A CANINE SIMULATED IN VITRO DIGESTION MODEL AND ITS VALIDATION FOR DIGESTIBILITY, INTESTINAL HEALTH AND MICROBIOME STUDIES.

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Abstract

Growing intertest in innovations for pet food and supplements is supported by increasing awareness of pet owners in healthy nutrition. However, there is a lack of pet-specific in vitro models to perform such research and support new product development. In vitro digestion is an important tool for analysing digestibility, nutrient bioavailability and preparing samples for functional testing. Therefore, this study aimed to further develop a static canine simulated digestion (CSD) model and validate it by assessing digestibility and functionality in vitro with dog nutrition-relevant ingredients. Spray Dried Plasma (SDP) and Fructo-Oligosaccharides (FOS) were selected as a high-quality protein and a prebiotic respectively, which in combination were hypothesized to demonstrate a superior effect for canine digestive health. The ingredients were added to a protein rich food (PRF) matrix for investigation.

CSD of the selected ingredients followed an INFOGEST protocol, adapted to reflect canine physiology and digestive conditions. Briefly, temperature, incubation time, gastric and intestinal pH, enzymes concentrations, and simulated fluids were adjusted, based on a small number of publications, which compared in vivo and in vitro digestibility data. Infant intestinal absorption model was used to simulate the canine intestinal barrier, reflecting higher permeability of the intestine in canine species compared to the adult human. Monolayer integrity was tested weekly using transepithelial electrical resistance (TEER). Protection against pathogen was tested by incubation of differentiated goblet-like epithelial cells (HT29-MTX) with digesta, followed by exposure to Salmonella enterica, and quantification of the pathogen adherence. Ex vivo faecal fermentation technology was applied to assess ingredient effects on canine gut microbiota and metabolite production.

Supplementation with 10% SDP significantly improved digestibility, calculated as remaining dry matter, when compared to the PRF alone. Treatments by individual ingredients and combination (SDP+FOS) significantly increased intestinal barrier integrity (TEER) when compared to the control. Furthermore, all treatments reduced the adherence potential of Salmonella enterica to HT29-MTX cells. Finally, ex vivo fermentation showed both PRF and SDP lowered pH when compared to the control, with the predominant acidification in presence of FOS. Combination of SDP + FOS resulted in the in the highest levels of the beneficial saccharolytic markers (acetate, propionate and butyrate) shifting system from proteolytic fermentation, conditioned by the presence of PRF.

Overall, CSD model was developed based on the INFOGEST protocol, reflecting physiological digestive conditions of a dog. New model was validated with a combination of SDP and FOS, which demonstrated potential benefits for intestinal health, protection against infection and microbiome health in a dog.

Topic: Advances in Digestion and Absorption Models

MODULATING EMULSION STRUCTURE AND B-GLUCAN CONTENT IN RAPESEED AND WHEY PROTEIN-STABILIZED SYSTEMS: INTERFACIAL PROPERTIES AND DIGESTIVE BEHAVIOUR

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Abstract

Dietary emulsions represent an important delivery system for lipophilic nutrients and bioactive compounds, and their interfacial structure plays a key role in digestion and bioaccessibility. This study investigates the formation and physicochemical properties of oil-in-water emulsions stabilized by either a commercial rapeseed protein (plant-based) or β-lactoglobulin (animal-based), with β-glucan incorporated as a functional polysaccharide.

Proteins were dissolved in the external aqueous phase, while β-glucan (commercial PromOat concentrate) was initially dispersed in the lipid phase. Emulsions containing 50 %(w/w) rapeseed oil were produced by high-shear mixing (20,000 rpm, 4 min), resulting in 0.6, 1.2 and 1.8 %(w/w) β-glucan. The transfer of fibre from the lipid phase to the continuous aqueous phase during emulsification was assessed, together with encapsulation efficiency, droplet size distribution, molecular weight distribution and bulk rheological properties.

All emulsions exhibited 100% lipid encapsulation efficiency, demonstrating the ability of both proteins to stabilize high-oil-load systems. Droplet size analysis revealed an increase in aggregation of droplets with increasing β-glucan content. Rapeseed protein-stabilized emulsions displayed sizes ranging from approximately 1 to 30 µm, whereas whey protein emulsions showed a broader distribution from about 0.8 to 60 µm when aggregates were included. The presence of β-glucan significantly increased the viscoelastic properties of both emulsion types, indicating the formation of a structured continuous phase.

Fractionation followed by size exclusion chromatography (SEC) analysis demonstrated that β-glucan was located exclusively in the aqueous continuous phase and not at the oil–water interface, regardless of the protein used. This suggests that β-glucan contributes mainly to bulk phase structuring rather than direct interfacial modification. The combined use of proteins and soluble fiber therefore results in composite systems where interfacial stabilization and continuous-phase rheology can be independently tuned.

Interfacial shear rheology is used to investigate the interface evolution during gastric digestion in vitro where the β-lactoglobulin layer was exposed to 0.4 mg/mL pepsin. First, the interfacial moduli decreased, indicating that the pepsin adsorbed to the interface and hydrolyzed the protein network to peptides. The layer was fully degraded after around one hour. After complete degradation of β-lactoglobulin, the peptides started to rearrange and build a new network with pepsin at the interface, which was indicated by the increase in interfacial moduli. During the enzymatic degradation, interfaces formed by animal protein tend to destabilize.

These findings provide a basis for designing plant- and animal-protein-based emulsions enriched with β-glucan for targeted modulation of digestive behavior and bioaccessibility of lipophilic compounds.

Keywords

Oil-in-water emulsions; plant and animal protein; β-glucan; Interfacial rheology; In vitro digestion.

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

COMPARATIVE ANALYSIS OF DIFFERENT IN VITRO DYNAMIC GASTRIC DIGESTION MODELS USING CARBOHYDRATE-BASED FOODS

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Abstract

There is increasing recognition that food structure plays a critical role in nutrient release and diet-related health outcomes. This has driven research into the structural changes that foods undergo during gastrointestinal digestion. In vitro digestion models have become essential tools for this purpose, being faster and more cost-effective than in vivo approaches.

Among the in vitro gastric digestion models, dynamic models are designed to replicate aspects of the contraction patterns of the stomach wall, the gradual acidification of the digestion mixture, and gastric emptying through an outlet simulating the pylorus. While there are numerous dynamic models, limited comparisons have been made between models to assess the importance of their features in food breakdown during gastric digestion. In this study, two dynamic gastric models with different geometry and contractions were utilized. The Human Gastric Simulator (HGS) v1 has a vertical, tapered cylindrical latex chamber with four small roller belts that do not encircle the latex chamber, while the HGS.v2 has a more anatomically accurate J-shaped chamber with circumferential contractions using c-clamps. The objective of this study was to compare the breakdown of cooked carbohydrate-based foods with different structures during gastric digestion, and to assess the impact of the gastric models on starch digestion in a static small intestinal model.

Cooked pasta, semolina, or rice (600 g) was digested in both HGS.v1 and HGS.v2. Samples were collected every 30 min over 240 min using a gastric secretion rate of 4.1 mL/min and a gastric emptying rate of 5.6 g/min. Emptied gastric digesta were analyzed for pH, dry matter gastric emptying, particle size distribution, and reducing sugar content. All emptied gastric digesta samples underwent static in vitro small intestinal digestion for 240 min, with samples taken each 30 min. The liquid phase of small intestinal samples was analyzed for reducing sugar content to quantify the hydrolyzed starch released during the 480 min digestion period.

Significant differences (p0.05) were observed between the HGSv.1 and HGSv.2 in pH, gastric emptying, and particle size distribution during pasta digestion. Gastric pH was higher in HGS.v1 at 60, 90, and 150 min (e.g., 3.09 vs. 2.53 at 60 min in the HGSv.1 vs. HGSv.2, respectively). Dry matter emptying rates showed no significant differences in the first 150 min (p>0.05) but were significantly higher in the HGS.v2 from 180 min onward (e.g., 0.40 vs. 0.28 g/min at 180 min). Particle size was larger in HGS.v1 (p0.05) at most time points, reflecting reduced mechanical breakdown. For example, particles were 3-fold larger at 30 min and 1.3-fold larger at 210 min compared to the HGS.v2.

These findings highlight that differences in dynamic model design can significantly influence digestion outcomes, highlighting the need for caution when comparing results from different in vitro models, even with identical protocols.

Keywords

Dynamic Gastric Model, Food Structure, Gastric Emptying, Starch Digestion

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

BIOACCESSIBILITY OF VITAMINS K AND D IN VARIOUS FOODS: DEPENDENCE ON LIPID MODIFIERS IN THE INFOGEST 2.0 MODEL

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Abstract

Vitamin K is well known for its roles in blood coagulation and bone health, yet emerging evidence suggests broader physiological functions. Vitamin D deficiency affects nearly half of the global population, and low vitamin D status is associated with increased risk of respiratory infections, bone disorders, and other health issues. For fat soluble vitamins, bioaccessibility is often the limiting factor for bioavailability. The standardized in vitro digestion model Infogest 2.0 was recently updated to better accommodate lipid soluble vitamins by introducing a standard meal designed to ensure a model independent of content of vitamin K (Infogest 2.0 – Vit K). This standard meal includes, among other components, chicken and cream. In the present study, we compared this meal with three other option of lipid codigested and we assessed the resulting bioaccessibility of vitamins K and D.

Method: Using the Infogest 2.0 – Vit K model, we examined whether replacing the standard animal based meal with a vegan meal matched for macronutrient composition but differing in fatty acid profile, or replacing it with either medium chain triglyceride oil or thistle oil, would affect the bioaccessibility of vitamin D vitamers in mushrooms, pork, and cracklings, as well as vitamin K vitamers in cheese and broccoli. Additionally, we assessed the bioaccessibility of vitamin D (vitamin D_3, 25 OH vitamin D_3, vitamin D_2, and 25 OH vitamin D_2) and vitamin K (PK, MK 4 to MK 10) in canola oil, eggs, and Nannochloropsis oceanica. All digestions were performed in triplicate.

Results: Co digestion of test foods with either the animal based or vegan meal—did not significantly influence the bioaccessibility of vitamins D or K. The results for the two different oils will be presented at the conference. Bioaccessibility of vitamin D and K in Nannochloropsis oceanica was the lowest bioaccessibility ($2.4\% \pm 0.3\%$ for vitamin K and $9.8\% \pm 5.0\%$ for vitamin D), whereas canola oil showed the highest ($88\% \pm 14\%$ for vitamin K and $91\% \pm 2.8\%$ for vitamin D). In addition, vitamin D bioaccessibility was lower in mushrooms than in pork, and vitamin K bioaccessibility was lower in broccoli compared to cheese.

Conclusion: With the data so far, the choice of lipid modifier in the Infogest 2.0 – vitamin K model—whether animal based or vegan and despirte differences in fatty acid profil—did not significantly affect the bioaccessibility of vitamins D and K. Although bioaccessibility varied across food sources, the overall pattern was consistent: Nannochloropsis oceanica showed the lowest bioaccessibility, while canola oil exhibited the highest.

Topic: Advances in Digestion and Absorption Models

THE IMPACT OF MYCOPROTEIN-BASED MEAT ANALOGUES ON EPITHELIAL BARRIER INTEGRITY, AS COMPARED TO THEIR ANIMAL-BASED COUNTERPARTS

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Abstract

Dietary proteins are essential for a proper functioning of the human body. Protein uptake is facilitated through gastrointestinal digestion into small peptides or free amino acids. Transport and absorption of these derived peptides and amino acids takes place by the epithelial barrier in the small intestine. The global consumption of "meat" protein currently totals 500 million tonnes per year, and less than 1% of this is derived from non-animal sources (PLENITUDE-project ID 838104). Therefore, meat replacers should contain at least an equal amount of protein that is both bioaccessible and bioavailable.

ABUNDA□, a mycoprotein fungal biomass containing essential amino acids and high Fiber content can be an important nutritionally relevant alternative when used as an ingredient in meat analogue products.

In this study we investigated and compared the impact of in vitro digested mycoprotein biomass, meat alternatives (fish flakes, beef burger, smoked or baked chicken filet) and animal-based counterparts on intestinal epithelial integrity using two different Caco-2 cell models. To mimic disruption of the intestinal barrier, Caco-2 were exposed to Clostridioides difficile toxin A (ToxA) and the digesta. In addition, a healthy Caco-2 layer was also exposed to the digesta.

The impact of the different digesta across an healthy and impaired Caco-2 layer were investigated by TEER measurements.

Our study showed that, except for the smoked chicken sample, none of the meat alternatives or animal-based counterparts showed disruption of the healthy epithelial barrier. Interestingly, mycoprotein improved epithelial barrier integrity after ToxA treatment.

From the results we conclude that the tested products did not show a drastic impact on epithelial integrity and that mycoprotein-based products themselves could improve or worsen a disrupted barrier, depending on the product formulation.

Keywords

Digest, Caco-2

Topic: Advances in Digestion and Absorption Models

PREDICTING PROTEIN HYDROLYSIS AFTER INFOGEST 2.0 PROTOCOL USING NEAR-INFRARED SPECTROSCOPY (NIR) AND MULTIVARIATE CALIBRATION

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Abstract

The INFOGEST 2.0 method has become a reference for the standardized assessment of in vitro digestion in food research. Despite its wide adoption, the methodology still relies on labor-intensive analytical procedures and requires multiple types of equipment and reagents, which may limit its routine application. In this context, near-infrared spectroscopy (NIR) emerges as a rapid, non-destructive alternative that has not yet been explored for predicting human digestion-related outcomes. Thus, this study aims to evaluate the feasibility of using NIR to predict free amine content, as an indicator of protein hydrolysis, after in vitro digestion following the INFOGEST 2.0 protocol using Pupunha (Bactris gasipaes) cookies as a model food. The pupunha fruit was previously cooked, oven-dried, and ground to obtain the flour, which was later applied in cookies with an increasing proportion of pupunha flour (12.5, 25, 50, 75, and 100%). A total of 105 cookie samples were analyzed using a NIR spectrometer (4000-10.000 cm-1/NIR Flex Solids FT-NIR spectrophotometer, BUCHI s.r.l, Flawil, Switzerland) and, subsequently, were digested, and the free amine (NH2) concentration was determined by the O-phthalaldehyde (OPA) method. The Partial Least Squares (PLS) algorithm was used to construct the prediction model. The accuracy of the proposed model was evaluated by the root-mean-square error of calibration (RMSEC) and cross-validation (RMSECV), linear determination coefficient for calibration (R2c) and cross-validation (R2cv). The release of NH2 during in vitro digestion increased significantly with the proportion of pupunha flour, reaching means values of 201a, 237.8b, 249.8b, 285.9c, and 359.8d mgNH2/g of protein for 12.5, 25, 50, 75, and 100% pupunha flour cookies, respectively, confirming that adding pupunha flour to cookies can offer greater nutritional value, in terms of protein bioaccessibility, compared to the wheat flour traditionally used. The best PLS model, developed using mean-centering preprocessing and 4 latent variables, showed RMSEC of 31.3 mgNH2/g of protein and R^2c of 0.74, while cross-validation yielded RMSECV of 33 mgNH2/g of protein and R^2cv of 0.71. The Variable Importance in Projection (VIP) analysis indicated that spectral regions around 10.000 and 6.494 cm-1 were most relevant for model development, which are consistent with spectral features associated with N–H overtones from amine-containing compounds. Beyond the nutritional relevance of pupunha flour, this study demonstrates that digestion outcomes generated by standardized in vitro protocols can be considered as moldable responses through spectroscopic data. These findings open new perspectives for integrating NIR spectroscopy with INFOGEST 2.0, enabling faster and more sustainable screening of digestion-related nutritional parameters without compromising analytical robustness.

Keywords

In vitro digestion, pupunha, cookies, chemometrics, free amines, prediction models

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

TRACING THE EMPTYING OF DIGESTA PHASES FROM THE HUMAN GASTRIC SIMULATOR FED DIETS DIFFERING IN PROPERTIES: COMPARISON WITH IN VIVO GASTRIC RETENTION TIMES

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Abstract

Dynamic gastric in vitro models can help understand the influence of diet properties on gastric emptying, a key modulator of nutrient digestion kinetics. We evaluated the capacity of the Human Gastric Simulator (HGS) to reflect gastric emptying behaviour of fibre-rich diets, known to induce variation in gastric emptying of digesta phases in vivo in pigs. Six diets contained different insoluble fibres: wheat bran, wood flour, sunflower hulls, fine-, medium- and coarse wheat straw. Two other diets contained either bran plus low-methylated pectin (LP), or coarse straw plus high-methylated pectin (HP). Tracers were used to follow digesta liquids (Cobalt- or Ytterbium-EDTA), fine (Titanium dioxide) and fibrous solids (Chromium mordanted fibres), and determine the in vitro emptying half time (T1/2) of digesta phases, which was compared to the in vivo mean retention time (MRT) of digesta phases. To understand the emptying behaviour of particles from the HGS, particle characterisation of the emptied digesta was performed using image analysis. Compared with the coarse diets (median particle area ≥ 0.87 mm2), fine diets (median particle area ≤ 0.22 mm2), accelerated gastric acidification, reaching an emptied digesta pH 2 within 210 vs. 300 min for coarse diets. The T1/2 of dry matter and fine solids was reduced for fine diets compared with medium and coarse straw (-73 to 136 min; P ≤ 0.05) but not with bran, while the T1/2 of fibrous particles was reduced for fine diets compared with coarse diets (-57 to 406 min; P ≤ 0.05). Particle characterisation of emptied digesta, (particle area, maximum Feret diameter, and eccentricity) highlighted that selective retention of coarser particles occurred in the HGS and was most pronounced for coarse diets compared to fine diets. Pectin addition to coarse insoluble fibres increased the T1/2 of liquids (+ 24 to 95 min; P 0.05) and decreased the T1/2 of fibrous particles (-27 to 78 min; P 0.05), reducing the separation among digesta phases. Reduced gastric sieving caused by the addition of pectin was reflected in the homogeneity of particle maximum Feret diameter and eccentricity in the emptied digesta. In vivo-in vitro relationships showed similar rankings for emptying of liquids and fibrous solids in vitro compared to in vivo (Spearman rank correlation coefficient between T1/2 and MRT of liquids of 0.88; P = 0.007). At lower MRT of liquids in vivo (40 min), the HGS could not discriminate differences between diets. The HGS underestimated gastric emptying of fine- and fibrous solids (Mean prediction error ≥ 108 min), particularly at large MRTs in vivo (> 250 min), underestimating digesta phase separation in vitro. This study illustrates the potential of the HGS to investigate the effects of diet properties on gastric emptying. It also highlights areas for model improvement to generate physiologically relevant in vitro estimates, which can be useful to design foods with specific rate of nutrient release.

Keywords

gastric emptying, in vitro-in vivo, fibres, tracers

Topic: Advances in Digestion and Absorption Models

MOLECULAR CHARACTERIZATION OF PORCINE PANCREATIN AND A-AMYLASE HIGHLIGHTS PROTEIN AND PEPTIDE COMPLEXITY IMPACTING IN VITRO DIGESTION ASSAYS

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Abstract

Commercial porcine pancreatin and its derivative α-amylase preparation are widely employed in in vitro digestion models and enzymatic assays, although their molecular composition is still poorly defined. SDS-PAGE and shotgun proteomic analyses of multiple commercial batches revealed that commercial “purified” α-amylase preparations are not single-enzyme products but partially enriched extracts that retain most of the proteomic heterogeneity of crude pancreatin. Functional classification analysis outlined the canonical repertoire of major pancreatic hydrolases in pancreatin, which was largely conserved in α-amylase preparations. In-depth proteomic analysis identified hundreds of additional gene products in both pancreatin and α-amylase preparations, including enzymes generally overlooked in in vitro digestion models or biochemical assays. Notably, the presence of enzymatically active aminopeptidase N derived from pancreatic granules was experimentally validated by Western blotting using a monoclonal antibody. LC-MS/MS analysis of the low-molecular weight (MW) fractions demonstrated extensive intrinsic proteolysis in both preparations, evidenced by an abundant pool of peptides (10 kDa) resulting from nonspecific endoproteolytic cleavages followed by secondary exopeptidase action. These low-MW components, together with free amino acids, constitute analytical interferents that contribute to background nitrogen and peptide signals, thereby biasing quantitative readouts of proteolysis in in vitro digestion assays, unless rigorous blank correction procedures are applied. Overall, this study establishes a comprehensive proteomic and peptidomic characterization of pancreatin-based enzyme products and underscores the need for more tightly controlled preparations to improve the reproducibility and physiological relevance of digestion models.

Keywords

Pancreatin, α-Amylase, Enzyme contaminants, Endogenous proteolysis, Proteomics

Topic: Advances in Digestion and Absorption Models

COLLABORATIVE TRIAL ON THE PERMEABILITY AND FUNCTIONALITY OF A CELL MODEL OF THE HUMAN EPITHELIUM (CACO-2/HT29-MTX), EMPLOYING CONSENSUS PROTOCOLS

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Abstract

One of the objectives of the INFOGEST Working Group 3-Intestinal Barrier Models is to define a standardized protocol to apply the popular in vitro gut barrier model, Caco2/HT29-MTX co-culture, for food-related applications. This will allow interlaboratory comparisons and will support the efforts to relate cellular transport to physiological absorption. Sixteen laboratories have conducted a collaborative trial with agreed conditions to investigate the permeability and functionality of Caco-2/HT29-MTX. Differentiation of co-cultures of ECACC-selected clones of Caco-2 and HT29-MTX (90:10) for 21 days recreated the small intestine enterocytes and goblet cell population under healthy conditions. The time evolution of monolayer transepithelial electrical resistance (TEER) over these 21 days allowed the definition of a confidence window by day 8, which can predict a mature differentiated barrier by day 21. An inverse relationship between the TEER value and the apparent paracellular permeability of the fluorescent dye, Lucifer Yellow, existed, but the weak correlation with non-linear monophasic decay (R2 = 0.318) indicated that the TEER value cannot be used as a sole predictor of monolayer integrity. Apparent permeability values for Lucifer Yellow (6.9 × 10-8 - 5.1 × 10-7 cm/s) and the drug Propranolol (1.4 × 10-5 - 2.6 × 10-5 cm/s) were in good agreement across the majority of laboratories, indicating that consensus conditions delivered consistent values in comparison to individual protocols previously tested in four laboratories. Permeability values for propranolol, which is highly permeable via uptake by passive transcellular transport, correlated well with the physiological situation reported in humans (Fa = 100%). To assess the model's responsiveness, a two-day challenge to our Caco2/HT29-MTX monolayers with an inflammatory cytokine cocktail induced an inflamed-like 'unhealthy' state. This resulted in a significant pro-inflammatory response according to gene expression or secretion of ROS, cytokines, chemokines, and mucins (p0.05), reduction in TEER values (P0.01), and increased Lucifer Yellow paracellular permeability (p0.05). In addition, initial pilot tests indicated that Caco-2/HT29-MTX monolayers created using the consensus protocols were suitable and responsive to digests and food components such as sugars, amino acids, vitamins, or toxins.

Overall, this collaborative study demonstrates that a standardized Caco-2/HT29-MTX co-culture protocol produces robust, reproducible intestinal barrier models suitable for food research, enables reliable interlaboratory comparisons, and provides a responsive platform for evaluating both physiological and inflammatory conditions.

Keywords

: intestinal human barrier, Caco-2, HT29-MTX, permeability, ring trial, inflammation

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Topic: Advances in Digestion and Absorption Models

QUANTIFYING INTESTINAL TRANSIT TIME IN INFANTS VIA NASODUODENAL AND ENTEROSTOMY MARKERS

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Abstract

Intestinal transit time (ITT) dictates the time available for the dissolution, solubilization, and absorption of nutrients during intestinal digestion. The length of ITT is especially critical in preterm infants as they can have functionally immature digestive capacities. Despite the recent increase in studies characterizing the biochemical parameters of preterm infant digestion (e.g., pH and fluid composition), ITT remains largely uncharacterized in the neonatal population. This study estimated ITT in a neonatal cohort by quantifying the progression of enteral feeds to distal digesta collection sites. Clinical data were reviewed for infants born preterm (less than 37 weeks' gestation) at the Oregon Health & Science University Doernbecher Hospital Neonatal Intensive Care Unit (n = 49, average corrected gestation age at time of collection (TOC): 39 1/6 weeks) utilizing nasoduodenal tubes or enterostomies for standard care. ITT was calculated as the interval between the midpoint of an enteral feed (human milk, formula, or combination) and its initial appearance in stoma effluent or nasoduodenal aspirates. Analysis included comparisons of transit duration across varying stages of digestion. A total of 317 data points were analyzed. The median ITT across the entire cohort was 71.8 minutes. Data were categorized by distal marker location: 25 infants from the duodenum (134 data points, avg CGA 36 5/7 weeks at TOC), 13 infants from the jejunum (83 data points, avg CGA 40 4/7 at TOC), 8 infants from the ileum (69 data points, avg CGA 38 2/7 at TOC), and 2 infants with samples from the proximal colon (25 data points, avg CGA 36 1/7 at TOC). Average transit times (minutes) from feeding to each segment were 49.4 to the duodenum, 59.7 to the jejunum, 81.9 to the ileum, and 96.2 to the colon. These findings provide baseline data for neonatal ITT. This data will inform the creation of more precise infant digestion models.

Keywords

Intestinal Transit Time, Preterm Infants, Neonatal Digestion

Topic: Advances in Digestion and Absorption Models

PEPTIDOMIC COMPARISON OF STATIC AND DYNAMIC IN VITRO MODELS WITH IN VIVO PRETERM GASTRIC AND INTESTINAL DIGESTA

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Abstract

Preterm infants frequently suffer from poor growth and gastrointestinal diseases, yet testing new nutrition interventions directly on these patients is logistically difficult and constrained to standard care of treatments. Ideally, these questions would be initially assessed via in vitro digestion (IVD) models which offer a replicable, cost-effective alternative to invasive human testing. While these models offer a safe, accessible alternative to human testing, these models have not been compared against in vivo preterm infant gastric and intestinal digesta. To address this, we compared 2 laboratory-based preterm infant digestion models against preterm infant in vivo gastric and intestinal aspirates fed the same human milk to determine if they accurately mimic the gastrointestinal system of a preterm infant in terms of peptide release. Pooled human milk was digested using a static preterm IVD protocol and a dynamic SHIME® IVD simulator programmed with preterm physiological parameters. Gastric and intestinal digesta were collected from preterm infants (n=3; mean GA 36 weeks, corrected GA 43 weeks) and were incubated ex vivo to match the digestion duration of the in vitro timepoints (gastric: 30, 60, 90, 120 min; intestinal: 30, 60, 90, 120, 180 min). Peptides from all samples were isolated via trichloroacetic acid precipitation and C18 solid-phase extraction, identified with liquid chromatography with tandem mass spectrometry, and analyzed with Thermo-Proteome Discoverer. We identified 1896 total unique peptides from in vivo samples, 1784 in the static model and 1803 in the dynamic model. Principal component analysis showed the dynamic model's peptide profile clustered closer to the in vivo samples than the static model, particularly during the gastric phase. Venn diagram analysis showed a 46% overlap in peptide count between the dynamic model and the in vivo aspirates, whereas the static model shared 33%. These findings provide direct clinical comparison of preterm digestion models using peptidomics. This comparison helps establish a more reliable preclinical platform for screening novel nutritional and pharmaceutical interventions.

Acknowledgements

Note: We are open to either an oral or poster presentation for this work.

Topic: Advances in Digestion and Absorption Models

DEVELOPMENT OF A DYNAMIC IN VITRO GASTRIC DIGESTION METHOD TO MIMIC ACID SUPPRESSION THERAPY: APPLICATION TO PROTEIN-BASED BEVERAGES

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Abstract

Dairy products are a source of high-quality protein, and milk exhibits slow protein release during digestion due to acid-induced coagulation. However, most digestion studies focus on healthy adults, with limited attention to vulnerable populations. Around 23% of adults worldwide use proton pump inhibitors to suppress gastric acid secretion, primarily for gastroesophageal reflux disease. Acid suppression may alter protein digestion due to higher gastric pH and lower pepsin activity. This study developed a dynamic gastric digestion protocol to mimic acid suppression and used this protocol to evaluate protein digestion kinetics and total protein hydrolysis in milk, kefir, and two plant-based milk-alternative beverages.

The Human Gastric Simulator (HGS) was used to simulate gastric digestion and was combined with a static small intestinal phase. Simulated gastric fluid at pH 0.8 with a secretion rate of 4.1 mL/min was used to mimic healthy adult digestion, whereas pH 2.5 with a secretion rate of 2.05 mL/min mimicked acid suppression. Gastric emptying was 5.3 g/min for both digestion conditions. For each digestion, a cup (240 mL) of dairy or plant-based beverage was added to the HGS. Samples were collected every 30 min for 3 h. Mass, pH, and moisture content were measured. Gastric digesta was mixed with simulated small intestinal fluid (1:1 w/w) and pH was adjusted to 7. Samples were taken every 30 mins for 3 h. Protein hydrolysis was evaluated in all gastric and small intestinal samples, and molecular weight profiles were analyzed by SDS-PAGE.

Gastric pH decreased to 1.4 ± 0.3 under healthy conditions and to 5.1 ± 0.5 under acid suppression conditions across all samples after 90 min digestion. The gastric emptying of dry matter was similar across samples and protocols, and no differences in gastric half-emptying time were observed (33 ± 4 min). Particle size distribution showed acid-induced protein aggregation and curd formation under healthy conditions, whereas acid suppression delayed these processes in dairy samples. For whole milk, aggregates appeared between 30–90 min gastric digestion under healthy conditions but were delayed to 60–120 min gastric digestion under acid suppression conditions. Similarly, low-fat milk formed curds between 30–60 min gastric digestion under healthy conditions and 60–90 min gastric digestion under acid suppression. In contrast, plant-based beverages showed no apparent curd formation under either digestion condition. Under healthy conditions, cumulative free amino group release increased rapidly within the first 120 min of total digestion, for all matrices, reaching 65% for low-fat milk, 76% for pea milk, and 73% for kefir, and plateaued after 180–240 min at approximately 95–99%.

This protocol provides a useful tool for studying protein-based beverages under acid suppression conditions and may contribute to the development of targeted nutritional products for populations with reduced gastric acidity.

Keywords

Human Gastric Simulator, gastric acidity, protein hydrolysis, liquid food matrices.

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

FROM PROTEIN ISOLATION TO CARBOHYDRATE BREAKDOWN: INSIGHTS INTO HUMAN MILK AMYLASE ACTIVITY

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Abstract

Human milk (HM) contains a broad spectrum of bioactive components that support the digestion and absorption of nutrients from milk in newborns, including digestive enzymes. Among them, α-amylase has been suggested to contribute to carbohydrate digestion in early life, particularly in newborns with immature pancreatic function. Interestingly, human milk does not contain classical α-amylase substrates such as starch, yet the enzyme is consistently detected, suggesting that its physiological role may extend beyond classical starch hydrolysis. Despite its potential physiological relevance, the presence and functional role of amylase in human milk remain insufficiently characterized and are often overlooked in in vitro digestion models.

The aim of this study was to isolate -amylase from human milk and to assess its potential activity toward selected carbohydrates. Milk samples were subjected to defatting and protein isolation, followed by preliminary purification using centrifugation and centrifugal filtration. The isolation protocol for human milk α-amylase was established by adapting purification strategies reported for milk and plant α-amylases, particularly those described by El-Fakharany et al. (2017), Amid and Manap (2014), and Azad et al. (2009). The obtained protein fraction was analyzed using SDS-PAGE to confirm presence amylase-like proteins. In parallel, the carbohydrate-degrading potential of the isolated enzyme under acidic and near-neutral pH conditions was evaluated by HPTLC using lactose, maltose, starch, and human milk oligosaccharides (HMOs) as substrates.

Electrophoretic analysis revealed the presence of a protein band with an apparent molecular mass of approximately 60 kDa in milk isolates, corresponding to α-amylase. HPTLC analysis demonstrated clear hydrolysis of starch, with the formation of dextrins, maltose, and glucose, as well as partial hydrolysis of maltose to glucose. No hydrolysis of lactose was observed. Incubation with HMO isolates resulted in subtle but reproducible changes in chromatographic profiles, indicating potential modification of oligosaccharide structures.

The findings highlight the importance of considering endogenous milk amylase when designing and interpreting in vitro digestion models for newborns, particularly in the context of carbohydrate availability and digestion.

References:

1. El-Fakharany, E. M., Uversky, V. N., & Redwan, E. M. (2017). Comparative Analysis of the Antiviral Activity of Camel, Bovine, and Human Lactoperoxidases Against Herpes Simplex Virus Type 1. Applied Biochemistry and Biotechnology, 182(1), 294–310.

2. Amid, M., & Manap, M. Y. A. (2014). Purification and characterisation of a novel amylase enzyme from red pitaya (Hylocereus polyrhizus) peel. Food Chemistry, 165, 412–418.

Keywords

human milk; α-amylase; neonatal digestion; carbohydrate hydrolysis; digestive enzymes; in vitro digestion

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

PHOSPHOLIPID OXIDATION MODULATES HYDROLYSIS KINETICS OF OIL-IN-WATER EMULSIONS IN A PHOSPHOLIPASE A2 AND PANCREATIC TRIACYLGLYCEROL LIPASE DIGESTION SYSTEM

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Abstract

Polyunsaturated fatty acids present in the structure of food lipids are highly susceptible to both enzymatic and non-enzymatic modifications. These alternations may arise at various stages, including food production, processing, storage, as well as gastrointestinal digestion [1–4]. The presence of additional oxygen-containing functional groups, including hydroxyl, hydroperoxide, epoxy, and ketone groups, at the lipid–water interface may alter interfacial properties and modulate interactions with lipolytic enzymes [5]. Nevertheless, experimental data on how oxidative modifications in the lipid structure influence digestion remain limited. Therefore, the aim of the study was to evaluate the impact of phospholipid oxidation on the kinetics of lipid digestion under simulated intestinal conditions.

Model oil-in-water emulsions were prepared by ultrasonication using highly refined olive oil, with either native or lipoxygenase-catalyzed oxidized phospholipids isolated from hen egg yolk serving as emulsifiers. The droplet size distribution and polydispersity index (PDI) were determined by dynamic light scattering (DLS). Lipid digestion was initiated by addition of either phospholipase A2 (Sigma–Aldrich, P6534) alone or in combination with pancreatic triacylglycerol lipase from porcine pancreatin (Sigma–Aldrich, P3292). The hydrolysis rate was monitored using a pH-stat method at pH 8.0 by continuous titration with 0.1 M NaOH to maintain constant pH and neutralize fatty acids released during digestion.

The results demonstrated that phospholipid modification significantly influenced lipid hydrolysis kinetics. In emulsions exposed to phospholipase A2 alone, phospholipid oxidation reduced the hydrolysis rate compared to systems stabilized with native counterparts. In contrast, in the two-enzyme system containing both phospholipase A2 and pancreatic triacylglycerol lipase, emulsions stabilized with oxidized phospholipids exhibited a slightly slower digestion rate during the initial phase (0–30 min). However, at later stages, hydrolysis was accelerated, as indicated by an increased rate of fatty acid release compared to emulsions containing native phospholipids.

Overall, our results indicate that oxidative modification of interfacial phospholipids alters the physicochemical properties of the oil–water interface, which may enhance enzyme accessibility and modulate lipolytic activity. However, further studies are needed to better understand the mechanisms underlying these effects.

References:

[1] Koch E. et al., Food Chem., 2024, 438, 137941.

[2] Harlina P. et al., Heliyon, 2024, 10(16).

[3] Rao D. et al., Trends Food Sci. Technol., 2025, 159, 104968.

[4] Nieva-Echevarría B. et al., Crit. Rev. Food Sci. Nutr., 60(3), 461-478.

[5] Parchem K. et al., Food Res. Int., 2023, 163, 112227.

Keywords

phospholipids; oxidation; emulsion; phospholipase A2; pancreatic triacylglycerol lipase

Topic: Advances in Digestion and Absorption Models

DOES INTESTINAL INFLAMMATION ALTER MYCOTOXIN PERMEABILITY? AN EVALUATION IN CACO-2/HT29-MTX MODEL

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Abstract

Intestinal inflammation is widely recognized to compromise epithelial barrier integrity through alterations in tight junctions, increased permeability, and dysregulated immune signaling [1]. Dietary intake is a major source of epithelial exposure to chemical contaminants, including mycotoxins, which target the gut by impairing epithelial integrity, modulating immune responses, and inducing oxidative stress [2].

Although inflammation is hypothesized to increase xenobiotic uptake by weakening the epithelial barrier, experimental data remain inconsistent [3]. This study aimed to validate an inflamed in vitro intestinal model and to assess whether acute inflammation modulates the impact and permeability of a food-relevant mycotoxin mixture. Caco-2/HT29-MTX cells (90:10) monolayers were differentiated for 21 days on 12-well inserts. An inflammatory phenotype was induced by IFN-γ, followed by IL-1β and TNF-α for 24 hours [4]. The apical medium was then replaced with DMEM (control) or a mixture of ochratoxin A (OTA), zearalenone (ZEN), and aflatoxin B1 (AFB1) (500 µg/L each, non-cytotoxic) for 3 hours. Barrier integrity was monitored in real-time (CellzScope) and gene expression related to inflammation (IL-1β, TNF-α, IL-8), oxidative stress (iNOS, COX2, SOD2) and intestinal-barrier-related markers (MUC2, ZO1, JAM1) were assessed by qPCR. Apparent permeability (Papp) of AFB1, OTA and ZEN were determined by measuring the fraction transported at 30, 60, 120 and 180 min.

The inflamed model was validated by a 23% reduction in TEER, alongside a marked increase in pro-inflammatory cytokines (i.e, IL-1β up 5-fold) and oxidative stress-related enzymes (i.e, SOD2 up 15-fold), confirming the physiological relevance of the induced phenotype. In contrast, exposure to the mycotoxin mixture did not further reduce TEER in either normal or inflamed conditions. Expression of barrier-related genes remained largely similar between normal and inflamed states, and mycotoxin exposure did not significantly modulate gene expression profiles.

All three mycotoxins were transported across the epithelial barrier, with AFB1 exhibiting the highest Papp in normal monolayers (3.36 × 10^−6 cm/s), followed by ZEN (2.61× 10^−6 cm/s) and OTA (9.27× 10^−8 cm/s), consistent with known intestinal absorption patterns [5]. However, no significant differences in transport were observed between normal and inflamed models.

These findings demonstrate that reduced TEER alone is not a reliable predictor of increased mycotoxin transport. While the model proved suitable for short-term evaluation of intestinal barrier integrity and biomarker responses, its ability to replicate chronic inflammation or predict long-term changes in contaminant absorption remains to be established.

[1] doi.org/10.3389/fimmu.2021.767456
[2] doi.org/10.1111/1541-4337.13242
[3] doi.org/10.1080/10717540490280345
[4] doi.org/10.1038/s41598-024-74802-w
[5] doi.org/10.1016/j.fct.2019.110595

Keywords

in vitro intestinal model; intestinal absorption; inflammation; mycotoxins

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Topic: Advances in Digestion and Absorption Models

PANCREATIN CONCENTRATION MODULATES IN VITRO DIGESTIBILITY OF PLANT PROTEINS: INFOGEST IN VITRO DIGESTION COMPARED WITH IN VIVO DATA

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Abstract

Interest in plant proteins as alternatives to animal proteins is increasing due to environmental, health, and animal welfare concerns; however, protein digestibility and the presence of antinutrients, remains a challenge. This study aimed to compare protein digestibility of selected protein sources with differing trypsin inhibitor activities using the INFOGEST static digestion model at two pancreatin concentrations (100 U/mL and 10 U/mL, based on trypsin activity), and to evaluate how these conditions align with previously reported in vivo digestibility data from a standardized pig model.

Six protein sources—Brown Rice Protein Concentrate (BRPC), Pea Protein Concentrate (PPC), Rapeseed Protein Isolate (RPI), Heated Rapeseed Protein Isolate (RPIH), Soy Protein Isolate (SPI), and Whey Protein Isolate (WPI) as a reference—were analyzed using the INFOGEST static in vitro digestion method. The rapeseed protein samples (RPI and RPIH) differed in trypsin inhibitor activity, with heat treatment reducing inhibitor levels and previously being associated with improved in vivo digestibility. Two pancreatin concentrations (100 U/mL and 10 U/mL) were applied to evaluate whether enzyme concentration influences the capacity of the model to reflect differences related to intrinsic trypsin inhibitor activity, and the results were compared with in vivo data previously reported.

In vitro digestibility values obtained with the INFOGEST protocol at 100 U/mL pancreatin concentration were in close agreement with in vivo data for BRPC, PPC, and RPI, with no significant differences observed ($p \geq 0.05$). In contrast, RPIH, SPI, and WPI showed significantly lower in vitro digestibility compared with in vivo values (p 0.05), indicating weaker method agreement for these proteins. In terms of absolute digestibility, BRPC exhibited the lowest in vitro protein digestibility using the 100 U method (71.31 ± 10.68%), whereas WPI showed the highest (91.89 ± 6.37%). When pancreatin concentration was reduced to 10 U/mL, a clearer differentiation between RPI and RPIH emerged, consistent with the improved digestibility of the heated sample reported in vivo. This suggests that lower enzyme concentrations may enhance the sensitivity of the model to intrinsic trypsin inhibitor effects, whereas higher pancreatin levels may attenuate or mask such differences.

These findings demonstrate that pancreatin concentration critically influences the ability of the INFOGEST static model to detect biologically relevant differences in plant protein digestibility. Protein sources containing intrinsic antinutrients, such as trypsin inhibitors, may exhibit significant effects in vivo that are not readily captured under high pancreatin concentrations (100 U/mL). Reducing enzyme concentration to 10 U/mL enhanced differentiation between rapeseed protein samples differing in trypsin inhibitor activity, suggesting increased methodological sensitivity to inhibitor-related effects.

Keywords

INFOGEST, plant proteins, in vitro protein digestion, trypsin inhibitors

Topic: Advances in Digestion and Absorption Models

A SNAPSHOT OF THE HUMAN JEJUNAL ENVIRONMENT: AN IN VIVO-LED APPROACH TO STANDARDISING ALTERNATIVE PROTEIN DIGESTION MODELS

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Abstract

The GIANT LEAPS project aims to accelerate the transition toward alternative proteins by characterising the digestive fate of sustainable ingredients. Addressing critical knowledge gaps regarding the nutritional quality of emerging proteinaceous matrices requires specialised methodologies to quantify food behaviour in the gastrointestinal tract. As part of this initiative, an in vivo digestibility study was conducted on two distinct food matrices, a yellow Chlorella smoothie and a faba bean protein extrudate, to establish definitive human protein digestibility scores.
A key focus of this work was the characterisation of the human jejunal enzymatic landscape via high-resolution mass spectrometry and enzyme activity assays. To isolate and quantify aminopeptidase activity, the study used Leu-pNA as a selective substrate. While Leu-pNA is resistant to major endopeptidases like trypsin, it can be susceptible to chymotrypsin-like proteases (Ruseler-van Embden & van Lieshout, 1988). We employed diisopropyl fluorophosphate (DFP), a potent inhibitor that rapidly deactivates serine-type endopeptidases while preserving the catalytic integrity of Aminopeptidase N (MEROPS J13.101). This dual-assay approach successfully suppressed background pancreatic activity, isolating the specific kinetic contributions of the jejunal enzymes. While these results are promising, the study highlights the need for expanding subjects cohort to distinguish matrix-specific effects from inter-individual variability. Mass spectrometry analysis of the proteins present in the fluid identified Aminopeptidase N as the sole peptidase. However, the presence of PEG (Polyethylene Glycol), the indigestible marker used to determine intestinal flow, significantly challenged both proteomic and peptidomic analyses (Itkonen et al., 2024). This interference necessitated exploring diverse methodological approaches to improve data quality.
This methodological pilot established foundational parameters for a physiologically relevant jejunal phase by using in vivo data from two volunteers. Rather than focusing on final digestibility, the study demonstrated the feasibility of using human clinical data to standardise the enzymatic units required for bench-top simulations, providing a blueprint for future research across diverse food matrices.

Itkonen, S. T., Calvez, J., Airinei, G., Chapelais, M., Khodorova, N., Sahaka, M., Benamouzig, R., Stoddard, F. L., Simojoki, A., Pajari, A.-M., & Gaudichon, C. (2024). True Ileal Amino Acid Digestibility and Protein Quality of 15N-Labeled Faba Bean in Healthy Humans. The Journal of Nutrition, 154(4), 1165–1174. https://doi.org/10.1016/j.tjnut.2024.01.030
Ruseler-van Embden, J. G. H., & van Lieshout, L. M. C. (1988). Increased Proteolysis and Leucine Aminopeptidase Activity in Faeces of Patients with Crohn's Disease. Digestion, 40(1), 33–40. https://doi.org/10.1159/000199640

Keywords

jejuanl digestion, aminopeptidase, mass spectrometry

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

IN VITRO DIGESTION OF LOCUSTA MIGRATORIA: A COMPARATIVE INVESTIGATION BETWEEN STATIC INFOGEST 2.0 AND DYNAMIC GATROMACHINE® MODELS

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Abstract

n the last decade, an increasing demand for sustainable protein sources was registered due to the growing global population, and edible insects received significant attention as high-quality alternative protein sources [1]. Following EFSA's classification of Locusta migratoria (LM) as novel food, investigating in more depth its protein quality when ingested as food or as food ingredient has become essential. As protein quality is related to the amino acid (AA) composition and digestibility [2], the aim of this study was firstly to investigate the AA profile after digestion using two different in vitro digestion approaches which have been applied to ground and oven-dried LM. Therefore, insects were digested by applying the static INFOGEST 2.0 protocol and using the dynamic Gastromachine® system [3,4]. Both methods simulated gastric and intestinal phases using pepsin, pancreatin, bile salts, and electrolyte solutions under controlled conditions. The resulting duodenal digested samples were treated with 80% (v/v) MeOH to separate the digestible from the indigestible fractions. A validated RP-HPLC-DAD method was set up and applied to the undigested and digested samples after a microwave-assisted acid protein hydrolysis to quantify the AAs [5]. The two digestion models produced similar results in terms of qualitative and quantitative AA profile. In fact, all AAs were quantified except Trp, which degraded during acid hydrolysis. Cys was the most abundant AA in the undigested insect; conversely, after the digestion, the most abundant was Glu. Similar bioaccessibility values were recorded for His, Gly, and Glu using both the digestion approaches. The concordance between static and dynamic digestion data highlighted the consistency of the two tested models in simulating human gastrointestinal conditions.
To investigate the potential of LM as an important protein source, the amino acid profile obtained after INFOGEST 2.0 digestion was used to evaluate the insect’s protein quality using the Digestible Indispensable Amino Acid Score (DIAAS). This demonstrated that the quality of Locusta migratoria protein was similar to that of traditional plant-based protein sources; however, it showed lower DIAAS values than animal-based protein sources.

References

[1] E. Ververis, G. Boué, Journal of Food Composition and Analysis, 2022, 114, 104859.
[2] A. Jonas-Levi, JJ. I. Martinez, Journal of Food Composition and Analysis, 2017, 61, 184
[3] A. Brodkorb, L.Egger, Nature Protocol, 2019, 14, 991.
[4] A. Fratter, A. Cignarella, European Journal of Pharmaceutical Sciences, 2024, 203, 106912
[5] L. Hammer, D. Moretti, Frontiers in Nutrition, 2023, 10, 1150581.

Keywords

INFOGEST 2.0, Gastromachine, Food digestion

Topic: Advances in Digestion and Absorption Models

DIGESTION MODEL MATTERS: IMPACT OF PANCREATIN VERSUS TRYPSIN/CHYMOTRYPSIN ON THE DIGESTIBILITY OF ALLERGENIC FOOD PROTEINS

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Abstract

Food allergy is a growing global health concern posing significant challenges to the food industry and regulatory authorities. The allergenic potential of food proteins is linked to their ability to bind IgE which can be modulated by gastrointestinal (GI) digestion. Understanding how different protein matrices behave under in vitro digestion conditions is essential to better predict allergenic risk and support the development of safer technological food ingredients. Although simplified enzymatic systems are often used, their comparability and suitability for allergenicity assessment remain unclear.

This work aimed to evaluate the influence of simulated GI digestion on the IgE-binding capacity of proteins from animal/legume matrices used as technological food ingredients and understand the use of pancreatin and simplified trypsin/chymotrypsin models for the intestinal phase.

The tested materials included lupine flour, green pea and soybean protein isolates, defatted roasted peanut powder, milk protein concentrate and whole egg powder. Simulated GI digestion followed the harmonized INFOGEST protocol [1], applying either pancreatin or trypsin/chymotrypsin in the intestinal phase. Protein profiles were evaluated by SDS-PAGE, and peptide released by high-resolution mass spectrometry (HR-MS), while their IgE-binding capacity by immunoblotting with sera from allergic patients. The degree of hydrolysis (DH) was determined by the OPA-NAC method at the beginning/end of gastric/intestinal phase.

SDS-PAGE and immunoblotting evidenced the effect of GI digestion by the degradation of protein bands and a reduction in the IgE-binding capacity along gastric and intestinal phases. Pancreatin and simplified trypsin/chymotrypsin models presented different patterns of protein degradation and IgE-binding capacity during the intestinal phase in all matrices, evidencing the importance of comparing their performance in allergenicity studies. In fact, the trypsin/chymotrypsin model produced a more gradual intestinal digestion pattern and enabled a clearer evaluation of residual IgE binding. Pancreatin, although leading to a higher extent of hydrolysis, was associated with a certain degree of unspecific IgE-binding after GI digestion, especially in legume matrices. HR-MS analysis showed a higher number of peptides released in the intestinal phase than in the gastric one. Intestinal digesta obtained with pancreatin contained shorter peptides than those generated by the simplified model, consistent with its broader enzymatic activity.

Overall, the two digestion models are not equivalent, and their results require careful interpretation when comparing allergenicity outcomes. These findings highlight the influence of the digestion model on protein degradation and IgE-binding, emphasizing the need for careful selection and standardization of in vitro protocols to evaluate the allergenic potential of food ingredients.

Ref.:

[1] Brodkorb A et al. 2019, 10.1038/s41596-018-0119-1

Keywords

Food allergy, food ingredients, pancreatin, trypsin/chymotrypsin, IgE-binding

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

ADVANCED METHODOLOGIES TO INVESTIGATE FOOD COMPOUNDS BIOACCESSIBILITY: MILLIFLUIDIC CELL-BASED SYSTEMS AS NEW PROMISING TOOLS

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Abstract

Millifluidic cell-based systems represent versatile in vitro platforms to reproduce dynamic simil-physiological conditions useful in the study of food and pharmaceutical compounds screening and testing1. We used a commercial millifluidic device (LiveFlow®, IVTech) to set-up a new multi-organ model to simulate the digestion process.

The gastrointestinal process was reproduced by two bioreactors constituted by chambers in which gastric (GIST-882) and intestinal (Caco-2) human cells were seeded. The first set-up was performed by testing methylglyoxal (MGO), a potential highly reactive and toxic compound, generated both endogenously and in food. MGO is a small dicarbonyl compound responsible for the formation of advanced glycation end products (AGEs), involved in oxidative and inflammatory processes related to age-related diseases (diabetes, cancer, cardiovascular and neurological disorders)2. Then, using the same set-up the effect of chlorogenic acid (CGA), a food-derived phenolic compound with anti-AGEs activity able to trapp MGO3 was investigated. MGO and CGA were also texted in mixture at different concentrations monitoring their concentration at different digestion steps by validated RP-HPLC-DAD methods. Toxicity studies were also carried out by cell-viability assays2,3. The results obtained using this new dynamic model were compared with those obtained by using static approaches (InfoGest protocol)4,5. The new dynamic system highlighted a potential role of gastric cells in the digestion, not evident using the static approach. In both dynamic and static approaches the results indicated that CGA lost the capacity to trapp MGO, as reported in our previous experiments, confirming that digestion experiments are fundamental for the prediction of the potential in vivo bioactivity of a compound. The only intestinal bioreactor was also used for testing different polyphenols' (caffeic, quinic, and rosmarinic acids, quercetin, rutin) bioaccessibility in comparison with literature data to validate the new system for screening experiments6. Preliminary experiments to add an oral cell-based compartment are also in progress.

Millifluidic cell-based systems already represent an important evolution of traditional in vitro tests with the possibility to recreate multi-organ platforms, but in the future they could be promising alternative to reduce animal testing, at least in preliminary experiments.

References

1. Cacopardo L. et al. Biosens Bioelectron 2019, 140, 111340.
2. Colombo R. et al. Food Funct. 2019, 10, 4330.
3. Colombo R. et al. Food Funct. 2023, 14, 541.
4. Minekus M. et al. Food Funct, 2014, 5,1113.
5. Hamzalıoğlu A. et al. Food Funct. 2016, 7, 2544.
6. Colombo R. et al. Appl. Sci. 2023, 13, 5033.

Keywords

Gastrointestinal models; dynamic approaches; millifluidic devices; digestion; food molecules.

Topic: Advances in Digestion and Absorption Models

INVESTIGATION OF THE STRUCTURE AND DIGESTION OF PLANT PROTEIN-STABILIZED LIPID EMULSIONS IN VITRO

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Abstract

Human lipid digestion begins at the interface of oil and water by interfacial adsorption of lipases. Tailoring the available surface area for lipase activity can lead to specific lipid sensing in the body, thus, tailored satiety hormone release.[1, 2] Therefore, we aim to explore the effects of plant proteins as the surface-active materials to modulate the gastric stability of oil-in-water emulsions.

Interfacial shear rheology was used to investigate the layer evolution during gastric in vitro digestion steps. Medium-chain triglyceride (MCT) oil was used for the oil phase. β-lactoglobulin (β-lg) and soy protein concentrate (SPC) were used for preparing protein solutions at pH 2. Interfacial storage Gi' and loss Gi'' moduli of adsorbed layers were measured over time at constant oscillation. Simultaneous subphase exchange was performed, based on the methodology described by Bertsch et al. [3]. After having equilibrated states with a protein solution, 0.4 mg/ml pepsin was injected into the subphase. This technique allows insights into viscoelastic layer evolution under various gastric conditions and thus an estimation of the emulsion destabilization process in the stomach.

Interfacial rheological measurements showed that Gi' > Gi'' for both β-lg and SPC, confirming that both proteins formed stable and viscoelastic layers at the interface. However, the viscoelasticity and stability were influenced by the protein source and the concentration of the protein solution. Besides, the interface type influenced the layer properties as well. Interfacial moduli were higher at the A/W interface compared to the O/W interface. It suggests that air provides a stronger driving force for protein adsorption and unfolding compared with the MCT oil phase.

The digestive behavior of pepsin also varied between proteins. For β-lg, the interfacial moduli decreased immediately after the injection of pepsin, indicating that the pepsin adsorbed to the interface and hydrolyzed the protein network to peptides. The layer was fully degraded after around one hour. After complete degradation of β-lg, the peptides started to rearrange and build a new network at the interface, which was indicated by the increase in interfacial moduli. The SPC layer was also digested by pepsin, which was characterized by a faster decrease in Gi' than Gi'' after adding pepsin. In contrast to β-lg, SPC exhibited a much slower reduction in the interfacial moduli. After around 28 hours, the condition Gi' Gi'' was reached, suggesting the complete breakdown of the interfacial network. These results suggest that interfaces stabilized by plant proteins may possess higher resistance to enzymatic degradation compared to animal proteins.

These findings provide fundamental information for designing plant-protein-based lipid emulsions to modify lipid digestion.

[1] Scheuble et al., Biomacromolecules, 2014

[2] Bertsch et al., Food Funct., 2022

[3] Bertsch et al., Soft Matter, 2019

Keywords

Interfacial rheology; plant protein; in vitro digestion

Topic: Advances in Digestion and Absorption Models

TOWARDS IN SILICO MODELS OF GASTRIC PROCESSING

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Abstract

Foods are amongst the most complex materials that one can study in terms of mechanics of materials. Yet if we are to deliver innovations in food production, we need understanding for how such complex materials behave rheologically under various loading and environmental conditions (strain rate/time, temperature). Only then we can provide solutions to the several, pressing challenges related to food, including a shift to sustainable, nutritious and enjoyable food products with a lower environmental impact, healthier human outcomes and positive sensory perceptions.

This talk will summarise our research related to the human digestion process of starch based foods which crucially affects the rate of release of nutrients in the gastrointestinal tract. We show how constitutive laws are calibrated through independent tests and then used as inputs in Finite Element (FE) models to simulate food structure breakdown during the gastric process. Parameters of the chemo-mechanical constutitve law related to diffusion, reaction and mechanical deformation parameters are calibrated using experimental data. Peristaltic waves are simulated via displacement boundary conditions on the stomach wall, while chemical loading is represented by a mass field of gastric fluids. The simulations are used to determine initial gatric emptying rates that can be linked to glycaemic index of foods.

Keywords

digestion, in silico models, Chemo-mechanical models, Diffusion

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

BEYOND INTESTINAL ABSORPTION: CAN FOOD CONTAMINANTS INDUCE NEUROTOXICITY THROUGH GUT-BRAIN AXIS SIGNALING?

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Abstract

Human exposure to food contaminants (FC) remains unavoidable due to environmental persistence and chronic low-dose dietary intake. Several FC, including pesticides and heavy metals, have been associated with neurodegenerative diseases (ND), particularly Parkinson's disease (PD) [1]. To date, research on FC in the gastrointestinal tract has primarily focused on intestinal transport, epithelial barrier integrity, and microbiota modulation following oral exposure. Yet the gut is more than an absorptive barrier; it is a neuroimmune-endocrine interface that communicates bidirectionally with the central nervous system through the gut-brain axis (GBA), with the enteric nervous system (ENS) serving as a direct neuronal link.

Given that both FC exposure and GBA dysfunction have been implicated in ND [2], this work aimed to understand the effect of FC in the ENS, and to which extent FC-induced alterations in the gut may exacerbate central neurotoxicity. Thus, a systematic review was conducted under PRISMA guidelines to compile and critically evaluate experimental evidence on intestinal and GBA involvement in FC-induced neurotoxicity. Twelve animal studies specifically evaluated gut-brain communication by assessing vagal involvement in central outcomes, while local intestinal and ENS effects were further characterized in 22 in vivo and 15 in vitro studies.

Pesticides, mycotoxins, bisphenols, acrylamide, manganese, and micro/nanoplastics (MNPs) exposure consistently induced ENS neurochemical remodeling, enteric glial activation, and intestinal inflammatory responses, often accompanied by epithelial barrier alterations. Rotenone, paraquat, and PS-MNPs promoted α-synuclein aggregation in enteric neurons and its propagation to the brain via the vagus nerve. Vagotomy attenuated central neurotoxicity, supporting a mechanistic role for GBA communication and reinforcing the body-first hypothesis of PD. These findings indicate that FC not only induce local alterations within the gastrointestinal tract but also contribute to central neurotoxicity through GBA-mediated signaling mechanisms.

Current intestinal in vitro models, particularly Caco-2/HT29 co-culture systems, are widely used to evaluate transepithelial transport and permeability, with harmonization efforts advancing within the INFOGEST framework [3]. Integrating ENS components into these models is essential to advance new approach methodologies (NAMs) and incorporate gut-brain mechanisms within adverse outcome pathway frameworks, enabling mechanistically informed toxicological assessment. Within the neuroNAMix project, we are developing in vitro platforms that combine epithelial and enteric neuronal elements to support mechanistic evaluation and prioritization of single and mixed FC exposures under physiologically relevant conditions, thereby linking intestinal absorption with neurotoxicity-relevant endpoints.

[1] 10.1016/j.cofs.2025.101369
[2] 10.1016/j.jpha.2025.101521
[3] 10.1021/acs.jafc.3c05479

Keywords

intestinal models; gut-brain axis; food contaminants

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

IMPACT OF PARTICLE DENSITY AND GASTRIC SECRETION RHEOLOGY ON GASTRIC EMPTYING

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Abstract

Background: Gastric mixing and emptying are influenced by meal properties like particle density and gastric secretion rheology but isolating their independent effects in vitro is challenging due to concurrent structural and biochemical breakdown of food. To eliminate these confounding factors, undigestible polymer beads (3 mm diameter) with densities representative of fats (Polypropylene, 0.90 g/cc), proteins (Polystyrene, 1.04 g/cc), and carbohydrates (Delrin, 1.42 g/cc) were used in model meals in a dynamic gastric digestion model, the Human Gastric Simulator (HGS). The rheological properties of in vivo (human) gastric secretions are significantly different from in vitro gastric fluid which may impact gastric emptying kinetics. Thus, a combination of particle densities representing different macronutrients and gastric secretions varying in rheology on gastric emptying was investigated.

Methods: A mixed model meal (400 g beads) was designed by combining beads in a 3:3:4 ratio for fats (Polypropylene) to proteins (Polystyrene) to carbohydrates (Delrin). To examine meal component mixing, beads were colored and separated into 3 fractions sequentially fed into the HGS: a bottom fraction (5% meal mass); a middle fraction (90% meal mass); and a top fraction (5% meal mass) maintaining the same ratio of bead types in each fraction. Prior to digestion, 200 mL of either water or 1% xanthan solution was added to the HGS representing fasting gastric secretions. After meal feeding, gastric contractions (3 per min) and secretions were started (4.86 mL/min) consisting of water or 0.3% xanthan solution to mimic in vitro or in vivo gastric fluid rheology, respectively. Gastric digestion continued for 180 min and 202 g of digesta emptied was every 30 min. The quantity of each bead type in each fraction was determined by density and color separation. In addition, model meals consisting of each of the three bead types in a single component model meal (400 g beads per meal) were tested with 3% of the beads in the top and bottom fractions and 97% in the middle fraction.

Results: In the mixed model meal, gastric emptying was significantly affected by secretion rheology, bead type and digestion time with significant interaction effects (p 0.001). Positional emptying of beads associated with total emptying ($r \geq 0.9$) with xanthan secretion significantly reduced the correlation between top fraction and total emptying for Polypropylene and Delrin (p 0.05). Gastric emptying half-time decreased from 108 to 36 min for Delrin; from 148 to 81 min for Polystyrene when xanthan was used instead of water as the gastric secretion in the mixed model meal.

Conclusion: Distinct emptying kinetics due to gastric secretion rheology (mimicking in vitro vs in vivo gastric fluid) highlights the need to reevaluate simulated gastric fluid formulation. Density driven differences in gastric emptying offers a framework to rationally engineer food structures for controlled gastric emptying.

Keywords

gastric secretion rheology, particle density, gastric mixing, gastric emptying

Topic: Advances in Digestion and Absorption Models

LINKS BETWEEN STATIC AND DYNAMIC GASTRIC DIGESTION MODELS IN SOLID DAIRY PRODUCTS

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Abstract

Background: Many dairy protein matrices are formed through aggregation of casein micelles into a continuous network causing macrostructural similarities across products. Although such matrices share comparable initial structure, variations in composition and processing can modulate mechanical properties, gastric softening, and gastric emptying. The Food Breakdown Classification System (FBCS) was developed to categorize products based on initial texture, macrostructure, and softening kinetics from static digestion as predictors of in vivo gastric breakdown. However, whether static digesta properties correspond to gastric emptying in a physiologically relevant dynamic digestion model remains unclear. Therefore, the objective of this study was to evaluate the relationship between digesta properties across static and dynamic in vitro gastric digestion models in 4 solid dairy foods with comparable protein structure.

Methods: Mozzarella, paneer, and haloumi were commercially procured, and freeze-dried haloumi was prepared from fresh haloumi. In the static model, mozzarella and paneer were sliced into 10 mm cubes and haloumi (fresh and freeze dried) into 12.7 mm cubes followed by a 30 s oral phase and 3 h gastric phase at pH 2 and 37°C in a shaking water bath. Digesta was collected every 30 min for analyzing dry matter loss, texture, and acid uptake. For dynamic digestion, the Human Gastric Simulator (HGS) was used, and samples were ground to 1-4 mm to simulate mastication. The gastric secretion rate was 4.1 or 4.86 mL/min and emptying rate at 5.67 or 6.73 mL/min, and digesta was emptied every 30 min up to 3 h and analyzed for pH, solid to liquid ratio of digesta and dry matter emptying.

Results: FBCS identified freeze-dried haloumi as Class 1 (high hardness, fast softening), mozzarella as Class 5 (medium hardness, slow softening), and paneer and haloumi as Class 6 (low hardness, slow softening). The gastric half-emptying times in the HGS were 69, 98, 107, and 133 min for paneer, mozzarella, haloumi, and freeze-dried haloumi, respectively. Despite fast softening, freeze dried haloumi had the slowest gastric emptying. This is likely because the low moisture content resulted in hydration being required prior to emptying. Softening kinetics strongly correlated with gastric emptying kinetics ($r = 0.93–0.98$), suggesting that structural weakening due to gastric fluid diffusion in the static model was associated with gastric emptying in the HGS. Except mozzarella, dry matter loss in the static model correlated strongly to solid phase emptied in the dynamic model ($r = -0.9$), suggesting surface erosion in the static model was associated with solid breakdown and release in the HGS.

Conclusion: These findings support the use of the FBCS to understand differences in gastric breakdown behavior of foods with varying structures, but complementary validation using dynamic gastric models enhances prediction of physiologically relevant gastric emptying behavior.

Keywords

Food Breakdown Classification System, Static vs Dynamic In Vitro Gastric Digestion Model, Dairy Food Matrices,

Acknowledgements

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Topic: Advances in Digestion and Absorption Models

SHEDDING LIGHT ON THE INTERACTION BETWEEN A FLAVAN-3-OL MICROBIAL METABOLITE AND COLON EPITHELIAL CELLS USING ADVANCED 2D INTESTINAL ORGANOID MONOLAYERS

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Abstract

Oligomeric polyphenols flavan-3-ols are present in many plant-based foods such as stone fruits and have been linked to beneficial health outcomes, including reduced risk of gastrointestinal diseases. Proanthocyanidins (PACs) are the major flavan-3-ols found in food, and after digestion, up to 90–95% of them reach the colon intact. The colonic microbiota transforms PACs into specific gut microbial metabolites, such as hydroxyphenyl-γ-valerolactones (PVLs). PVLs like the 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (3’4’ PVL) are produced in the proximal colon, where they may act locally, interacting with colon epithelial cells (CEC). Recent findings highlighted that 3’4’ PVL may inhibit proliferation and promote apoptosis in colon rectal cancer cells. However, to date, there remains a knowledge gap regarding the interaction between PVLs and healthy CEC. To explore this interaction, 3D and 2D colon organoid models, bulk RNA sequencing, and mass spectrometry imaging (MSI) approaches were used to advance the understanding of the effect of 3’4’ PVL on healthy CEC. Firstly, a screening of different physiological concentrations of 3’4’ PVL (0-150 µM) was performed on 3D-mouse colonoids. Results showed that 3’4’ PVL didn’t affect cell viability, but inhibited apoptosis in a dose-dependent manner, 51-58% at the highest concentrations. Next, 2D colon organoid monolayers were seeded from dissociated 3D colonoids in wells of 48-well microplates. After 5 days, monolayers reached confluence and were treated with 3’4’ PVL at 100 µM. A single exposure for 72h and a sequential exposure, 3 times every 72 h, were performed. RNA-seq results showed that 3’4’ PVL shifted the gene expression profile of CEC compared to the control. Moreover, Differential Gene Expression analysis revealed 4872 and 5488 genes differentially expressed genes (DEGs) in PVL/Control single and sequential exposures, respectively. Interestingly, PVL treatment downregulated DEGs associated with the negative regulation of apoptosis. Additionally, gene set enrichment analysis (GSEA) revealed significant activation of the P53 pathway and mTORC1 signaling in PVL-treated monolayers, indicating that PVL enhanced stress responses and supported controlled growth, metabolism, and survival in colon cells. These findings were confirmed and supported by MSI results, showing that 3’4’ PVL modulates the spatial distribution of intracellular lipids, particularly phosphatidic acid, which may regulate apoptosis and proliferation via the mTOR pathway. Additionally, 3’4’ PVL was biotransformed into other metabolites inside colon cells that may further contribute to cellular homeostasis. Overall, these findings suggest that 3’4’ PVL protects healthy colon cells by regulating key signaling pathways, gene expression, and lipid metabolism. Thus, this highlights its beneficial role in maintaining colon cell homeostasis and epithelial integrity.

Topic: Advances in Digestion and Absorption Models

COMPARING THE DEGREE OF PROTEOLYSIS AND LIPOLYSIS OF HUMAN MILK DIGESTED BY PRETERM INFANT IN VITRO DIGESTION MODELS AGAINST PRETERM INFANT DIGESTA

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Abstract

Preterm infants face significant health risks due to their underdeveloped gastrointestinal systems, which compromises their digestive function. A protective factor to preterm infants from poor health outcomes is feeding human milk (HM). HM contains nutrients and bioactive components such as proteins and lipids that build immunity, support digestion, and improve absorption in the preterm infant gut. Current understanding of digestion and the degree to which digestion of HM occurs within the preterm infant gastrointestinal tract remains limited. One way to study how HM is digested by a preterm infant is through in vitro digestion models where HM feeds are digested under standardized conditions and parameters simulating the preterm infant. In vitro digestion models also offer a solution to the technical challenges of in vivo studies, as in vivo sampling is time consumptive, expensive, and limited in both sample volume and types of feeds.

We recruited 3 preterm infants (mean GA 36 weeks, average CGA 43 weeks) from the Oregon Health & Science University neonatal intensive care unit who received a donor HM feed. Digesta samples were collected from the stomach from indwelling naso-gastric tubes, and intestinal samples were collected via nasal-jejunal tubes (30 min post-feed) and frozen at –80°C until analysis. The same donor HM was used for static and dynamic in vitro digestion models with matched sampling timepoints to preterm infants. The digestion profiles of in vivo and in vitro samples will be compared across digestion in terms of degree of proteolysis and lipolysis. We will measure the total degree of proteolysis and lipolysis through an OPA (O-phtalaldehyde) assay and a free fatty acid analysis kit, respectively. We hypothesize total proteolysis and free fatty acid release will increase across digestion. We also hypothesize that the dynamic in vitro digestion model will align more with in vivo data in comparison to static as it considers secretion and gastric emptying rates.

Comparing digestion profiles by examining protein and lipid hydrolysis patterns from in vivo and in vitro samples across gastric and intestinal timepoints will help validate these in vitro digestion models. Beyond model validation, this comparison will inform improvements in donor HM processing and support the optimization of nutritional interventions for preterm infants.

Keywords

preterm infant, digestion, proteolysis, lipolysis, human milk

SESSION

4 Physicochemical and Imaging Techniques for Characterising Food Digestion

Werner Weitschies

Professor of Biopharmaceutics

Institute of Pharmacy, University of Greifswald, Germany

Professor Werner Weitschies is the Chair of Biopharmaceutics at the Institute of Pharmacy at the University of Greifswald. With a background in academic and industrial pharmaceutical research, he specialises in developing advanced oral drug delivery systems and applying in vivo imaging technologies and predictive biopharmaceutical testing methods to these systems.

He has authored over 300 publications and holds more than 30 patents in this area. His primary research interest lies in understanding gastrointestinal drug transit and absorption. To this end, he has developed innovative tools such as magnetic marker monitoring and ingestible systems to track the behaviour of dosage forms in humans. He is also involved in designing and evaluating patient-centred, physiologically relevant dosage forms, particularly for ageing populations.

He is a founding member of the interfaculty Centre for Drug Absorption and Transport (C_DAT) at the University of Greifswald. He has repeatedly been listed among Clarivate's Highly Cited Researchers, reflecting his significant impact on the field of pharmaceutical sciences.

ABSTRACT

Food-Drug Interactions: Lessons learned from MRI Imaging

The interaction between food intake and orally administered drugs in the gastrointestinal tract is not well understood, making it difficult to predict the effects of food intake on the extent and time course of drug absorption. This is why we have been investigating this topic for many years. The use of magnetic resonance imaging (MRI) and, more recently, real-time MRI now makes it possible to gain in-depth insights into gastrointestinal processes such as transport and mixing of gastrointestinal contents and to a limited digestion. This lecture will present data obtained using these techniques. Their significance for understanding the gastrointestinal processing of food will be discussed. Furthermore, examples will be presented how these insights can be applied to improve physicochemical and in silico simulation models.

ORAL PRESENTATIONS

Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

PROBING DIGESTION OF BREAD AND FRUIT AT THE SCALE OF A FOOD PIECE USING MRI

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Abstract

Upon ingestion, solid foods reach the stomach as an aggregated bolus composed of particles with sizes up to 5 mm. Digestion kinetics are governed by structural and compositional features of these particles, that can limit enzymatic action, either by reducing the diffusion of digestive fluids within the matrix or by the inherent resistance of specific microstructures, such as gluten and cellular walls. Gaining insight into how solid foods are digested calls for piece-level, spatially resolved characterization of digestion phenomena. Owing to its ability to non-destructively characterize food composition and structure, MRI is well-suited for such purposes.

This study aimed to investigate the mechanisms occurring at the scale of food pieces that govern their kinetics of digestion. To explore different matrix effects, three foods belonging to two main categories were selected: bread, as a model of starchy products, and apples and tomatoes, as models of fleshy fruit with distinct microstructures. Using an original in vitro digestion setup designed for imaging of large solid food pieces, a 1.5 T whole-body MRI imager was used to characterise changes in composition and multiscale structures during gastrointestinal digestion. The adapted INFOGEST static in vitro protocol was applied to ~15 mm cubic food pieces, sized to match the millimetric resolution of MRI images. 3D morphometric imaging, transverse relaxation time (T_2) and micro-porosity (fruits only) mapping were performed throughout digestion and complemented by chemical analyses of the digestion fluid.

The bread volume remained stable during gastric digestion but decreased during intestinal phase, while the surface became roughened and fluid penetrated the pores, consistent with gluten hydrolysis. T_2 analysis distinguished two water fractions in bread: one interacting with the solid crumb matrix and the other corresponding to the digestion fluid within pores smaller than the voxel. Temporal changes in these fractions may indicate internal enzymatic attack and nutrient hydrolysis. Notably, starch hydrolysis occurred independently of gluten network breakdown and could be monitored via changes in the T_2 of the digestion fluid. In apples, volume reduction and the decrease in peripheral mean T_2 and microporosity during intestinal digestion, reflected tissue erosion and fluid penetration. Similar behaviour was observed with both active or denatured enzymes, suggesting that the effects were mainly driven by pancreatin and bile diffusion, cell disintegration and local pH variation rather than enzyme activity. No changes in peripheral T_2 or volume reduction comparable to those in apples were observed in tomatoes, suggesting that their internal microstructure is more resistant to digestive fluids.

In conclusion, this MRI-based approach provides piece-scale, spatially resolved insights into the digestion of solid foods and highlights the influence of food microstructure on digestion behaviour.

Keywords

Magnetic Resonance Imaging; structure; food matrix; NMR relaxation;

Acknowledgements

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Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

UNRAVELLING THE SPECIATION AND STRUCTURAL DYNAMICS OF HEME IRON DURING DIGESTION BY X-RAY ABSORPTION SPECTROSCOPY

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Abstract

The World Health Organization (WHO) estimates that iron deficiency affects more than 1.2 billion people worldwide, highlighting the urgent need for the development of effective nutritional strategies. While iron bioaccessibility is commonly assessed using different in vitro digestion models, the speciation of iron during gastrointestinal digestion -a key determinant of its absorption- remains poorly characterized and understood. Therefore, gaining insight into these transformations is essential for the design of more effective nutritional interventions.

To address this, we applied a comprehensive methodological framework to investigate iron speciation during gastrointestinal digestion. Myoglobin was employed as a standard due to its well-established high absorption efficiency. Samples were subjected to in vitro digestion following the harmonized INFOGEST protocol, and iron speciation was characterized using synchrotron-based X-ray absorption spectroscopy (XAS). Cellular iron uptake was evaluated using a Caco-2/HT-29MTX (90:10) co-culture model.

XANES analysis confirmed the maintenance of the ferrous (Fe^{2+}) state throughout digestion. Interestingly, EXAFS revealed dynamic and reversible structural adaptations during digestion. While non-digested myoglobin exhibits octahedral coordination, gastric conditions induced a five-coordinate geometry, which reverted to octahedral coordination after the intestinal phase. This structural flexibility suggests a protective mechanism that may prevent iron release and oxidation in the stomach, thereby facilitating its controlled delivery for absorption. Consistent with this, cellular assays demonstrated efficient iron uptake from the digested myoglobin.

Overall, this study establishes XAS as an indispensable tool for mechanistic digestion research, providing evidence of reversible heme iron coordination changes during digestion that underpin its enhanced bioavailability.

Keywords

iron deficiency, digestion, XAS, iron speciation

Acknowledgements

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Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

RETHINKING LACTOSE MALABSORPTION: DIGESTIVE RESPONSE TO A LOW-LACTOSE, HIGH GALACTO-OLIGOSACCHARIDES MILK ASSESSED BY MRI IN HEALTHY CHINESE ADULTS

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Abstract

Background:
Lactose malabsorption is highly prevalent in China and often leads to reduced dairy intake due to “lactose avoidance” which can compromise intake of essential nutrients provided by dairy products such as calcium, vitamin D, and high-quality proteins. Fibre intake is also suboptimal, with >95% of adults below recommendations. The MilkRI clinical trial examined whether a low-lactose milk in which most lactose is converted to galacto-oligosaccharides (GOS) could provide a digestively tolerable milk alternative for Chinese adults.

Methods:
This randomised, double-blind, controlled, 2×2 crossover study enrolled 24 healthy Chinese adults with 96% confirmed lactose malabsorption. Following an overnight fast, participants consumed a single 400 mL dose of either the investigational product (IP; low-lactose milk with 12.3 g GOS and 2.6 g lactose) or the control product (CP; regular skimmed milk with 24.2 g lactose, no GOS). Postprandial responses over 315 min were assessed by magnetic resonance imaging, breath hydrogen testing, and symptom questionnaires. Primary outcomes were small bowel water content (SBWC; AUC0–315 min) and maximum small bowel motility (Cmax0–315 min). Secondary outcomes included perceived digestion, appetite and symptoms, gastric emptying, colonic volume and breath H2.

Results:
SBWC increased only modestly with CP, with no significant difference between products (AUC ratio IP/CP: 1.12 [95% CI 0.95, 1.33], p=0.16). IP produced a modest rise in maximum small bowel motility (+8% above CP; Cmax difference IP–CP: 28.73 a.u. [3.17, 54.30], p=0.03) and accelerated gastric emptying (T1/2 difference IP–CP: –9.79 min [–17.88, –1.70], p=0.02) compared to CP. Both products were well tolerated; no adverse events and only infrequent, mild symptoms were reported with no differences between products. No differences were observed in perceived digestion or appetite. IP led to higher breath H2 (AUC ratio IP/CP: 1.36 [95% CI 1.02, 1.82], p=0.04) and colonic volume (AUC ratio IP/CP: 1.09 [95% CI 1.04, 1.15], p = 0.001), reflecting increased microbial fermentation.

Conclusion:
Milk in which lactose was converted into prebiotic GOS was well tolerated and promoted microbial fermentation in the colon of Chinese adults. No significant increase in small bowel water content was observed after milk ingestion compared with low-lactose milk. This challenges the long-held assumption that lactose-containing dairy markedly elevates luminal osmotic flux and contributes to digestive symptoms in lactose malabsorbers. Future investigations will be required to understand how lactose and fibers interact with the microbiome and link to potential digestive symptoms in lactose intolerant individuals.

Keywords

Lactose malabsorption; Galacto-oligosaccharides; Digestive tolerance; Magnetic Resonance Imaging; Prebiotic; Fiber

Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

MEASUREMENTS OF REDOX BALANCE AND PH ALONG THE GUT USING A MINIATURIZED INGESTIBLE SENSOR

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Abstract

Monitoring conditions in the gastrointestinal tract (GIT) is challenging due to its length and inaccessibility. Current methods such as endoscopies in humans and T-cannulas in animals are invasive, while alternative biospecimen like fecal samples only provide insights into the final end of the GIT. To overcome these challenges, a Gastrointestinal Smart Module (GISMO) was developed, a highly miniaturized ingestible with multiple sensors. This ingestible can measure pH, temperature, and oxidation-reduction potential (ORP) throughout the GIT at 20 second intervals. Redox balance is important for maintaining the intestinal barrier and facilitating interactions between the host, immune system, and GIT microbiota. Disruptions in immune function and microbiome composition can destabilize the balance of oxidants and antioxidants, which can be detectable through altered redox signals.

After technical validation in lab and pre-clinical models, GISMO was tested in a first-in-human study with 66 capsules administered to 15 healthy participants, with maximum 5 capsules per participant and maximum 2 capsules per day. These miniaturized, easy-to-swallow capsules (size 0) continuously measured body temperature, pH, and redox balance across the GI tract for up to 7.5 days. All capsules, but one, were retrieved for post-ingestion lab analysis.

The GISMO was well tolerated and easy to use, with no adverse events reported. Sensor data were annotated to divide the profiles into stomach, small intestine, and large intestine based on pH profiles following commonly used thresholds in literature. The data showed that ORP decreased from an oxidative environment with 162 ± 70 mV (mean ± SD) in the stomach, -126 ± 60 mV in the small intestine, and a strongly reducing environment of -360 ± 16 mV in the large intestine. The redox profiles showed low intra- and inter-subject variability.

This study marks the first in vivo measurement of redox balance along the human GIT, creating a new way to get insights into the role of redox balance in maintaining GIT homeostasis, inflammation, and the microbiome. The ability to conduct frequent and simple monitoring could revolutionize the way gastrointestinal diseases are tracked and managed. Further clinical studies will assess GISMO's diagnostic potential in patient populations.

Keywords

ingestible sensor, gastrointestinal tract, redox balance, diagnostic tool

Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

WHEN MILK MEETS COCOA, COFFEE AND TEA: HINDERED COAGULATION AND FAVORED GASTRIC EMPTYING OF MILK PROTEINS IN A BIOMIMETIC IN VITRO DIGESTION SYSTEM (NERDT)

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Abstract

Mixing cocoa, coffee or tea with milk is a widespread practice worldwide. However, little is known about how milk proteins behave in the stomach in the presence of these rich sources of polyphenols. This study investigated the effect of mixing whole milk with hot water extracts of cocoa, coffee and tea on intragastric coagulation during dynamic in vitro digestion.

Milk was mixed with water (control) or with extracts from cocoa, coffee and tea at a 70:30 (v/v) ratio, and preheated to 60 °C to mimic the consumption conditions of hot beverages. The effect of adding cocoa, coffee or tea extracts on acid- and pepsin-induced milk coagulation was first studied using real-time monitoring on a rheometer. In acid-induced coagulation, the control exhibited a significantly shorter gelation time than the milk mixtures containing extracts (P 0.01). In the case of pepsin-induced coagulation, the inhibitory effect of extracts was even more pronounced, with coffee further delaying gelation compared to the control, while tea and cocoa completely prevented gel formation under our experimental conditions.

The milk mixtures were then studied during simulated gastric digestion using the NEar Real Digestive Tract (NERDT) dynamic in vitro digestion system. This system closely mimics the biochemical environment, anatomical and biomechanical features of the human stomach. Samples collected at the stomach exit at different time points were investigated for pH, dry matter, microstructure (confocal microscopy), particle size distribution (laser light scattering), and protein emptying (SDS-PAGE).

The average pH of the digesta decreased from 6.46 ± 0.04 to 2.15 ± 0.23 during digestion (from 0 to 105 min, respectively) with no significant differences across all milk mixtures. During gastric digestion of the control, protein coagulation occurred between 5 and 18 min (pH 6.3 ± 0.01 and 5.7 ± 0.28, respectively), whereas in the presence of cocoa, coffee or tea, milk coagulation was observed between 18 and 27 min (pH 5.6 ± 0.07 and 4.84 ± 0.22 respectively). Particle size distribution and confocal micrographs confirmed a delayed protein aggregation and larger aggregates with entrapped milk fat clearly visible in samples containing extracts. The gastric emptying of proteins was also consistent with these findings, with a strong decrease of protein concentration in the emptied digesta from the onset of intragastric milk coagulation. This difference disappeared toward the end of digestion.

The observed effects are likely due to the high polyphenol content of the extracts, which are known to interact with milk proteins. Our findings therefore suggest that protein–polyphenol interactions delay casein coagulation in the stomach and may alter gastric emptying and subsequent nutrient absorption along the small intestine.

Keywords

Milk, Protein, Polyphenols, Rheology, Gelation, Coagulation, Digestion

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ISOTHERMAL TITRATION CALORIMETRY AS AN INNOVATIVE METHOD TO DETERMINE ACTIVITY OF DIGESTIVE ENZYMES AND THE DIGESTIBILITY OF MACRONUTRIENTS IN FOOD

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Abstract

Common methods to assess digestive enzyme activities typically rely on simplified model systems and artificial or highly specific substrates (e.g., UV/Vis based assays, pH-stat assay). [1] Consequently, these approaches have limited potential to be transferred to experiments evaluating the digestibility of macronutrients, such as starch, proteins, and triglycerides, in their food-relevant structures and within complex matrices.

To allow the comparability of findings across different studies, a standardized in vitro digestion protocol has been established. [2] This protocol primarily quantifies endpoint product concentrations, however, does not provide information on hydrolysis kinetics, such as the digestion rate and the proportion of digested substrate. In order to assess the impact of the specific substrate structure and its modifications, as well as the effect of antinutritional factors on digestion, continuous and time-resolved monitoring of the hydrolysis of macronutrients is required.

In addition to conventional analyses of hydrolysis products, an innovative approach based on isothermal titration calorimetry (ITC) is proposed, which enables real-time evaluation of enzyme activity under simulated gastric and intestinal in vitro digestion conditions. [3] By measuring heat release, ITC enables continuous monitoring of enzymatic reactions and provides robust quantification of digestion rates, including Michaelis-Menten and inhibition parameters, and the degree of hydrolysis. Importantly, the method is independent of substrate- or product-specific properties and offers a highly flexible system that can be tailored to specific experimental needs.

Using potato starch, hemoglobin, and tributyrin as model substrates, the ITC-based method was benchmarked against the standard enzyme activity assays for amylase, pepsin, and lipase. [4, 5] In addition, the impact of specific parameters (e.g., temperature, substrate to enzyme ratio, buffer) were evaluated and exemplary digestion rates and degrees are presented for different grain flours, legume proteins, and oils. [5, 6]

References:
[1] Minekus et al., Food Funct. 2014, 5, 1113–1124. [2] Brodkorb et al., Nat Protoc. 2019, 14, 991–1014. [3] Xiong et al., Curr. Res. Food Sci. 2026, 12, 101266. [4] Xiong et al., Lebensmittelchemie 2025, 79, S3-121. [5] Plambeck, Buchweitz, Lebensmittelchemie 2025, 79, S3-069. [6] Xiong et al., Lebensmittelchemie 2024, 78, S3-194.

Keywords

enzyme activity assays, digestion rate, inhibition parameters, degree of hydrolysis

Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

(SUB)SECOND BILE-DRIVEN INTERFACIAL DISPLACEMENT REVEALED BY MICROFLUIDICS

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Abstract

Lipid digestion is initiated at the oil–water interface, where bile salts displace pre-existing interfacial structures and enable subsequent lipase adsorption. While these interfacial processes are known to be critical for intestinal lipid digestion, their early-stage dynamics remain poorly resolved due to the limited temporal resolution of conventional techniques.

We used microfluidic techniques to elucidate the hierarchy of process as they occur at the interface. The benefits of using these techniques are related to very precise control of digestion conditions (exact droplet size) as well as analysis of very short time scales that are much shorter than is possible with classic techniques such as the droplet volume tensiometer (>3 seconds). We systematically evaluate the effects of bile salt, buffer, and lipase at concentrations used in the INFOGEST protocol, and we do that for the individual components as well as combinations thereof. As reference we use an oil that cannot be digested to gain understanding of possible effects of digestive products.

By isolating the intestinal phase and decoupling interfacial effects from lipolytic product formation, this study provides direct, time-resolved insight into early intestinal interfacial processes that are inaccessible to traditional diffusion-based methods. The ongoing research aims to clarify the relative roles of individual digestive components and their mutual interactions at the oil–water interface, providing a basis for critically assessing hypotheses proposed in the literature regarding early-stage intestinal interfacial processes.

Keywords

in vitro digestion, bile, lipase, interface, microfluidics

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Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

TRACKING NANOSTRUCTURAL CHANGES DURING GASTROINTESTINAL DIGESTION OF ALTERNATIVE FOOD SYSTEMS USING A MULTI-SCALE STRUCTURAL ANALYSIS APPROACH

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Abstract

Understanding the structural transformations occurring during gastrointestinal digestion is essential to rationally design food systems with improved nutritional quality and controlled nutrient release. During digestion, food components are progressively disassembled and reorganized, leading to the formation of complex nano- and micro-structures that strongly influence nutrient bioaccessibility and intestinal transport.

In this work, a multi-technique approach was applied, combining advanced structural tools such as small angle X-ray and neutron scattering (SAXS and SANS) with microscopy, spectroscopy, rheology and compositional analysis, to investigate the multi-scale structural evolution of different food systems derived from alternative biomass sources during in vitro gastrointestinal digestion.

Our results demonstrate that the initial structural architecture, as well as protein-polysaccharide interactions, play a crucial role in modulating the digestion pathway and the structural organization of the released digestion products. In particular, cell wall polysaccharides were identified as one of the main factors limiting protein digestibility in alternative sources like seaweeds. On the other hand, interactions between the released peptides, soluble polysaccharides and bile salts promoted the formation of distinct nanostructures, such as mixed micelles and lamellar phases. This nanoassembly process was shown to be affected by the type and concentration of soluble polysaccharides present in the digestion medium, as well as the rheological properties of the digesta, ultimately influencing intestinal transport mechanisms of nutrients.

Overall, this work highlights the power of combining advanced structural characterisation techniques with in vitro digestion models to achieve a mechanistic understanding of food digestion. Such knowledge is crucial to support the development of next-generation food products with tailored nutritional functionality.

Keywords

nanostructure, proteins, polysaccharides, scattering, X-rays

Acknowledgements

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Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

FOLLOWING STRUCTURAL DEGRADATION OF CHICKPEA MEALS DURING IN VIVO DIGESTION USING CONFOCAL MICROSCOPY

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Abstract

Understanding how the microstructural organisation of plant-based foods changes during digestion is essential for explaining their diverse metabolic effects. Pulses such as chickpeas possess cell walls that encapsulate starch granules and proteins, forming natural barriers that influence nutrient release. As these structural features break down in the gastrointestinal tract, their transformation affects the rate and extent of starch gelatinisation, protein hydrolysis, and cell-wall disintegration, thereby shaping postprandial glycaemic responses and satiety signals.

In this work, we monitored the structural changes occurring during digestion of meals containing broken, intact, or clustered chickpea cells. Samples were obtained in vivo from aspirates collected from the mouth, stomach, duodenum, and ileum of participants after consumption of meals containing these structures [1,2]. Using confocal laser scanning microscopy with fluorescent staining of cell walls, proteins, and starch, applied directly to gastrointestinal aspirates, we observed the structural features of the samples and visualised their digestion. This approach enabled us to observe how intact cells, partially disrupted cells, and fully broken matrices transform under physiological digestive conditions.

Tracking the disappearance of cell-wall integrity, the swelling and enzymatic digestion of starch granules, and the progressive solubilisation of protein bodies provided detailed insight into the sequential liberation of nutrients. Importantly, the imaging data revealed that nutrient release was strongly dependent on whether cellular boundaries remained intact, with entrapped starch and protein showing delayed digestion compared to material exposed after mechanical or enzymatic disruption.

By directly linking microscopic structural changes to biochemical digestion profiles in aspirated samples, this work highlights the critical role of food microstructure in governing nutrient bioaccessibility. These findings underscore the importance of incorporating structural analyses into digestion research, as they offer mechanistic explanations for the variable metabolic responses observed with pulse based foods. Such understanding is essential for guiding the design of minimally processed foods that utilise natural cell structures to modulate glycaemia, promote sustained satiety, and support healthier metabolic outcomes.

[1] Cai, M., Tejpal, S., Tashkova, M., Ryden, P., Perez Moral, N., Saha, S., Garcia Perez, I., Serrano Contreras, J., Wist, J., Holmes, E., Edwards, C., Frost, G., et al. (2025). Upper gastrointestinal tract metabolite profile regulates glycaemic and satiety responses to meals with contrasting structure: a pilot study. Nature Metabolism, 7, 1459–1475. https://doi.org/10.1038/s42255-025-01309-7

[2] Fernandes, N., Cai, M., et al. (2025). Dietary modulation of the human small intestine metabolome and microbiome. medRxiv. https://doi.org/10.1101/2025.11.25.25340785

Keywords

Food microstructure, Nutrient release, Confocal microscopy, Digestion

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Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

POSTPRANDIAL AMINO ACID PROFILES AND GASTRIC BEHAVIOUR AFTER CONSUMPTION OF MILK AND PLANT-BASED ALTERNATIVES: A RANDOMIZED CROSS-OVER STUDY

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Abstract

Background: The availability and demand for plant-based drink alternatives to bovine milk are increasing. However, plant-based drinks are not a complete nutritional substitute for bovine milk, because of their lower protein quantity and quality, as well as further differences in macro- and micronutrient composition. Knowledge on the impact of these differences on digestion-related outcomes, including postprandial amino acid profiles, glucose and insulin levels, and gastric emptying is still limited. In addition, insights into the effects of plant-based milk alternatives enriched by adding plant protein on these outcomes are lacking.

Objective: To compare the differences in postprandial amino acid profiles, glycemic response, and gastric emptying between bovine milk and plant-based drinks with or without added protein.

Methods: 12 healthy males (age 27±7.4 years; BMI 22.8±2.3 kg/m2) participated in a randomized crossover study with three treatments. After an overnight fast, participants consumed 750 mL of semi-skimmed milk, a standard oat-based drink (OBD) or an oat-based drink enriched with pea protein (OBD+Pea). These drinks are commercially available products, not matched for macronutrient composition and OBD + Pea had a higher calculated protein quality than OBD. Bovine milk contains more protein compared to the OBD+Pea and the OBD (3.7, 1.7 and 0.7 g/100 ml respectively), while the OBD+Pea and the OBD contain more total carbohydrate than bovine milk (6.2, 5.7 and 4.8 g/100 ml, respectively). Blood samples were taken to measure plasma amino acids, glucose, and insulin levels up to t=300 min after the start of ingestion. Gastric content volume was measured using Magnetic Resonance Imaging (MRI) for bovine milk and OBD+Pea up to t=120 min.

Results: Bovine milk showed the highest postprandial total amino acid (AA) and essential amino acid (EAA) concentrations, followed by the OBD+Pea, while the OBD induced the lowest responses (OBD vs. Milk: 819 ± 33 µM; OBD+Pea vs. Milk: 535 ± 32 µM; OBD vs. OBD+Pea: 284 ± 32 µM; all P0.001 for total AA; OBD vs. Milk: 425 ± 14 µM; OBD+Pea vs. Milk: 293 ± 14 µM; OBD vs. OBD+Pea: 132 ± 14 µM, all P0.001 for EAA). Gastric emptying of milk was significantly slower than OBD+Pea after the first 60 minutes (all P0.006). Postprandial glucose was higher for the two OBDs compared to milk during the first 45 minutes (all P0.001) and insulin concentrations during the first 60 minutes (all P0.05).

Conclusion: Protein enrichment in plant-based milk alternatives can be a valuable strategy to increase protein content and improve amino acid composition. However, these products still cause faster gastric emptying and higher postprandial glycemic and insulinemic responses compared to bovine milk, because of the remaining differences in carbohydrate and protein content, and because milk protein coagulation can slow down gastric emptying which might contribute to the more prolonged amino acid response.

Keywords

Protein digestion, MRI, gastric emptying, amino acids, plant-based milk alternatives.

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Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

STUDY OF RHEOLOGICAL PROPERTIES OF CURD FORMED DURING GASTRIC PHASE OF GOAT MILK INFANT FORMULA AND CORRELATION WITH MACRONUTRIENT DIGESTION

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Abstract

The increasing interest in goat infant formulas (IF) as an alternative to cow IF is driven by their favorable digestibility and nutritional properties. Understanding the gastric phase is crucial, as it plays a key role in the digestion of macronutrients, which is essential for optimizing the nutritional profile of infant foods. This study aims to explore i) how digestive gastric enzymes can modulate the structural properties of gels formed in the stomach and ii) how the casein: whey protein ratio of IFs can modify the structural properties of gels formed in gastric phase and the correlation with the protein and lipid digestion.

To achieve our objectives, 2 commercial goat IFs with distinct casein: whey protein ratios (40:60 and 80:20) were used and 3 gastric digestion modalities were chosen: 1) glucono delta-lactone (GDL) alone to simulate acid-induced gel formation; 2) GDL + porcine pepsin; 3) GDL + pepsin and gastric lipase, both provide by rabbit gastric enzyme (RGE). The gelation process and variations in the storage modulus (G') of gels were monitored for 2 hours at 37°C using dynamic oscillatory rheology. To initiate gel formation, GDL (2.5%) was directly added to IFs within the rheometer with either porcine pepsin or RGE at a final concentration of 10U/ml of pepsin. A digestion protocol at the infant stage was used to study the macronutrient digestion of IFs during the gastric phase using an acidification curve from pH 6.6 to 4. Samples were collected at different time points to quantify the protein digestion (SDS-PAGE, degree of proteolysis and size exclusion chromatography), the degree of lipolysis, as well as the evolution of particle size.

The addition of pepsin enhanced gel firmness beyond that achieved by GDL acidification alone. Notably, gels formed with RGE demonstrated lower G' values and delayed curd formation compared to those formed with pepsin. Rheological results indicated that casein-dominant IF exhibited greater gel firmness compared to whey-dominant IF across all modalities.

Given the rheological findings, we aimed to determine whether the casein: whey ratio affects nutrient digestion under the most physiologically relevant condition, using RGE. At each digestion time point, casein-dominant IF showed larger particle sizes and reduced protein digestion relative to whey-dominant IF. Despite differences in rheological behavior, lipolysis during the gastric phase remained unaffected by gel structure, suggesting that lipolysis is independent of gel formation. While higher gel firmness may lead to a larger particle size distribution and reduced proteolysis during the gastric phase.

This study underscores the importance of understanding the impact of the gastric curd structure on the macronutrient's digestibility of IFs, providing insights into optimizing infant nutrition.

Keywords

Goat infant formula, rheology, gastric phase, proteolysis, lipolysis

Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

SIMULTANEOUS MEASUREMENT OF GASTRIC EMPTYING AND HALF-EMPTYING TIME T50 IN THE UPRIGHT POSITION USING MAGNETIC RESONANCE IMAGING AND 13C BREATH TEST

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Abstract

Background

Gastric Emptying (GE) is a key physiological food digestion step. The stomach half-emptying time T50 is the main endpoint commonly used to characterise GE. Conventional techniques to measure this have limitations. Magnetic resonance imaging (MRI) has many advantages and can provide unique insight into food digestion, but conventional MRI requires to image participants inside a horizontal bore, which complicates concomitant use of other techniques.

Objectives

1) To measure GE of an oral glucose tolerance drink in the upright position using an open-design MRI scanner and simultaneous sodium [13C]-acetate GE breath test

2) To explore correlation of T50 derived by MRI and by 13C breath test

Methods

Twelve healthy volunteers participated. They were positioned seated in a 0.5T ASG Paramed MROpen scanner and imaged at fasting baseline using a coronal, breath-hold, multi-slice, fast spin-echo sequence with TR=3192ms and TE=102ms and an acquired resolution of 3.0mm×2.6mm×10.0mm, with the field of view covering the entire stomach.

They were then asked to drink 300mL water containing 75g glucose and 150mg sodium [13C]-acetate. MRI scans were taken for 2h together with simultaneous breath samples. Intragastric meal volumes were measured using manual image segmentation. Breath samples were analysed for 13CO2 enrichment by mass spectrometry. Stomach half-emptying times T50 were then calculated for both techniques. Traditional breath test modelling [1] and Wagner-Nelson modelling [2] were used for T50 breath test. Data shown as mean±SD.

Results

The study protocol was well tolerated by the participants. The upright position allowed the participants to drink the intervention whilst seated in the scanner with the receiver around the abdomen, and to start imaging immediately after they finished the glucose drink.

Breath test T50 values depended strongly on the metabolic modelling used. Traditional breath test modelling yielded a T50 (144±28min) that was 33% slower than T50 MRI (108±28min). Using Wagner-Nelson modelling, breath test T50 (93±25min) was 55% faster than that from the traditional modelling and closer to the MRI T50 values. Regardless of the modelling used, breath test T50 individual values correlated weakly and not significantly with the corresponding MRI T50 values (R2=0.16, P=0.1904 for traditional modelling and R2=0.18, P=0.1731).

Conclusion

MRI in the upright position provides a direct measurement of gastric contents, and participants can easily be fed test interventions inside the scanner. Breath test T50 are cheaper and widely available, but values can vary dramatically depending on the metabolic modelling used, and correlation with direct imaging measurement was poor. An improved understanding of upright intragastric food distribution and GE could increase in vitro-in vivo relevance of bench digestion models.

References

1. B Braden et al. Gastroenterology 108,1995

2. M Sanaka et al. Clin Exp Pharmacol Physiol 34,200

Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

INFLUENCE OF THE ANALYTICAL METHOD ON MEASURED LIPOLYTIC ACTIVITY IN WHOLE HUMAN MILK

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Abstract

Lipids are the main source of energy provided by human milk (HM), and their digestion requires the release of free fatty acids (FFAs) from triacylglycerols. This process depends on the presence and, ideally, high activity of lipolytic enzymes, which is challenging in the context of the immaturity of the infant digestive system [1]. HM contains endogenous lipolytic enzymes, primarily bile salt–stimulated lipase (BSSL/BSDL) and, to a smaller extent, lipoprotein lipase (LPL) [2]; therefore, the lipolytic activity of the starting material can represent an important variable in in vitro HM digestion studies. At the same time, a reliable assessment of this activity depends on the specificity of the assay used: signals obtained with DTNB-based methods (commercial assay: QuantiChrom) may reflect the activity of other hydrolases/esterases rather than exclusively BSSL/LPL, which complicates comparisons across studies and may lead to different conclusions. In this work, the QuantiChrom thiol assay (DTNB) was compared with a p-nitrophenyl–based method in the presence of bile salts (pNP(+)) and without bile salts (pNP(−)) [3] to demonstrate that assay choice affects the estimated lipolytic activity of HM and the interpretation of differences between preterm vs full-term and colostrum vs mature milk.

Whole milk was collected after preterm (n=15) and term delivery (n=15) at colostrum (days 1–5) and mature milk (~day 30), forming PC, PM, FC and FM. Technical repeatability was assessed on pooled PC/PM/FC/FM (10 technical replicates per method; CV%). Individual samples were measured in triplicate and averaged per donor. Methods were compared using Spearman correlation, linear regression and ratio Bland–Altman analysis; group, stage (repeated factor) and interaction were tested per method in a 2×2 model with Šídák correction.

Both pNP(+) and pNP(−) showed lower CV% than the thiol assay across all pooled samples. In individual samples, the thiol assay consistently produced higher values than the pNP_total method (pNP_total = pNP(+) + pNP(−)), with a positive correlation (Spearman $r = 0.405$; $p = 0.0013$) but a clear proportional bias and lack of interchangeability between methods (mean thiol assay/pNP_total ratio = 2.253; 95% limits of agreement: 0.510–3.996). The 2×2 analyses were interpreted separately for each method; however, both statistical significance and the magnitude of observed effects could differ between assays.

These findings support that thiol assays may overestimate physiologically relevant HM lipolytic activity and underline the need to report assay, substrate and conditions precisely and to compare results across methods cautiously.

References:

[1] Manson & Weaver, 1997, Arch Dis Child Fetal Neonatal Ed, doi:10.1136/fn.76.3.F206.

[2] Koh et al., 2020, Front Nutr, doi:10.3389/fnut.2020.552362.

[3] Bertino et al., 2013, J Pediatr Gastroenterol Nutr, doi:10.1097/MPG.0b013e31827af155.

Keywords

human milk, lipases, lipolytic activity, assay comparison

Acknowledgements

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Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

TECHNO-FUNCTIONAL AND PROTEIN NUTRITIONAL QUALITY OF AMARANTH AND HEMP-BASED MILK ALTERNATIVES

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Abstract

Plant-based milk alternatives formulated from emerging proteins are gaining popularity due to shifts in human dietary patterns and environmental concerns, such as resource use and biodiversity loss. Amaranth and Hemp are highly valuable resources with high protein content, with market potential for innovative product development. This study focuses on assessing techno-functional and nutritional properties of a milk alternative (MA) made from amaranth protein concentrate (APC) and hemp protein concentrate (HPC). Protein concentrates were prepared by defatting seed flour following pH-shifting to solubilize and subsequently precipitate proteins. The protein content of APC and HPC was 74.1 ± 0.0 and 71.1 ± 0.2, respectively. The MA was standardized to 3.5 % (w/w) protein and 1.5 % (w/w) fat. Physicochemical characterization of MA included particle size distribution, rheological measurements, and confocal laser scanning microscopy. The developed products were further subjected to the INFOGEST standardized gastrointestinal digestion. The peptide and protein profile of the digests was characterized by nanoLC-Orbitrap MS/MS and SDS-PAGE. The initial particle size (D3,2) of APC-MA and HPC-MA was 0.57 ± 0.00 µm and 0.64 ± 0.03 µm, respectively. Global stability after 1 week of storage at 4°C demonstrated no phase separation in both emulsions. Apparent viscosity at a shear rate of 100 s-1 showed a higher viscosity in HPC-MA (35.02 ± 5.29 mPa*s) compared to APC-MA (17.51 ± 2.09 mPa*s). Flow curves were adjusted to the power Law model to obtain rheological parameters. The index flow behavior associated with parameter n showed pseudoplastic behavior. The consistency index (K) was higher in HPC-MA than in APC-MA. Confocal microscopy was used to visualize protein distribution dye with Fast Green in the MA, as well as oil drops using Nile red. Both MA showed a homogeneous distribution of oil, with no evident flocculation of the drops. According to the particle size measured in the presence of 1% w/v SDS, no flocs were observed either. SDS-PAGE protein profiles under reducing conditions of APC and HPC showed bands between 55 and 10 kDa that were characterized by in-gel digestion, in order to identify the specific proteins. The gastric digestion produced several bands below 10 kDa, while the intestinal phase led to the disappearance of most bands. The physicochemical structure of the digests was followed by particle size, rheology, and confocal microscopy. Peptidomic analysis revealed peptides from the major storage proteins like 11S globulin for APC-MA and Edestin for HPC-MA. Protein digestibility of both MA was above 80%. In conclusion, MA formulated from APC and HPC demonstrated good physical stability, suitable apparent viscosity, and high protein content with high digestibility. Consequently, these findings could serve as a seed to develop a high-nutritional MA with emerging plant sources.

Keywords

Amaranth, Hemp, Plant-based milk alternatives, Techno-functional characterization, Nutritional evaluation

Acknowledgements

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Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

EFFECT OF PH AND TARA GUM INCORPORATION ON THE MULTI-SCALE STRUCTURE AND DIGESTION-RELEVANT STABILITY OF MICELLAR CASEIN SYSTEMS

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Abstract

Tara gum (TG) is a non-ionic galactomannan widely used as a stabiliser in dairy products. Its influence on the behaviour of micellar casein (MC) under varying pH conditions was evaluated. The aim of the study was to determine how TG incorporation and environmental acidification modify the colloidal stability, physicochemical properties, and microstructure of MC–TG systems in the context of simulated digestion. Mixtures with MC:TG ratios of 95:5, 85:15 and 75:25 were analysed over the pH range 2–8. Turbidity, zeta potential, particle size distribution and confocal laser scanning microscopy were employed.

MC stability at near-neutral pH was associated with the presence of colloidal calcium phosphate nanoclusters. Progressive acidification reduced electrostatic repulsion and promoted aggregation, particularly close to the isoelectric point. Due to its non-ionic character, TG did not significantly alter surface charge trends. Its action was primarily physical. TG increased the viscosity of the continuous phase and limited particle mobility. In the pH range 3-5, higher turbidity and reduced sedimentation of aggregates were observed. Microscopy confirmed the formation of extensive aggregated structures near the isoelectric region and a more homogeneous dispersion at pH 6.0.

pH regulation remained the dominant factor governing microstructural changes, while TG acted as a kinetic stabiliser. The extent of aggregation and matrix densification is directly relevant to enzymatic accessibility during digestion. More compact, precipitated networks may restrict enzyme penetration and slow proteolysis, whereas well-dispersed systems favour more uniform protein hydrolysis. Therefore, modulation of MC–TG ratios and environmental pH represents a potential strategy for structuring dairy protein matrices with controlled digestion kinetics and tailored release of bioactive peptides under gastrointestinal conditions.

The research was funded by the Minister of Science and Higher Education Republic of Poland, task entitled: The Research Network of Life Sciences Universities for the Development of the Polish Dairy Industry

Keywords

Microstructure, Colloidal stability, In vitro digestion

Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

DEVELOPMENT OF AN HPTLC WORKFLOW FOR TRACKING LIPASE AND PHOSPHOLIPASE A2 ACTIVITY IN A LIPID EMULSION DIGESTION MODEL

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Abstract

Understanding lipid digestion at the interfacial level is essential for evaluating nutrient bioaccessibility and the modulation of enzymatic activity in complex food systems. The efficiency of intestinal lipid hydrolysis directly affects fat absorption, postprandial metabolism and the development of metabolic disorders including obesity, making digestive enzymes important therapeutic targets [1]. Consequently, analytical tools that enable detailed monitoring of lipid transformation and enzyme specific activity under physiologically relevant conditions are critical for mechanistic research and the development of dietary and therapeutic strategies. This study presents the development of a high-performance thin layer chromatography (HPTLC) method for product-based profiling of lipid emulsion digestion driven by lipase and phospholipase.

A model lipid emulsion was designed to mimic intestinal conditions by reproducing the interfacial environment of dietary fat droplets using refined olive, lecithin isolated from hen egg yolk, and bile salts. Enzymatic digestion was carried out under controlled in vitro conditions in the presence of pancreatic lipase from porcine pancreatin (PPL) alone or in combination of phospholipase A2 (PLA2). The resulting lipids were extracted with chloroform:methanol and reaction products were monitored using HPTLC. Two different solvent system were used: chloroform:methanol:water:ammonia 25% (60:34:4:2), and n-hexane:diethyl ether:acetic acid (70:30:3) for profiling of polar and neutral lipids, respectively. To visualize the reaction products on a TLC plate, two derivatizing reagents were used: 0.01% primulin in aqueous acetone (80%), and copper sulphate (VI) in orthophosphoric acid. Additionally, enzymatic reactions were performed using synthetic inhibitors of PLA2 (varespladib), PPL (orlistat), and extracts of leaf, steams and flowers of Amaranthus cruentus as a natural source of potential inhibitors.

The selected mobile phases allowed clear separation of lipid digestion products, with primuline proving the most effective derivatization reagent. Changes in product distribution were observed over time, highlighting the role of PLA2 in facilitating lipase access to triacylglycerols. The digestion profile was also altered in the presence of synthetic inhibitors and plant matrices. Overall, the proposed HPTLC strategy offers a rapid and cost-effective platform for monitoring lipid emulsion digestion, characterizing enzyme specific activity.

[1] Wit, M., Trujillo-Viera, J., Strohmeyer, A., Klingenspor, M., Hankir, M., & Sumara, G. (2022). When fat meets the gut-focus on intestinal lipid handling in metabolic health and disease. EMBO molecular medicine, 14(5), e14742. https://doi.org/10.15252/emmm.202114742

Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

MONITORING IN VITRO GASTRIC BOLUS DIGESTION WITH ULTRASOUND

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Abstract

Background

Monitoring structural degradation of food bolus during gastric digestion is essential to understand how bolus properties influence digestion processes and subsequent nutrient absorption. However, real-time, non-invasive monitoring of these changes remains challenging.

Aim

This study aimed to validate, for the first time, the use of ultrasound (US) for tracking changes in food bolus characteristics during in vitro gastric digestion.

Methods

To allow US imaging of the gastric content, a static in vitro digestion model (Gastric Ultrasound Model (GUSMO)) was designed. It consists of a water bath and a repositionable frame that holds a polyethylene bag, simulating the stomach. A rectangular “acoustic window” sealed with thin plastic film was incorporated, creating an almost transparent acoustic interface between the US probe and the water in the bath. Bread boli (total n=9) treated with amylase, pepsin, or no enzyme underwent 90 minutes of static digestion in the waterbath based on a modified INFOGEST protocol, while recording US images at 0, 10, 30, 60 and 90 min. The boli were segmented on the images and the number of bolus pixels was calculated. In addition, five Haralick texture features were calculated to characterize the internal bolus structure. On each image, average grey value was calculated in 20 regions of interest (ROIs) positioned at linearly increasing distance from the bolus edge.

Results

Bolus area increased over 90 min. From 10-60 min, amylase-treated boli were larger than pepsin-treated (ratios 1.79-2.35) and no enzyme boli (ratios 1.43-2.42). Regarding image texture, the bolus area of amylase-treated boli had 1.21-1.30 times higher homogeneity, and 2.5-4% lower entropy than pepsin-treated boli from 30 min onward. From 10 min, the mean grey value for amylase-treated boli decreased less fast with echo depth than for the other treatments, which indicates a reduced ultrasound attenuation. In the amylase-treated boli, an increase in homogeneity (rho=0.8) and decreases in entropy (rho=-0.81) and slope (rho=-0.85) were associated with increasing degree of starch hydrolysis. These results suggest that the amylase-treated boli transitioned from a heterogeneous structure with entrapped small air pockets, to a more uniform texture as digestion progressed, due to fluid infiltration and filling of air pockets. In contrast, the no enzyme boli and pepsin-treated boli did not expand rapidly and had a more heterogeneous structure with higher ultrasound attenuation.

Conclusion

This study shows the capacity of US imaging for quantifying bolus degradation in vitro. Future work could explore this novel application of US imaging in dynamic in vitro models and in vivo.

Keywords

In vitro gastric digestion, Bolus breakdown, Ultrasonography, Image texture features

Topic: Physicochemical and Imaging Techniques for Characterising Food Digestion

TRACKING BOLUS BREAKDOWN USING ULTRASOUND IMAGING IN A DYNAMIC IN VITRO GASTRIC DIGESTION MODEL

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Abstract

Dynamic in vitro gastric models have been developed to reproduce physicochemical aspects of food digestion under controlled conditions. However, non-invasively monitoring food bolus structural changes during gastric digestion remains challenging. In addition, under dynamic conditions, mechanical disintegration and enzymatic hydrolysis occur simultaneously, making it difficult to distinguish their contributions to food bolus structural changes. We previously established that ultrasound imaging (US) can be used to monitor changes in bread boli in a static gastric digestion model. This study aims to integrate a dynamic in vitro digestion model with US to monitor bolus breakdown and establish how image features relate to biochemical and structural changes.

In this study, the near-real dynamic in vitro digestion system, the NEar Real Digestive Track (NERDT), will be used. The NERDT system consists of a silicone stomach model compatible with US, a mechanical contraction device to mimic gastric peristalsis, and a controlled gastric emptying unit, enabling dynamic digestion, sampling and imaging. Masticated spit-out boli collected from human mastication will be used as test samples to provide realistic bolus structure. Three experimental conditions will be tested in triplicate: 1) peristalsis only, isolating physical shearing; 2) enzymes only, assessing enzymatic degradation; and 3) combined peristalsis and enzymatic digestion. Particle size and enzymatic hydrolysis will be measured before and after digestion. The US probe is placed at the antrum region and images will be captured at baseline and during 90 min digestion. Bolus and chyme areas will be segmented as regions of interest (ROI). ROI area expansion (nr of pixels) and fluid infiltration characteristics (grey value slope across longitudinal sub-ROIs at increasing distance from probe, reflecting soundwave attenuation). In addition, image texture analysis will be performed using Haralick texture features calculated based on Grey-Level Co-occurrence Matrix (GLCM) and grey value distribution histograms. These US image features will be correlated with particle size and the degree of starch and protein digestion, and compared across the three conditions.

Our previous static in vitro gastric digestion study with the use of US showed that bolus swelling correlated with bolus area expansion, increased GLCM homogeneity and decreased GLCM entropy, attributed to enzymatic matrix breakdown which allowed fluid penetration. Pilot measurements combining mechanical shearing and enzymatic effects have shown that US images can capture the bolus-to-chyme transformation, which is visible as echo region expansion and grey value reductions. This has set the stage for data collection, which is now ongoing. Results for the planned experimental conditions will be presented at the conference.

Keywords

Dynamic in vitro digestion, Bolus breakdown, Ultrasound imaging, Image texture features

SESSION

5 Role of Gut Microbiota in Digestion

Kieran Tuohy

Chair in Energy Metabolism and Microbiome

School of Food Science & Nutrition, University of Leeds, United Kingdom

Prof Kieran Tuohy is a microbiologist working in the field of nutrition, food science and biotechnology. He received his PhD from the University of Surrey (UK) in 2000 after graduating from University College Dublin, Ireland (BSc, Industrial Microbiology) and from the University of Aberdeen (MSc in Environmental Microbiology). After training as a post-doctoral researcher, Kieran was appointed lecturer in Food Metabolomics at the Department of Food Science and Nutrition, University of Reading. Kieran led the Nutrition and Nutrigenomics Group at the Fondazione Edmund Mach in Trento, Italy, between 2010- 2022 and in February 2022 was appointed chair in Energy Metabolism and Microbiome at the School of Food Science & Nutrition, University of Leeds, UK.
His work focuses on how microbiomes within the gut and along the food chain influence human nutrition, metabolic health, and disease risk. In particular, his group used in vitro colonic models and human dietary interventions to investigate how different functional foods (e.g. probiotics, prebiotics, polyphenols), whole foods (fruit, whole grains) and diets, shape the gut microbiota and its metabolic output. The aim is to identify how dietary modulation of the gut microbiota regulates host health through the flux of metabolites absorbed form the colon. As part of the National Alternative Proteins Innovation and Knowledge Centre at Leeds (NAPIC https://napic.ac.uk/), his team also investigates how different alternative proteins impact on the gut microbiota, their production of bioactive compounds and potential to improve host health. He is also part of the Irish Co-Centre for Sustainable Food Systems (https://foodcocentre.org/) where his team explores the potential of non-invasive biomarkers of nutrition and their potential application to measure the physiological consequences of transition to a more environmentally friendly diet.

ABSTRACT

Role of Gut Microbiota in Digestion and its potential to influence "Nutri-Kinetics"

The intestinal tract is colonised by a diverse collection of microorganisms dubbed the gut microbiome. Dominated by anaerobic bacteria, distinct microbial communities reside in different anatomical regions of the gut with local physicochemical and biological factors determining species diversity, abundances and metabolic activity. The colon harbours the most diverse microbial community and is also the region with the highest density of microbial cells (up to 1011 CFU/g contents). This colonic microbiota plays a critical role in recovering energy and cell signalling molecules (short chain fatty acids, SCFA) from non-digested carbohydrate (e.g. fibre, prebiotics, resistant starch), production of vitamins (e.g. vitamin K, B2, B6 and B12), influencing amino acid metabolism (notably aromatic amino acids involved in neurotransmitter production) and conversion of complex plant polyphenols into more bioavailable and sometimes more biologically active smaller phenolic derivatives. However, with the exception of SCFA and polyphenols, few studies have directly measured the contribution of colonic metabolites to circulating levels, and the influence of the colonic microbiota to nutrient kinetics or nutri-kinetics largely remains undetermined. Moreover, recent studies have shown that the small intestine too can harbour complex microbial communities, albeit at lower population levels. Here, resident microorganisms have potential to directly influence nutrient metabolism given the high absorptive capacity of this region of the gut, and indeed bacteria in the small intestine, including ingested probiotic bacteria, have been shown to influence postprandial levels of biomolecules absorbed from the distal ileum such as bile acids. However, here too we lack mechanistic studies measuring the microbial contribution to nutrient metabolism and kinetics largely due to the inaccessibility of the small intestine and difficulty in collecting postprandial blood samples at scale in human nutrition studies. Recent advances, both in technologies to directly sample the small intestinal microbiome non-invasively and the emergence of wearable, continuous and remote nutrient sensors are now producing the means to tackle such knowledge gaps. This study will discuss the current evidence demonstrating the role of the gut microbiome in digestion and go on to explore how new technologies may soon revolutionise how we measure the metabolic output of the gut microbiome and its contribution to human nutrition, health and disease.

ORAL PRESENTATIONS

Topic: Role of Gut Microbiota in Digestion

IN-VITRO DYNAMIC GASTROINTESTINAL MODELS FOR THE PREDICTION OF COMPLEX GUT MICROBIOME-PRODUCT INTERACTIONS IN MICROBIAL, FOOD AND PHARMACEUTICAL RESEARCH.

Brunette Katsandegwaza [1] , Irma Gonza [1] , Céline Antoine [1] , Manon Gresse [1] , Margot Gillis [2] , Cindy Staerck [3] , Bernard Taminau [4] , Caroline Douny [5] , Martine Schroyen [2] , Patrice Filee [3] , Véronique Delcenserie [1]

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Abstract

The complex interaction between the gut microbiome and the host is significant to gut health research. The gut microbiome is not only demonstrated to play a big role in modulating gut health but is also indicated pivotal to systemic conditions that include dermatitis, vaginosis, mental health and respiratory conditions. A better understanding of how the gut microbiome directly, or indirectly, impacts different conditions is of major interest to parties from different aspects of research. Equally important is elucidating how internal and external factors shape the microbiome itself, knowledge that could ultimately enable the manipulation of the gut microbiome for therapeutic or preventive purposes.

Complex in vitro dynamic gut models enable a controlled peek into how the gut microbiome reacts to interventions that include exposure to different foods, probiotics, postbiotics and prebiotics, vegetal extracts and any other interventions of interest; hence providing a robust pre-clinical platform before going for human trials. Although a wide range of models are currently available, our research laboratory has great experience in using the SHIME® gut model. The Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) enables the mimicking of physiologically relevant gastrointestinal conditions, that include temperature and pH regulation, enzyme and bile acid digestion as well as anerobic conditions necessary for colonic fermentation during digestion. It is set up of interconnected double jacket fermenters, allowing a potential simulation from oral to distal colon digestion transit. The system also enables consistent formulation administration, as well as sampling throughout the whole experiment, without the need to stop the fermentation assay. We can also modify the system by including a mucosal compartment (M-SHIME®) to simulate the colonic mucus layer, supporting the mucin adhering microbiota. The major output from the SHIME system includes discovering the biocomplex evolution of the gut microbiota in response to an intervention through metagenomics, quantification of microbial metabolites that are demonstrated to have direct impact on host health, as well as a downstream assessment of the impact of microbial metabolites on immunology through inflammation and cytotoxicity assays.

We collaborate with many partners from academic institutions, independent research institutions, industry as well as government research institutions, to assist in answering diverse questions that focus on the gut microbiome across different species that include human (adult or infant), horses as well as pigs. We demonstrate the ability of the SHIME platform and its derived models, to characterize the impact of interventions on the host's gut microbiota and health.

Keywords

Digestion models, SHIME, Gut models, Gut microbiome, Probiotics, Vegetal extracts, Postbiotics

Acknowledgements

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Topic: Role of Gut Microbiota in Digestion

PRODUCTION OF COLONIC MICROBIAL METABOLITES FROM DIFFERENT PROTEIN SOURCES USING HUMAN ILEAL DIGESTA AND A DYNAMIC MODEL OF COLON FERMENTATION

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Abstract

Protein fermentation in the large intestine have been generally associated to poor health outcomes, because of the production of potentially harmful microbial metabolites. However, there is little knowledge on how the production of such microbial metabolites is influenced by the protein source as well as by the effect of the composition of the ileum effluents, i.e., the fraction of dietary and endogenous material escaping digestion in the small intestine and entering the large intestine.

In this study, we explored the relationship between ileal effluents amino acid composition and the production of protein fermentation metabolites by using a hybrid in vivo/in vitro approach, whereby ileum effluents from ileostomy patients, who ingested nine meals with different protein sources, were incubated for 56 h in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®), a dynamic in vitro model of colon fermentation. NH3, short-chain fatty acids, branched-chain fatty acids (BCFA), H2S, tryptophan derivatives, and biogenic amines were measured in the proximal (PC) and distal (DC) sections of the SHIME.

The relative changes in NH3 was positively correlated with the total concentration of N in the ileal digesta; A significant positive correlation was also observed for isobutyrate, isovalerate, cadaverine, spermidine and H2S with the corresponding precursor(s). A significant negative correlation was found between the concentration of the sum of the monitored Trp metabolites and the concentration of Trp in the ileal digesta in the DC vessel of the SHIME. In both PC and DC, the relative change of NH3 was a good predictor for the production of other metabolites such as BCFA and H2S. In DC, NH3 was a good predictor of SCFA. Consumption of zein, whey and pigeon peas may produce relatively higher levels of protein fermentation metabolites.

This study represents an example of successful application of an hybrid approach where relevant ileum effluents collected in vivo are directly used in an in vitro model of colon fermentation for further mechanistic investigations. Our results provide insights into the effect of the composition of the ileum effluent on protein fermentation metabolites, which may help to predict the effect of dietary proteins on fermentation outcomes in the large intestine.

Keywords

Protein Fermentation, SHIME, Short Chain Fatty Acids, Ammonia, in vitro models, Gut Microbial Metabolites

Acknowledgements

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TARGETING THE GUT-BONE AXIS: IN VITRO EFFECTS OF PROBIOTIC S. CEREVISIAE CNCM I-3856 ON THE MICROBIOTA OF PATIENTS WITH OSTEOPOROSIS AND HEALTHY CONTROLS

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Abstract

Introduction

Through the gut-bone axis, the gut microbiome is believed to be involved in bone health. Saccharomyces cerevisiae CNCM I-3856 (SC) benefits gut health, previously shown in patients with irritable bowel syndrome (IBS). IBS is more prevalent in women and seems to be associated with bone related diseases. This study evaluated the effect of SC on the gut microbiota of patients with osteoporosis and healthy donors and its potential impact on the gut-bone axis.

Materials and methods

The validated Colon-on-a-plate™ model was used to perform short-term in vitro colonic simulations. Fecal samples from 12 healthy elderly individuals (11 female and 1 male, >55y/o) and 12 individuals diagnosed with osteoporosis (all female, >55y/o) were used as a bacterial source. Two conditions were tested: untreated control and treatment with probiotic SC. Colonic supernatants were collected after 48h and evaluated for microbial metabolic activity using conventional methods (SCFA, lactate, ammonium) and untargeted metabolomic analysis was performed using laser-assisted rapid evaporative ionization mass spectrometry (LA-REIMS). Microbial community composition was also assessed using Shotgun sequencing.

Results

SC induced significantly higher acetate, propionate, butyrate, and lactate production versus untreated control for both the healthy and osteoporosis groups (p 0.001). SC supplementation reduced the species richness, but not the species evenness compared to the untreated control. Beta-diversity showed distinct separation of conditions representing the healthy population and conditions representing the osteoporosis population, implying a distinct microbial community composition for both donor groups. In addition, within each donor group, SC clustered separately than the untreated control. There were statistically significant changes in 11 taxa with SC supplementation, of which 4 were healthy-specific, 6 osteoporosis-specific and 1 in both populations. Some enriched bacterial species are known short-chain fatty acid (SCFA) producers. Furthermore, LA-REIMS demonstrated significant differences in metabolic profile between the SC supplemented samples and the untreated control, for both donor groups, indicating high treatment robustness.

Conclusion

A single administration of S. cerevisiae CNCM I-3856 significantly increased levels of beneficial SCFAs, supported by enrichment of SCFA-producing bacterial species, with significantly diverse metabolic profiles detected between osteoporosis patients and healthy donors.

Keywords

microbial community composition, microbial metabolic profile, osteoporosis, Saccharomyces cerevisiae CNCM I-3856

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HUMAN MICROBIAL BIOTRANSFORMATION OF BRAZILIAN BERRY ANTHOCYANINS UNDER OBESE AND EUTROPHIC CONDITIONS: AN INFOGEST-BASED MS/MS METABOLOMIC APPROACH

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Abstract

The study of polyphenols gastrointestinal digestion and their microbial metabolites in obesity are essential due to their function in modulating oxidative stress, systemic inflammation, carbohydrates and lipid metabolisms. Brazil holds the greatest biodiversity in the world, being rich in native fruits that are excellent sources of anthocyanins (ACNs), which are still underexplored scientifically and commercially. Additionally the microbiota's metabolism of berry ACNs are still not well understood, even more in the case of disease driven differential biotransformations, like in obesity. Therefore, the objective of this work is to assess the obese vs eutrophic human microbial biotransformation of brazilian berries Grumixama and Black Pitanga ACNs. We applied a metabolomic approach based on INFOGEST protocol coupled to MS/MS analysis. The INFOGEST protocol was applied to the fresh fruits and anthocyanidic extracts, in order to evaluate the fruit matrix effects. Bioacessible fractions were subjected to in vitro batch colonic model inoculated with fecal microbiota pools from 5 eutrophic vs 5 obese individuals. After 48 hours bioaccessible microbial metabolites were collected through ultracentrifugation. Metabolites were analyzed by UHPLC-TQD-MS/MS in MRM mode, looking for 23 expected metabolites. Glycosylated ACNs from grumixama and black pitanga were completely depleted after colonic fermentation, indicating enzymatic hydrolysis followed by microbial consumption of the bioaccessible compounds. Aglycone forms became predominant in the bioaccessible fractions, suggesting anthocyanidins as transient intermediates during intestinal exposure. Microbial metabolism generated low-molecular-weight phenolics, mainly gallic acid, protocatechuic acid and phloroglucinol aldehyde, which accumulated in the final bioaccessible fraction and are consistent with cyanidin-type anthocyanin ring-fission pathways. Qualitative metabolic profiles were similar between obese and eutrophic microbiota. However, eutrophic microbiota produced higher levels of protocatechuic and gallic acids, particularly when whole fruits were fermented compared to extracts, indicating an effect of the food matrix and metabotype on metabolite formation. In summary, ACNs from grumixama and black pitanga were extensively biotransformed by gut microbiota into low-molecular-weight phenolic acids that represent the main bioaccessible metabolites. Although obese and eutrophic microbiota exhibited qualitatively similar metabolic pathways, eutrophic microbiota produced higher levels of key phenolic metabolites, particularly in the presence of the whole fruit matrix, highlighting the combined influence of metabotype and food matrix on ACN biotransformation. These findings contribute to the understanding of disease-driven differences in microbial metabolism and reinforce the potential of underexplored Brazilian berries as relevant sources of microbiota-derived bioactive compound

Keywords

Anti-obesity effect; Gut microbiota; Anthocyanins; Phenolic Compounds; UHPLC-MS/MS

Acknowledgements

We thank FAPESP for funding Paulo Berni research project

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ADVANCING IN VITRO COLON MODEL FOR UNDERSTANDING GUT MICROBIOTA-HOST INTERACTIONS: THE INFOGUT COST ACTION CA23110

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Abstract

Background Recent scientific literature underscores the crucial role of the gastrointestinal tract in human health, with the effects of nutrients, bioactives, and toxic compounds being influenced by their interactions with gut microbiota. Clinical testing of food, feed, supplements and pharmaceuticals raises ethical concerns, while the transferability of animal models to humans remains challenging due to physiological and metabolic differences.

Methods In order to address these gaps, the COST Action INFOGUT aims to advance in vitro human colon models by providing standardized and reproducible protocols for both regulatory and research applications. The COST Action brings together around 300 participants from academic and private sectors, experts in gastroenterology, microbiology, physiology, nutrition, food science, biochemistry, bioinformatics, and also biotechnology from over 30 countries to generate robust data sets that will elucidate the complex interactions between the microbiota and the host.

Results With five working groups focusing on the review of existing models and standardization, extension to other gut compartments than the colonic one, diseased conditions, data management, and regulatory/education efforts, INFOGUT is positioned to contribute to the development of healthier food systems and the prevention of related diseases while relying less on animal studies. This initiative also aims to promote the development of educational tools for young researchers and raise societal awareness to prevent misleading consumer choices related to gut health.

Conclusions INFOGUT aims to fill the scientific gap related to completing the simulation of the whole digestion/colonic fermentation process with an optimized standardized simple system. The network shall share the latest technical advances in in vitro gut model development within the scientific community, with the aim to improve and develop standard protocols and generate from results clear messages to disseminate to all stakeholders.

Keywords

COST action, in vitro gut model, microbiota, dissemination, training

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GLYCATION OF MEAT DURING PROCESSING AND GASTROINTESTINAL DIGESTION MODULATES DIGESTIBILITY AND GUT MICROBIAL COMPOSITION, FERMENTATION AND IMMUNE RESPONSES

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Abstract

Research question

Glucose addition and thermal processing are common practices in the culinary and industrial preparation of meat, promoting protein glycation, better known as the Maillard reactions. However, it remains unclear whether protein glycation continues during gastrointestinal digestion and what the subsequent implications are for gut health, following the intake of meat.

Materials and methods

Four nutritionally complete and balanced experimental diets differing in glycation status were formulated. The diets contained pork that was either moderately heated without glucose (MH-G), moderately heated with 5% glucose (MH+G), intensively heated without glucose (IH-G) or intensively heated with 5% glucose (IH+G). Moderate heating was performed at 180 °C in the oven until core 70 °C was reached, and intensive heating at 110 °C until core 100 °C. The diets were subjected to simulated gastrointestinal digestion and were fed ad libitum to male Sprague Dawley rats (10 per group) for 3 weeks. Dry matter digestibility of the heated pork was evaluated. Glycation levels (pentosidine) were assessed (HPLC-FLD) in diets, in vitro gastrointestinal digesta and rat stomach digesta from the pyloric region. Faeces were collected on day 14 to assess colonic microbial fermentation. At the end of the trial, distal colon content was assessed for microbial composition (16S rRNA-Seq) and calprotectin levels (ELISA), and colon mucosa was collected for transcriptomics analysis (mRNA-Seq).

Main findings

As a marker of glycation-derived protein cross-linking, pentosidine levels were found 1.2–1.7-fold higher in IH+G diet than the other diets. Such differences were markedly amplified after simulated gastrointestinal digestion (2–10-fold higher) and in rat stomach digesta (3–11-fold higher), indicating that glycation continued during digestion. Increased glycation was linked to reduced digestibility, as IH+G pork exhibited 26-50% reduced dry matter digestibility than the other treatments. And rats fed IH+G diet produced 2.5-fold higher faecal mass than rats fed the other diets, despite similar feed intake. This high-glycation diet also markedly shifted the colonic environment. Rats fed IH+G diet showed distinct shifts in microbiota composition, with 38 bacterial taxa significantly enriched, whereas only 6–9 taxa were distinct in the other diet groups. This was accompanied by markedly higher faecal levels of SCFAs (2–12-fold), while no clear increased levels of several protein fermentation markers, in rats fed IH+G diet than the other diets. In addition, 30 KEGG pathways related to adaptive immunity were significantly downregulated in colon tissue of rats fed IH+G diet compared to the other diets. This was accompanied by 4–6-fold higher levels of faecal calprotectin, and 2.8-fold upregulation of NADPH oxidase 1 expression and 1.3-fold higher levels TBARS in colon tissue, indicating stimulated epithelial oxidative stress and innate immune response.

Keywords

Pentosidine, melanoidin, short chain fatty acids, calprotectin, gut health

Acknowledgements

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PROTEIN FERMENTATION BIOMARKERS IN PLASMA AND URINE VARY BETWEEN PROTEIN SOURCES DURING A RANDOMIZED FULLY CONTROLLED DIETARY INTERVENTION

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Abstract

Microbial fermentation of undigested proteins in the large intestine may result in production of metabolites that are potentially harmful to gut epithelium and host health. An increase in protein intake is known to increase the flow of proteins into the large intestine, and in turn, protein fermentation. Yet, the relation between protein substrate flowing into the colon and metabolites therefrom remains largely unexplored in humans.

We studied the effect of protein composition and digestibility on protein fermentation biomarkers, digesta transit, and GI microbiome in humans. Two purified protein sources differing in amino acid (AA) composition and presumed digestibility were used to create a contrast in protein inflow (quantity and composition) into the large intestine. We hypothesized that consuming bovine plasma protein (BPP, low digestible) would increase protein fermentation and related metabolites, compared to whey protein isolate (WPI, high digestible).

Fifteen participants consumed either BPP or WPI (30 g/d divided over 3 meals) during a 7-day fully controlled cross-over dietary intervention, with a 7-day washout period between. Macronutrient content was identical for both diets with 12% proteins, 35% lipids, 51% carbohydrates, and 2% fiber (kcal:kcal) of total energy intake. Postprandial plasma, morning urine spot samples, and fecal samples (quantitative collection for ≥ 2 days) were analyzed for protein fermentation biomarkers including ammonia, branched chain fatty acids (BCFA), urea, p-cresol, indoles, and phenols.

In vitro degree of hydrolysis was 32.6±1.38% for BPP and 26.3±0.05% for WPI (P=0.10). Total postprandial AA concentrations in plasma(p) did not differ by protein source (Pp=0.64). In plasma and urine(u), consumption of BPP tended to result in higher concentrations of p-cresol sulfate (Pp=0.05, Pu=0.09), p-cresol glucuronide (Pp=0.07, Pu=0.08), phenyl sulfate (Pp=0.09, Pu=0.09), and phenylacetylglutamine (Pp=0.05, Pu=0.07), compared to WPI. Test protein source did not affect fecal(f) ammonia (Pf=0.84), short-chain fatty acids (Pf=0.96), and branched-chain fatty acids concentrations (Pf=0.63). Transit time of an ingestible capsule tended to increase in the large intestine (Δ11.9h, P=0.07) and total GI tract when consuming BPP (Δ12.2h, P=0.07). Microbial alpha- and beta diversity were unaffected by BPP or WPI. Plasma and urine metabolites were correlated with each other but not with metabolites measured in feces.

Incorporation of 30g/d BPP or WPI in a fully controlled diet produced differences in the urine and plasma levels of tyrosine-, phenylalanine- and tryptophan-derived microbial metabolites mainly due to protein composition differences as digestibility differences were smaller than expected. However, not all metabolite concentrations were directly related to amino acid composition of the protein source. Markers measured in plasma and urine may better reflect protein fermentation than markers measured in feces.

Keywords

protein fermentation, amino acid composition, protein digestibility, metabolites, ammonia, phenol, p-cresol, indole

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ENZYMATIC HYDROLYSIS EXTENT OF PEA PROTEINS SHAPES GUT MICROBIOTA METABOLISM AND SHORT-CHAIN FATTY ACID PRODUCTION AFTER SIMULATED DIGESTION

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Abstract

Diet-microbiota interactions are strongly influenced not only by nutrient composition but also by food processing. While plant proteins are increasingly consumed, how technological modifications affect their gastrointestinal fate and interactions with the microbiota remain poorly understood.

This study investigated whether controlled enzymatic hydrolysis of pea protein concentrate (PPC) can steer the composition of the gut microbiota and metabolic outputs following *in vitro* gastrointestinal digestion and colonic fermentation. PPC was hydrolysed with Alcalase for 5, 60, and 240 min to obtain hydrolysates (PPH5, PPH60, PPH240, respectively) with increasing degrees of hydrolysis, and then simulated digestion was performed using the INFOGEST protocol. Protein digestibility was measured in the bioaccessible fraction by the OPA assay. Extensive hydrolysis significantly improved intestinal protein digestibility (PPC 80%; PPH240>90%). The bioaccessible fraction was added to Caco-2 monolayers to evaluate epithelial viability, potential pro-inflammatory responses, and amino acid transport across the intestinal barrier. At the same time, the non-bioaccessible residues underwent 24 h *in vitro* colonic fermentation using pooled human faecal inoculum. None of the samples impaired cell metabolic activity or increased IL-6 and IL-8 secretion, confirming epithelial compatibility. Moreover, branched-chain amino acid transport across the epithelial monolayer remained unchanged regardless of the extent of hydrolysis, indicating that controlled protein hydrolysis enhances digestibility without affecting intestinal barrier integrity or amino acid bioavailability. Colonic fermentation revealed that the extent of hydrolysis markedly shaped microbial outcomes. Compared with non-hydrolysed PPC, PPH samples promoted beneficial taxa, including *Bifidobacterium longum*, *Anaerostipes hadrus*, and *Acidaminococcus intestini*, with a clear separation between samples in multivariate analysis. Metabolically, short-chain fatty acid production was also modulated by hydrolysis extent: limited hydrolysis (5 min) favoured acetate generation (PPC 45.7 mM; PPH5 49.6 mM), whereas extended hydrolysis (240 min) significantly increased propionate levels (PPC 21.4 mM; PPH240 25.9 mM). Positive correlations were observed between acetate levels and *A. hadrus*, as well as propionate production and *B. longum* and *A. intestini*, supporting a microbiota-mediated metabolic shift.

These findings demonstrate that controlled enzymatic pea protein hydrolysis is not merely a strategy to enhance digestibility, but a technological lever capable of modulating diet-microbiota interactions and steering microbial metabolite production. Therefore, this process could represent a novel approach to design plant-based ingredients with targeted prebiotic-like functionality.

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MYCOPROTEIN INCLUSION IN HYBRID MEAT PRODUCTS ATTENUATES OXIDATIVE REACTIONS DURING DIGESTION AND MODULATES COLONIC MICROBIAL ACTIVITY IN RATS

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Abstract

The replacement of animal-derived proteins with alternative protein sources may influence gastrointestinal chemistry and gut health. This study investigated how partial (30% or 70%) or full (100%) replacement of dietary pork with Quorn mycoprotein affects oxidative reactions during digestion, intestinal redox status, and gut microbial ecology. In vitro gastrointestinal digestion was combined with a three-week rat feeding trial. In vitro, inclusion of mycoprotein markedly attenuated lipid oxidation during thermal processing and simulated digestion, with pork-based products showing 8- to 14-fold higher levels of malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), and hexanal compared to hybrid products and mycoprotein digests. In vivo, higher dietary levels of mycoprotein were associated with lower oxidative stress in colonic tissue, as indicated by a significant reduction of MDA and glutathione peroxidase (GSH-Px) activity. These effects coincided with shifts in microbial metabolism, including significantly increased short-chain fatty acid (SCFA) production and reduced levels of sulfur-containing microbial metabolites. Gut microbiota composition also differed between dietary groups. The pork-based diet was characterized by a large outgrowth of mucin-degrading (e.g. Akkermansia) and sulfate-reducing (e.g. Desulfovibrionaceae) bacteria, whereas hybrid and mycoprotein-rich diets showed a more balanced microbial profile. These microbial changes suggest that mycoprotein inclusion may help limit excessive degradation of the protective mucin layer in the large intestine. Furthermore, higher dietary mycoprotein levels were associated with increased colon length, supporting an overall shift toward a more favorable colonic environment. Together, these findings indicate that partly replacing pork with mycoprotein attenuates diet-induced oxidative reactions during digestion and is linked to alterations in gut microbial activity and host intestinal redox status. These findings highlight mycoprotein as a promising functional component in hybrid meat products, where partial replacement of pork can reduce oxidative reactions during digestion and steer microbial activity toward a colonic environment less prone to oxidative stress and mucus barrier disruption.

Keywords

Mycoprotein, Hybrid meat, Lipid oxidation, Gastrointestinal digestion, Colonic microbiota, Short chain fatty acids

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MODELING MICROBIOTA – HOST INTERACTIONS USING ANAEROBIC GUT-ON-CHIP SYSTEM

Arturs Ābols (Co-founder & CSO)

Cellbox Labs Ltd.

Abstract

A significant proportion of gut microbiota consists of strict anaerobes, which are difficult to maintain in conventional in vitro systems together with gut epithelial cells, limiting the ability to study physiologically relevant microbiota - host interactions.

To address this challenge, we have developed an industrial- grade, mucus-producing, vascularized Gut-on-Chip (GoC) platform designed for scalable co-culture of human intestinal cells with anaerobic microbiota. The system integrates a differentiated intestinal epithelium with a perfused endothelial compartment, enabling controlled simulation of the intestinal barrier under dynamic flow and oxygen-gradient conditions.

The platform supports the evaluation of food derived compounds, probiotics, and microbiota driven metabolism through real-time barrier function assessment and multi-omics analysis. Using donor derived microbiota, we demonstrate stable maintenance of anaerobic communities for up to 72 hours, enabling monitoring of microbial composition, metabolic activity, and translocation across the epithelial - endothelial interface.

This approach provides a physiologically relevant and scalable framework for investigating microbiota behavior and host responses applicable in functional food development, probiotic validation, and personalized nutrition.

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POSTER PRESENTATIONS

Topic: Role of Gut Microbiota in Digestion

SYSTEMATIC LITERATURE REVIEW PROPOSAL TITLE: THE IMPACT OF NUTRITIONAL INTERVENTIONS ON COGNITIVE FUNCTION IN ADULTS WITH IRRITABLE BOWEL SYNDROME (IBS):

Zoe Rutter Kelly [1 2 3]

[1] Dr Amalia Tsiami, [2] Dr Neda Sattarzadeh, [3] Dr Miguel Toribio Mateas

Abstract

Abstract Introduction & Objective Irritable Bowel Syndrome (IBS) is a common gastrointestinal condition often accompanied by cognitive symptoms such as poor memory, concentration, executive function, brain fog and neurocognition. Recent evidence suggests that diet may play a key role in managing both gut and cognitive symptoms. This systematic review aims to examine the impact of nutritional interventions on cognitive function in adults with IBS.

Methodology

This review will follow the Joanna Briggs Institute (JBI) methodology for systematic synthesis of quantitative evidence, with a focus on meta-analysis to integrate findings across studies. A comprehensive search will be conducted across databases including PubMed, CINAHL and PsycINFO for studies published. Keywords will include “IBS,” “nutrition,” “diet,” “cognition,” “memory,” and “adults” with additional key words used. Titles and abstracts will be screened, followed by full-text assessment for eligibility. Data will be extracted and quality assessed using the JBI critical appraisal tools JBI (2020). A narrative synthesis will be used due to expected study heterogeneity.

Inclusion and Exclusion Criteria

Studies must include adults (18+) diagnosed with IBS and assess the effect of nutritional interventions (e.g., low-FODMAP, Mediterranean Diet and Personalised Diets) on cognitive outcomes. Exclusion, studies involving children, non-nutritional interventions, non-English language papers or unrelated outcomes will be excluded.

Discussion

This review will summarise current evidence linking diet to cognitive function in people with IBS, potentially guiding dietary recommendations and future research. It aims to highlight the importance of integrative care that considers both physical and cognitive symptoms. Findings may inform healthcare professionals on effective, nutrition-based strategies to improve quality of life for individuals with IBS.

Keywords

IBS, Microbiome, Gut mircobiota, Cognition, Well-being, Diet, Dietary Intervention, low-FODMAP, Adults with IBS

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INULIN MITIGATES PFAS-INDUCED DYSBIOSIS DURING GASTROINTESTINAL DIGESTION

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are chemically robust environmental contaminants that accumulate in ecosystems and human tissues, posing risks to physiological homeostasis. This study explored the interactions of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) with inulin, a prebiotic fibre, and evaluated the subsequent fate in the gastrointestinal (GI) track and the effects on the human gut microbiota. PFAS binding to inulin was investigated across a range of polymer concentrations (5-50 mg L-1) and pH values (3, 5, and 7), revealing enhanced sequestration at higher polymer levels and acidic conditions, consistent with electrostatic and hydrogen bonding interactions. Stability of PFOA under simulated GI conditions was assessed using the INFOGEST 2.0 protocol, demonstrating minimal chemical transformation throughout oral, gastric, and intestinal phases. In vitro faecal fermentation assays with microbiota from five healthy donors showed that PFOA concentrations gradually decreased, indicative of microbial uptake or partial modification, with inter-individual variability in removal rates. PFOA exposure led to significant shifts in microbial composition, including depletion of beneficial genera (Bifidobacterium, Ligilactobacillus) and proliferation of opportunistic taxa (Escherichia, Shigella, Segatella, Megamonas). Supplementation with inulin partially restored microbial community structure and enhanced production of short-chain fatty acids, such as lactate, acetate, and formate, reflecting improved metabolic activity. These results highlight the capacity of dietary inulin to counteract PFAS-induced dysbiosis, offering mechanistic evidence for nutritional interventions to mitigate health risks associated with persistent fluorinated pollutants.

Keywords

Gut microbiota; Gastrointestinal simulation; Inulin supplementation; Microbiome dysbiosis; Perfluoroalkyl substance

Acknowledgements

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Topic: Role of Gut Microbiota in Digestion

GUT MICROBIOME AND METABOLITE RESPONSES TO HIGH-MOISTURE EXTRUSION OF PLANT-PROTEIN INGREDIENTS

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Abstract

Plant-based meat analogues (PBMAs) can be consumed as sustainable alternatives to animal protein. Due to their processed nature and ingredient composition (protein and dietary fibres), there is an increasing interest in understanding how PBMAs and their production influence gut health outcomes. Therefore, this study investigated how high-moisture extrusion (HME), a common method for PBMA production, and plant ingredient selection influence metabolite production and microbial composition during in vitro colonic fermentation of five commercial protein ingredients: fava bean isolate (FBI), pea protein isolate (PPI), soy protein concentrate (SPC), soy protein isolate (SPI), and vital wheat gluten (VWG). The ingredients were analysed before and after HME for physical properties relevant for microbial fermentation: dietary fibre distribution, water-holding capacity (WHC), and nitrogen solubility. After colonic batch fermentations up to 48 hours, short-chain fatty acids (SCFAs), ammonia and microbial shifts composition and functional activity were quantified. HME did not alter the soluble-to-insoluble fibre ratio, indicating preserved substrate composition for saccharolytic fermentation. However, HME reduced overall fermentability across all ingredients, reflected in lower SCFA and ammonia production. This reduction was linked to decreased nitrogen solubility and protein aggregation, which likely restricted microbial access despite increased WHC. Ingredient refinement strongly modulated outcomes: SPC, which retains dietary fibre and has lower protein content, supported a more favourable metabolic profile with higher SCFA and lower ammonia compared with SPI. However, macronutrient composition or single structural parameters alone did not consistently predict fermentability across the various ingredients, underscoring the role of intrinsic ingredient properties and matrix effects. These findings highlight that ingredient choice, rather than extrusion structuring alone, is central to optimizing PBMA formulations for gut health. This work provides evidence on impact of protein sources and processing. It establishes a foundation for future considerations for the production of healthy and sustainable PBMAs.

Keywords

Plant-based meat analogues; protein transition; extrusion; gut health

Topic: Role of Gut Microbiota in Digestion

INFLUENCE OF RESISTANT STARCH ON HUMAN GUT MICROBIOTA AND IMMUNE RESPONSE USING IN VITRO COMPLEMENTARY APPROACHES

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Abstract

Introduction

Resistant starch is a prebiotic insoluble fiber mainly found in cooked and cooled starchy food. RS can be provided by High Amylose Wheat (HAW) for which gut microbiota modulation has been documented in humans but without being linked to immune responses so far. The aim of this study was to evaluate the anti-inflammatory properties of RS 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.

Methods

An original approach combining the human ARtificial COLon (ARCOL) model and leukocytes isolated from human blood was used. ARCOL model was used to reproduce the main nutritional, physicochemical and microbial parameters of the large intestine of healthy human adults. Three bioreactors were run in parallel, one used as a control and two daily supplemented with 15 g/L of RS 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 (SCFAs) measurement. Bacterial composition was assessed by 16S Metabarcoding. Supernatants from ARCOL fermentations were diluted and incubated with human leukocytes to measure pro-inflammatory cytokines after cell stimulation with lipopolysaccharides.

Results

Supplementation with RS or inulin led to significant increases in main SCFAs concentrations (mainly acetate and butyrate) and gas production, with donor-dependent effects on profiles. The impact of RS and inulin on microbiota composition was also different depending on the donors. However, both prebiotics led to an increase in Ruminococcus abundance. Interestingly, Blautia was more prevalent with all donors only when fermentative media was supplemented with RS. Supernatants from ARCOL bioreactors led to a decrease in some pro-inflammatory cytokines by human leukocytes. Yet, no significant difference was observed between the control and treated (both RS and inulin) conditions.

Discussion

RS and inulin supplementation induced donor-dependent modulation of microbiota composition and metabolic activities. However, in our study, gut metabolites produced after fiber supplementation did not exhibit any effect on immune pathways. In a next step, it would be of great interest to investigate if the two prebiotics have more marked effects when simulating in vitro diseased situations associated with microbiota perturbations. Hence, the impact of RS and inulin will be soon assessed using the ARCOL model set up to reproduce the specific nutritional, physicochemical and microbiological parameters found in the colon of obese patients, coupled with human immune cells and adipose tissue.

Keywords

resistant starch, inulin, in vitro colon model, human leukocytes, inflammation

Topic: Role of Gut Microbiota in Digestion

IN VITRO ASSESSMENT OF MICROBIAL LIPID CONVERSION PATHWAYS RELEVANT TO THE SMALL INTESTINE

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Abstract

Background: Fatty acids are essential components of membranes and important sources of metabolic energy in all organisms. Combined with other dietary energy sources, fats have a large impact on energy balance and metabolic health in humans. Lipid digestion in humans results in more than 90 % lipid uptake in the small intestine, but a minor proportion can be used as substrate by the intestinal microbiota during small intestinal transit or in the colon. Based on in silico analysis of two lipid conversion pathways, two small intestine-relevant microbiota species were identified: Lactiplantibacillus plantarum and Citrobacter freundii. The former may impact host health by converting linoleic acid to conjugated linoleic acid (CLA) and the latter may impact dietary energy and host metabolism through β-oxidation of free fatty acids (FFA).1-3

Method: Sunflower digest and control digests (made with adapted INFOGEST protocol standardized on lipase activity) and FFA linoleic acid dissolved in control digest, were added to warm BHI media (w/o glucose and w galactose) in a 24 deep-well screening plate with constant pH measuring. Aerobic fermentation was started after adding the bacteria in mid-log growing phase at 2.5 % (v/v). Samples were taken at start and after 24 h of incubation and were analyzed for SCFA and sugar content via HPLC-RI/UV, and long chain fatty acid including CLA via LC/ESI-MS/MS

Results: Linoleic acid residues, as FFA or bound to a glycerol backbone, decreased strongly after 24 h incubation with C. Freundii. For L. Plantarum no or only a mild decrease was observed. Glycerol was metabolized fully by C. Freundii and only partially by L. Plantarum, and SCFA patterns differed as well between both strains and between digested sunflower oil and linoleic acid as FFA. L. Plantarum showed rapid CLA production, occurring within the first hour of incubation with digested sunflower oil and, to a lesser extent, with linoleic acid as FFA. CLA is decreased at 24 hours, due to further conversion or consumption by the L. Plantarum.

Conclusion: The observed results are consistent with the disappearance of fatty acid residues by beta-oxidation by C. Freundii, and with rapid CLA conversion by L. Plantarum. This demonstrates that the described in vitro approach can be applied to functionally study biologically relevant fat conversion pathways relevant to the aerobic conditions of the small intestine. The setup allows the study of both FFA and lipids that need pre-digestion and the results suggest that these are not the same functionally.

1. Fujita, Y et al., 2007
2. Iorizzo, M et al., 2024
3. Kim, J. H et al., 2016

Keywords

Lipid fermentation, Single strain, Lipid pathway, Small intestine

Acknowledgements

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Topic: Role of Gut Microbiota in Digestion

INTERACTION BETWEEN FOOD MATRICES AND THE WEANING INFANT GUT MICROBIOME USING A NEW IN VITRO WEANING INFANT DIGESTION MODEL

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Abstract

The gut microbiome plays an important role in human health regulation, which can be affected by multiple factors, such as prebiotics/fibre, antibiotics and probiotics. Among them, the vitality of diet's effect on the gut microbiome has been highlighted and focused on due to its long-term regulatory effects on the human gut microbiome. Individual nutrients had been emphasised previously due to their capability to sustain the growth or modulation of the human gut microbiome. However, the physical forms they present to the gut microbiome have been neglected and rarely studied. The physical form may be altered by protein cross-linking, polysaccharides matrix and emulsion formation, which shows various food matrix structures (liquid, gel or solid). Therefore, its bioavailability and bioaccessibility may be altered after human digestion, especially when experiencing an impaired/immature digestion system. The weaning infants' gut model was chosen as a representation for establishing the in vitro digestive and colonic fermentation systems to study the food matrix impact on the human gut microbiome due to the importance of solid food introduction at this stage. INFOGEST had been developed for many years as a mature adult in vitro digestion model. Several infant INFOGEST models have also been proposed and in the literature, but as yet no consensus model has been proposed . Moreover, there has been limited focus on the conditions required to simulate the digestive conditions of a weaning infant. Hence, a modified in vitro digestion model is proposed herein to represent the physiology of weaning infants, including an in vitro gut microbiome consortia model to simulate processes in the lower gut. This model will allow us to examine the digestion kinetics, metabolism behaviour and innate interactions between the nutrients within foods of varying matrices. Comparison between the existing and proposed model has been performed to demonstrate the efficiency of weaning infant digestion and interactions between food matrices and the gut microbiome will be presented afterwards.

Keywords

Food Matrix, Gut microbiome, Digestion models, Weaning infant digestion

Topic: Role of Gut Microbiota in Digestion

DYNAMICS OF GLUCOSINOLATE/ISOTHIOCYANATE PAIRS FROM MUSTARDS IN LARGE INTESTINE USING AN IN VITRO COLONIC FERMENTATION AND INFLUENCE ON GUT MICROBIOTA

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Abstract

Isothiocyanates (ITCs) are bioactive compounds [1, 2] derived from glucosinolates (GLS) present in some mustard varieties that are produced from myrosinase hydrolysis during mastication and additionally in large intestine [3]. The metabolism of glucosinolates and ITCs production in the distal part of the digestive tract have been understudied.

This study analyses the hydrolysis of the two main glucosinolates (glucotropaeolin and sinigrin) present in the green parts of two mustard varieties (white mustard (Sinapis alba) and Ethiopian mustard (Brassica carinata)) in the large intestine by the colonic microbiota. The starting point was the non-bioaccessible fraction of these mustard varieties, obtained after the static gastrointestinal digestion INFOGEST protocol.

The samples were placed inside an anaerobic jar using the AnaeroGen system and incubated at 37 ◦C for 24 h under mild oscillation (60 rpm) in an incubator. The supernatant and the solid residue of each sample were collected and analysed. To develop the in vitro colonic fermentation a culture medium and a faecal inoculum from five healthy human donors was also used. The GLS hydrolisis and formation-degradation of ITC in the in vitro colonic fermentation assay was monitored at 1, 3, 8, 12, 16 and 24 hours.

The initial sinigrin content (3.52 µmol) decreased slightly to 3.26 µmol during the first 3 h of colonic fermentation, fell to 2.45 µmol at 8 h, and decreased dramatically to values of 0.60 µmol after 12 h, disappearing at 24 h. On the other hand, allyl-ITC content, the main breakdown product of sinigrin, was formed from 1 h of colonic fermentation (0.05 µmol), increased until 0.10 µmol at 3 h, and this content remained constant until 16 h, decreasing to negligible values in parallel with the decrease in sinigrin. These results suggest the formation of ITC from GLS by the gut microbiota, although the ITC formed is a highly reactive molecule with a short half-life [4].

With regard to the glucotropaeolin present in white mustard, the initial amount of glucotropaeolin (1.25 µmol) decreased sharply after 1 hour of the colonic fermentation assay, resulting in the formation of 0.90 µmol of benzyl-ITC. This concentration remained constant until 8 h when it decreased to negligible values from 12 h onwards.

The microbiota exposed to these compounds, during colonic fermentation, exhibited changes in the relative abundance of the Operational Taxonomic Units (OTUs,) compared to sample controls. Overall, results indicated changes in microbiota richness, with a reduced alpha diversity in the treated faecal samples. Interestingly, treated samples presented high abundance of Enterobacteriaceae at the initial times (12 h) that declined during the last phase of colonic fermentation.

References:

[1] Wang and Bao. Free Radic. Biol. Med. 167 (2021) 125–140

[2] Dinh et al. Pharmacol. Res. 169 (2021) 105666

[3] Angelino and Jeffery. J. Funct Foods 7 (2014) 67–76

[4] Andernach et al. 434 Food Ch

Keywords

Colonic fermentation, bioaccessibility, gut microbiota, bioactive compounds

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Topic: Role of Gut Microbiota in Digestion

ATHLETES AND PROBIOTICS: A CASE FOR PRECISION BIOENGINEERING?

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Abstract

A better understanding of how the gut microbiome directly, or indirectly, impacts different conditions is of major interest to parties from different aspects of research. Equally important is elucidating how internal and external factors shape the microbiome itself, knowledge that could ultimately enable the manipulation of the gut microbiome for therapeutic or preventive purposes. Although studies demonstrate significant differences in the gut microbiome of individuals clustering along lifestyle, diets, genetic background, health conditions and extent of physical activity, studies into how these different clusters respond to an identical microbial modulating treatment (probiotic) are becoming of great interest in the microbiome field.

Through use of a human gut simulation model, we recently demonstrated a differential response to a probiotic, L.p.plantarum, from 3 donors meeting a ‘healthy’ donor criterion. Donor 2 presented with a significantly different microbial profile compared to Donor 1 and Donor 3 at baseline. Furthermore, samples from Donor 2, who comes from an athletic background, clustered separately from the other 2 donors after probiotic treatment. The same donor exhibited increased propionic and lactic acid production before and after probiotic treatment, with a significantly truncated butyrate acid signature. Interestingly, fermentation of Donor 2 fecal material after probiotic treatment using the BATCH derived model of the SHIME system resulted in statistically reduced colony forming units on MRS agar, suggesting that the metabolic profile of Donor 2 resident microbes did not support the growth and colonization of L.p.plantarum, as well as other microbes capable of growing on MRS agar.

In conclusion, although probiotics are designed and optimized to effect a standard beneficial impact on a wide range of individuals and gut microbiomes, certain specific clusters that include high intensity athletes will benefit from precisely designed probiotics, given their highly modified gut microbiome that has evolved to support intense physical activity.

Keywords

SHIME, BATCH, Donors, Gut microbiome, Probiotics, Athletes

Acknowledgements

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Topic: Role of Gut Microbiota in Digestion

MICROBIAL AND METABOLIC RESPONSES TO SIMULATED GASTROINTESTIONAL DIGESTION AND COLON FERMENTATION OF PROTEIN- AND FIBER-RICH PLANT CELL CULTURES

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Abstract

Diet is a key modulator of human gut microbiota composition, function, and microbial metabolite production, influencing host physiology and health. Understanding the interactions between different food sources and the complex microbial community is important to promote and maintain overall human health. Plant cell cultures (PCCs) have long been used in the production of secondary metabolites for pharmaceuticals and food additives, however, they also represent a promising, nutritionally balanced source of whole-food ingredients. The aim of this study was to investigate how PCC powder derived from scurvy grass (Cochlearia danica) and rowan (Sorbus aucuparia) modulate gut microbiota composition and are metabolized in the gut. Both rowan and scurvy grass PCCs are rich in protein (21.3 g and 32.2 g/100 g of dry mass, respectively) and dietary fiber (48.9g and 29.2g/100g of dry mass, respectively). In this study, the PCC samples were first subjected to the simulated gastrointestinal digestion via the INFOGEST method, followed by an in vitro colonic fermentation. Full length 16S rRNA sequencing and LC-MS-based untargeted metabolomics analyses were performed to evaluate the microbial composition and metabolite profile during the colonic fermentation at 0, 4, 6, and 24h. Short-chain fatty acids in the samples were analyzed using UHLPC method. These results indicated no changes in the overall alpha-diversity after 24 hours of colonic fermentation of the digested PCC. However, some changes in the differential abundance of certain taxa were observed, notably an increase in Bacillota_I during rowan PCC fermentation, and significant increases in the relative abundance of the genus Ligilactobacillus ($p = 0.0495$), in particular in the Ligilactobacillus salivarius group. The alterations were already evident after 6 hours of fermentation, and similar trends were observed following the scurvy grass PCC fermentation. Both PCC fermentations resulted in decreases in Streptococcus during the 24 hours, but this change did not reach statistical significance in rowan PCC ($p=0.495$ for rowan PCC). We observed similar time-dependent shifts in the metabolite profiles of both PCCs, while the metabolite profile of control showed no such changes. After the 24h fermentation period, there were significant changes in 732 and 721 metabolites for rowan and scurvy grass PCCs, respectively. Metabolites with the highest influence on principal component analysis included cytidine, 1,2-dimethoxy-3-propylbenzene, aspergillic acid, sebacic acid, and cymatherelactone. Following fermentation of both PCCs, enriched pathways included lysine degradation, arginine and proline metabolism, and the urea cycle. These findings suggest that rowan and scurvy grass PCCs can influence gut microbiota by affecting microbial composition and metabolic activity.

Keywords

Gut microbiota, colonic fermentation, protein fermentation

Topic: Role of Gut Microbiota in Digestion

INVESTIGATING THE ROLE OF CHICKPEA COTYLEDON INTEGRITY IN GUT MICROBIOME–HOST INTERACTIONS

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Abstract

Pulses such as chickpeas are starch-rich legumes high in fibre and protein, and their intake is recommended for sustainable and healthy diets. Pulse consumption supports cardiometabolic health and elicits low glycaemic responses, influencing satiety-promoting gut hormones; however, the recommended effective intake is substantially higher than typical UK consumption. Food processing methods can improve pulses palatability and facilitate their use to increase dietary fibre intake, but disrupting pulses cotyledon integrity negatively affects glycaemic and gut hormone responses, suggesting implications of nutrient delivery to the gut microbiome. Furthermore, the impact of long-term pulse consumption on the gut microbiome, and its relationship with physiologically relevant outcomes such as appetite regulation and metabolic health, remains uncertain due to limited human evidence and inconsistent study designs.

The present project investigates how transitioning to a chickpea-enriched diet affects host metabolism by modulating gut microbiome composition. The study aims to address the question: How does the cellular integrity of chickpea cotyledons after upper gastrointestinal digestion influence substrate availability in the colon, thereby affecting gut microbiota composition and function? Understanding how whole chickpea cellular structures deliver fermentable substrates to the colon is essential for clarifying the role of food structure on diet–microbiome interactions.

To address this objective, processed chickpeas with either intact or disrupted cotyledon structures were subjected to simulated upper gastrointestinal digestion using the INFOGEST protocol, followed by dialysis and transfer to an in vitro colonic fermentation model. Undigested components, including proteins and resistant starch, were quantified to characterise colonic substrate availability. Microbial fermentation will be assessed by measuring short-chain fatty acids, amino acids, and other metabolites using nuclear magnetic resonance spectroscopy. Gut microbiota composition and function will be characterised using DNA extraction, shotgun metagenomic sequencing, and bioinformatic analyses.

Differential delivery of fermentable substrates to the colon is expected to result from intact chickpea cotyledon cells resisting upper gastrointestinal digestion more than disrupted cells. This is expected to cause changes in the composition of the microbial community and the production of metabolites during colonic fermentation.

These findings will improve understanding of how pulse food structure influences microbiota-mediated digestion and bioavailability, supporting pulse-based dietary strategies to modulate the gut microbiome. Future work will extend this research to assess long-term gut microbiome and host responses by analysing blood and tissue samples from mice fed a chickpea-enriched diet, alongside human faecal samples collected following a pulse-enriched dietary intervention.

Keywords

Pulses, chickpeas, in vitro digestion, gut microbiome, in vitro colon models

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Topic: Role of Gut Microbiota in Digestion

PREDICTING MICROBIAL COMMUNITY SHIFTS: AN ABM OF DIETARY IMPACT ON COLONIC BACTEROIDES AND FIRMICUTES SPATIAL ECOLOGY

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Abstract

This study aimed to develop and validate a spatially explicit Agent-Based Model (ABM) to simulate and predict how dietary nutrient availability shapes the population dynamics and spatial distribution of Bacteroides and Firmicutes in the colonic lumen. A novel ABM in MATLAB® was developed, following the ODD protocol for transparency. The model simulated a 2D cross-section of the proximal colon (24,000 μm²). Key components included: 1) Individual bacterial agents (Bacteroides spp. and Firmicutes spp.) with species-specific parameters for growth, metabolism, and motility calibrated from in vitro data; 2) dynamic nutrient particles (fibers, proteins) representing dietary input; 3) a diffusive nutrient grid; and 4) simplified immune cell agents. Simulations were run for up to 500 temporal steps (simulating ~25 h). Scenarios of high/low fiber and protein availability were tested. Model outputs (population sizes, Firmicutes-to-Bacteroides (F/B) ratio, spatial clustering) were compared against established ecological principles and empirical data from dietary intervention studies The model successfully generated emergent spatial heterogeneity. Under high-fiber conditions, Bacteroides formed denser clusters near nutrient sources, while Firmicutes showed broader dispersion, illustrating niche differentiation. A high-fiber diet led to a significant increase in the absolute abundance of both phyla but decreased the F/B ratio from a baseline of ~2.1 to ~1.4, aligning with metagenomic observations from human cohorts. Protein-rich, low-fiber scenarios promoted a higher F/B ratio (>2.5) and reduced overall diversity. Spatial analysis revealed that local nutrient depletion by fast-metabolizing Bacteroides created competitive exclusion zones, indirectly affecting Firmicutes distribution—a non-linear outcome highlighting the importance of spatial context in microbial interactions. This spatially explicit ABM demonstrates that dietary nutrients directly influence not only the abundance but also the spatial organization and competitive interactions between major colonic phyla. The model predicts that dietary shifts alter the F/B ratio through mechanisms of localized spatial competition and resource partitioning. It provides a computational framework to bridge the gap between dietary inputs and microbial ecology, offering testable hypotheses for personalized nutritional strategies aimed at modulating gut microbiota composition and function for improved health outcomes, as suggested by diet-microbiota research.

Berry, D., & Widder, S. (2014). Frontiers in Microbiology, 5, 219.
Donaldson, G.P., Lee, S.M., & Mazmanian, S.K. (2016). Nature Reviews Microbiology, 14(1), 20–32.
Grimm, V., et al. (2006). Ecological Modelling, 198(1-2), 115–126.
Sonnenburg, J.L., & Bäckhed, F. (2016). Nature, 535 (7610), 56–64.
Yatsunenko, T., et al. (2012). Nature, 486(7402), 222–227.

Keywords

microbiota, simulation, agent-based model

Topic: Role of Gut Microbiota in Digestion

EFFECTS OF HYDROGELS CONTAINING B-CAROTENE-LOADED NANOEMULSIONS ON SELECTED GUT MICROBIAL POPULATIONS AND SHORT-CHAIN FATTY ACID PRODUCTION

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Abstract

Nanoemulsions (NE) are effective delivery systems for lipophilic bioactive compounds, such as β-carotene (βC), into aqueous food matrices, enhancing their stability and bioavailability. Embedding NE within polysaccharide-based hydrogels can further protect encapsulated compounds from degradation during gastrointestinal transit and increase the delivery of both βC and polysaccharides to the colon, where they may act as fermentable substrates for the gut microbiota. Thus, this study aimed to evaluate the impact of alginate- and carrageenan-based hydrogels containing βC-loaded nanoemulsions on selected gut microbial populations and short-chain fatty acid (SCFA) production using an in vitro colonic digestion model.

Six hydrogel formulations were prepared using alginate or carrageenan at a final concentration of 1% (w/w). Each polysaccharide solution was mixed with one of three filler phases: water (W), a nanoemulsion without βC (NE), or a βC-loaded nanoemulsion (βC-NE). Hydrogels were subjected to static in vitro gastrointestinal digestion following an INFOGEST-based protocol. After digestion, the non-absorbable fraction was recovered and subjected to in vitro colonic digestion with human faecal inoculum. The Firmicutes/Bacteroidetes (F/B) ratio and relative abundances of Lactobacillus and Bifidobacterium were assessed by qPCR. SCFAs (acetic, propionic and butyric acids) were quantified by gas chromatography.

Regardless of nanoemulsion incorporation or polysaccharide type, all digested hydrogels reduced the F/B ratio compared to the control without an added carbon source. This shift, often linked to metabolically favourable gut microbiota profiles, suggests that the polysaccharide matrices were preferentially fermented by Bacteroidetes, a phylum recognized for its ability to degrade complex polysaccharides. At the genus level, Bifidobacterium relative abundance increased only in digested βC-NE hydrogels, particularly in those containing alginate. In contrast, Lactobacillus populations were unaffected by the presence of βC in the hydrogels, but increased in NE formulations, most notably in carrageenan-based hydrogels. These genus-specific responses likely reflect differences in βC affinity and polysaccharide degradation capacity. Regarding SCFAs, acetic and propionic acid levels increased only in digested NE hydrogels. This may be explained by microbial cross-feeding, as lactobacilli produce acetic and lactic acids, the latter serving as a substrate for propionic acid formation. Butyric acid levels remained unchanged across all formulations, suggesting that, despite the reduced F/B ratio in digested hydrogels, butyric acid-producing lineages within Firmicutes were not selectively affected. Overall, these findings indicate that nanoemulsion-filled hydrogels may differentially modulate gut microbial populations and their metabolic activity, supporting their potential as targeted delivery systems for bioactive compounds to the colon.

Keywords

hydrogels, alginate, carrageenan, polysaccharides, nanoemulsions, β-carotene, gut microbiota, SCFAs

Acknowledgements

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Topic: Role of Gut Microbiota in Digestion

ELUCIDATING THE PROTEIN FATE OF PARMIGIANO REGGIANO CHEESE: A MULTI-STAGE APPROACH COUPLING IN VITRO STATIC DIGESTION AND DYNAMIC SHIME® GUT SIMULATION

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Abstract

Parmigiano-Reggiano (PR) is a PDO cheese produced by using raw cow milk and a natural whey starter [1]. It is highly appreciated for the nutritional value of its protein content, which boasts a high biological value with a complete essential amino acid profile. Furthermore, the breakdown of milk proteins by gastrointestinal enzymes (i.e., pepsin, trypsin and chymotrypsin) generates peptides which can exhibit interesting biological properties [2]. This process is further enhanced by microbial enzymes present in the gut, which target distinct cleavage sites to generate unique peptide varieties [3]. Despite the growing interest in elucidating this complex phenomenon of digestion in such a matrix, there is limited research exploring the comprehensive fate and the released products through the gastrointestinal tract including small and large intestines. Herein, this study investigated the molecular behavior of PR cheese proteins during digestion. To do so, 12-month-ripened PR cheese was subjected to the in vitro static digestion protocol (validated INFOGEST method), filtered through a 3 kDa ultrafiltration membrane, and the obtained retentate was further submitted to in vitro colonic fermentation in the dynamic model SHIME® (Simulator of Human Intestinal Microbial Ecosystem). This multi-stage approach was employed to fully simulate the three primary phases of human digestion: i) enzymatic hydrolysis during the gastroduodenal phase; ii) absorption within the small intestine; iii) microbiota dynamics within large intestine. During each phase, the kinetic of the released products was monitored and the latter evaluated for their free amino acid content, degree of protein hydrolysis, peptide composition, and solubilized proteins. As expected, the results showed a different release of free amino acids and peptides depending on the stage of digestion considered. In addition, some peptides were identified in the three different sections of the large intestine. These peptides are being investigated in detail to evaluate the mechanism of the interaction with the gut microbial communities, their characteristics and potential biological properties. This will contribute to understanding the potential health benefits associated with the consumption of these high nutritional food products.

[1] Solieri, L., Bianchi, A. & Giudici, P. Inventory of non starter lactic acid bacteria from ripened Parmigiano Reggiano cheese as assessed by a culture dependent multiphasic approach. Syst. Appl. Microbiol. 35, 270–277 (2012). https://doi.org/10.1016/j.syapm.2012.04.002.
[2] Castellone, V. et al. Peptide profile of Parmigiano Reggiano cheese after simulated gastrointestinal digestion: From quality drivers to functional compounds. Front. Microbiol. 13, (2022). https://doi.org/10.3389/fmicb.2022.966239.
[3] Sultan, S., Huma, N., Butt, M. S. & Aleem, M. Therapeutic potential of dairy bioactive peptides : A contemporary perspective. 839https://doi.org/10.1080/10408398.2015.113

Topic: Role of Gut Microbiota in Digestion

FERMENTED MYCELIUM-BASED MEAT ALTERNATIVES: LINKING FUNGAL FERMENTATION TO NUTRITIONAL QUALITY, FLAVOUR, DIGESTIBILITY, AND GUT MICROBIOME INTERACTIONS

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Abstract

To reduce the environmental impact of our global food system, a transition towards a more plant-based diet is essential. Plant-based meat alternatives (PBMAs) play a major role in the protein transition, but face challenges like a low consumer acceptance due to the presence of antinutrients and off-flavours, a high degree of processing, and the reliance on unsustainable raw materials such as soy. While some PBMAs use more sustainable plant ingredients, like pea or flava beans, they do not meet the nutritional quality and content of soy or meat. Fermentation using edible fungal mycelia shows great potential to enhance both nutritional properties of PBMAs by reducing off-flavours and maintaining a high nutritional composition, thereby also improving digestion.

This study aims to develop and characterize fermented mycelium-based meat alternatives produced from locally sourced Danish substrates and mycelia isolated from 32 edible mushroom species. We hypothesize that fermentation by edible fungal strains can improve flavour and protein quality, while producing a food product that supports digestion and beneficial microbial activity in the gut.

Our new mycelium-based meat alternatives will be tested for a) their nutritional content, b) their flavour profile by a trained sensory panel, c) their ability to produce mycotoxins through whole genome sequencing, d) their digestibility in the upper-gastrointestinal (GI) tract using INFOGEST 2.0 and e) their effect on the faecal microbiome composition and functionality. To explore the effect on the microbiome, digested mycelium products will be fermented in colonic batch cultures inoculated with faecal microbiota from omnivore (“traditional Danish diet”) and plant-based consumers. Microbiome composition and metabolic outputs will be profiled using 16S rRNA long-read sequencing (Oxford Nanopore GridION) and short-chain fatty acid profiling via gas chromatography-mass spectrometry.

By combining nutritional, sensory, digestibility, and microbiome outcomes, this study will create a scientific foundation for the development of sustainable fermented mycelium-based meat alternatives. The findings are expected to improve understanding of how fungal fermentation can contribute to both sustainable food innovation and positive modulation of the gut microbiome, supporting dietary strategies that promote human and planetary health.

Keywords

Mycelium, novel foods, digestion, gut health

Topic: Role of Gut Microbiota in Digestion

INVESTIGATION OF THE BIDIRECTIONAL RELATIONSHIP BETWEEN GUT MICROBIOTA AND DIOSPYROS KAKI LEAF EXTRACT BY IN VITRO COLONIC FERMENTATION

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Abstract

Diospyros kaki is an edible plant known for its health-promoting properties due to the high content in bioactive compounds [1]. However, polyphenols have low bioavailability, as only 5-10% of the ingested compounds are absorbed in the small intestine because of their low stability and solubility under gastrointestinal conditions [2]. The non-digestible fraction is metabolized in the colon by the gut microbiota into low-molecular-weight, potentially bioactive compounds. However, a bidirectional relationship exists between polyphenols and the gut microbiota, as phenolic compounds and their metabolites can modulate gut microbiota composition and function [3].

In this study, an in vitro colonic fermentation was performed to evaluate the impact of gut microbiota on the phenolic composition of D. kaki leaf ethanolic extract, as well as on low-molecular-weight catabolites formation, antioxidant activity, and potential prebiotic properties. The phytochemical profile of the extract was characterized by UHPLC-HRMS-MS and the non-digestible fraction obtained after in vitro digestion (Infogest 2.0) was submitted to in vitro colonic fermentation with human faecal samples [4,5]. Aliquots were collected at 0, 4, 8, 24, and 48 h to evaluate the change in phenolic composition, catabolites formation, and gut microbiota composition changes [6]. Antioxidant properties were also evaluated by DPPH, ORAC and ABTS assays [7], while short-chain fatty acids (SCFAs) were quantified by GC-FID [8].

Several flavonoids, mainly kaempferol and quercetin derivatives, were identified, with most compounds being present in the non-digestible fraction. Gut microbiota modified the phytochemical profile, promoting the formation of nine catabolites. An increase in antioxidant capacity was only observed by the ORAC assay after 2 and 4 h of fermentation. Additionally, the fermentation promoted SCFAs production, including hexanoic and heptanoic acid, indicating an enhanced microbial fermentation activity. Differently, the gut microbiota diversity unchanged, as the treatment with D. kaki modulated specific microbial taxa. Therefore, in vitro colonic fermentation significatively affected the composition of the non-digestible fraction of D. kaki, leading to the formation of bioactive catabolites with antioxidant properties. In addition, D. kaki showed potential prebiotic properties without altering gut microbiota diversity.

1. Ann, N. et al. Int. J. Nutr. Pharmacol. Neurol. Dis. 2025, 15, 159–163.
2. Rasera, G. B. et al. Compr. Rev. Food Sci. Food Saf. 2023, 22, 260-286.
3. Valdés, L. et al. Food funct. 2015, 6, 2424-2439.
4. Brodkorb, A. et al. Nat. Protoc. 2019, 14, 991-1014.
5. Pérez-Burillo, S. et al. Nat. Protoc. 2021, 16,3186-3209.
6. Curry, K.D. et al. Nat. Methods. 2022, 19, 845-853.
7. Pereira-Caro, G. et al. Foods 2021, 10, 457.
8. Baenas, N. et al. Lwt 2020, 134, 110140.

Keywords

edible plant; in vitro digestion, colonic fermentation; gut microbiota; antioxidant; prebiotic effect.

Topic: Role of Gut Microbiota in Digestion

IN VITRO EVALUATION OF THE POTENTIAL PREBIOTIC EFFECT OF COOKIES FORMULATED WITH COFFEE HUSK

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Abstract

Introduction: Coffee and its by-products are rich sources of fiber and polyphenols. These compounds are responsible for the health benefits associated with coffee, exhibiting anti-inflammatory and antioxidant properties. In this regard, the fiber from coffee husk can modulate the composition of the intestinal microbiota, exerting prebiotic mechanisms. Both fibers and polyphenols generally possess anti-inflammatory and antioxidant properties.
Objective: The main objective of the project is the in vitro evaluation of the health potential of coffee husk as an ingredient in bakery matrices and its ability to modulate the intestinal microbiota, using a semi-dynamic colonic fermentation model.

Methodology: The in vitro effect of cookies formulated with coffee husk on the composition of human colonic microbiota and its metabolic activity related to short-chain fatty acids has been evaluated. To this end, a semi-dynamic in vitro assay was conducted using the in vitro Dynamic Colonic Fermentation Digestor (ColonSim), which simulates gastric and intestinal digestion (small intestine), as well as the conditions and bacterial flora composition present in the colon of a healthy adult. The colonic fermentation assay was carried out with the daily incorporation, for 7 days, of two cookies formulated with coffee husk, developed within the framework of the project. Throughout the colonic fermentation process, viable microbial populations in the colonic microbiota were monitored through microbiological counts using selective culture media, and the production of short-chain fatty acids (acetic acid, propionic acid, and butyric acid) was monitored and analyzed by gas chromatography with FID detection.

Results: The results reveal that overall fermentation becomes activated after the colonic fermentation of the cookies formulated with coffee husk, likely due to the presence of insoluble fibers, fermentable phenolic compounds, and changes in microbial composition that favor SCFA-producing groups. Treatment with coffee husk cookies generates a highly fermentative environment, markedly increasing total SCFA production.

Conclusions: Coffee husk cookies act primarily as a metabolic modulator rather than as a selective stimulator of beneficial bacteria. Their impact on SCFA production is highly significant, indicating active fermentation and a substantial metabolic shift in the intestinal ecosystem.

Keywords

Semi-dynamic, coffee husk, microbiota, metabolic modulation

Topic: Role of Gut Microbiota in Digestion

IMPACT OF LONG-TERM SUPPLEMENTATION WITH A MILK PROTEIN–XANTHAN GUM COMPLEX ON GUT FERMENTATION PARAMETERS AND MICROBIAL ENZYME ACTIVITY IN RATS

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Abstract

The increasing prevalence of metabolic disorders and the growing importance of functional foods require verification of whether modification of the food matrix structure through the formation of protein–polysaccharide complexes affects the intestinal environment and microbiota activity in an in vivo model.

The aim of this study was to evaluate the effect of long-term supplementation of a standard “Western-type” diet with a protein–polysaccharide complex on intestinal peristalsis, intestinal content mass, the profile of volatile fatty acids (VFA) in the caecum, and the activity of selected bacterial enzymes as functional indicators of the gut microbiota.

The experiment was conducted on 32 Sprague–Dawley rats divided by sex into control groups fed a standard diet and experimental groups receiving the same diet enriched with a milk protein–xanthan gum complex. The dietary intervention lasted for three months.

The inclusion of the milk protein–xanthan gum complex induced consistent and directionally uniform changes in the intestinal environment in both sexes. The most pronounced effect was an increase in intestinal content mass. In the small intestine, content mass increased by approximately +91% in females and +120% in males, while in the caecum the increase reached +167% and +193%, respectively. Changes in the colon were moderate and more sex-dependent.

Despite the increased content mass, the total VFA pool per gram of caecal content decreased by approximately 25% in females (76.784 → 57.342 µmol/g) and 28% in males (73.838 → 52.817 µmol/g). The reduction primarily involved propionate and butyrate, accompanied by an increased relative proportion of acetate. The fraction of putrefactive VFA was also reduced (−46% in females; −36% in males). The direction of changes was consistent in both sexes, with slightly greater reductions in selected fractions in males.

The enzymatic profile of the microbiota indicates a substantial reorganization of metabolic activity. A marked increase in β-glucosidase activity was observed, together with a pronounced decrease in β-glucuronidase activity (−63% in females; −66% in males) and a reduction in ammonia concentration (−9% and −13%, respectively). These changes are consistent with reduced markers of proteolytic fermentation, observed in parallel with the decrease in putrefactive VFA. Sex-specific differences were selective: the decline in α-glucosidase was stronger in males, whereas a pronounced reduction in β-galactosidase was mainly observed in females.

The protein–polysaccharide complex increased intestinal content mass and altered fermentation conditions in the caecum. The reduction in putrefactive VFA and ammonia suggests attenuation of proteolytic fermentation, while changes in bacterial enzyme activity indicate that the structural organization of the protein–hydrocolloid matrix may serve as a tool for modulating gut microbiota function.

Keywords

caecal fermentation, intestinal content mass, enzyme activity, proteolytic fermentation

Acknowledgements

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Topic: Role of Gut Microbiota in Digestion

IN VITRO DIGESTION AND COLONIC FERMENTATION OF FIG PHENOLICS: EXPLORING THE INTER-INDIVIDUAL DIFFERENCES IN METABOLIC FATE

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Abstract

Figs (Ficus carica L.) are a rich source of phenolic compounds recognized for their antioxidant and anti-inflammatory properties [1]. However, their health-promoting effects depend largely on their transformation and bioavailability after reaching the colon, where they are extensively metabolized by the gut microbiota [2]. Understanding the colonic transformation of individual phenolics is crucial to elucidate their biological potential and inter-individual variability in their metabolic fate. The aim of this study was to evaluate the bioaccessibility and metabolism of fig polyphenols following an in vitro simulated gastrointestinal process and subsequent fecal fermentation. Fig, “Brown Tukey” variety, was submitted to the INFOGEST 2.0 digestion protocol [3], followed by an in vitro fecal fermentation performed individually using fecal samples from six healthy donors over 48 hours. Samples were collected at 0, 2, 4, 8, 24 and 48 h and analysed using ultra-high-performance liquid chromatography coupled with high-resolution tandem mass spectrometry (UHPLC-HRMS/MS), enabling the identification and quantification of both parent phenolics and their transformation products. The in vitro digestion and colonic fermentation markedly affected the fig polyphenol content. While after the intestinal phase, the total polyphenol content remained relatively stable compared to the initial fig sample, a deep biotransformation of native compounds was observed after 48 h of fecal incubation. UHPLC-HRMS/MS analysis revealed a time-dependent shift from complex flavonoids to simpler phenolics. Notably, a high degree of inter-individual variability was observed among the six donors, particularly regarding the kinetics of metabolite formation and the specific metabolic profiles generated. These findings demonstrated that although the overall quantity of polyphenols was preserved during digestion, figs polyphenols underwent substantial colonic transformation, generating diverse potentially more bioactive metabolites. The prevalence of hydroxyphenylacetic acids and hydroxybenzoic acids as major fermentation products suggests they may be the key drivers of the potential health effects associated to fig consumption. Moreover, the observed inter-individual variability suggests that the health benefits of fig consumption may be modulated by the specific composition of the individual´s gut microbiota.

References:
[1] Alzahrani, et al., (2024). Food Safety and Health, 2(2), 179-195.
[2] Williamson, G. (2025). Annual review of food science and technology, 16(1), 315-332.
[3] Brodkorb, et al., (2019). Nature protocols, 14(4), 991-1014.

Keywords

Fig, in vitro fermentation, gut microbiota, phenolic catabolites, inter-individual variability, phenolic

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Topic: Role of Gut Microbiota in Digestion

EXPLORING THE ASSOCIATIONS BETWEEN FIG PHENOLIC-DERIVED METABOLITES, MICROBIOTA COMPOSITION, AND SHORT-CHAIN FATTY ACIDS

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Abstract

Dietary intake of polyphenols, particularly from fruits like fig (Ficus carica L.), has been linked to various health benefits mediated by the gut microbiota1. While figs are rich in flavonoids and phenolic acids, many of these compounds reach the colon unchanged, where they undergo extensive microbial derived transformations, generating metabolites with potential health relevance. Simultaneously, gut bacteria ferment dietary components into short-chain fatty acids (SCFAs), key molecules involved in gut and systemic health. This study aimed to investigate the complex interplay between fig-derived phenolic metabolites, gut microbiota composition and SCFAs production. In vitro fecal fermentations were performed individually using fecal samples from six healthy donors, each incubated with the digested fig sample over 0-48 h. Phenolic acids were analysed using UHPLC-HRMS/MS, microbial communities were assessed by Nanopore-based 16S rRNA gene sequencing 2 and SCFAs measured by gas chromatography-flame ionization detector (GC-FID)3. Our results revealed specific associations between phenolic metabolites and bacterial genera. Indeed, catechin and paeonol were positively correlated with Bifidobacterium and negatively correlated with Lacrimispora. Additionally, epicatechin, gallic acid, apigenin-7-O-glucoside, quercetin, and paeonol were also negatively associated with Lacrimispora, while quercetin-3-arabinoside displayed a negative correlation with Desulfovibrio. After 48 h, a marked shift toward beneficial genera, such as Bifidobacterium and Faecalibacterium, was observed, alongside a reduction in potentially pathogenic taxa like Desulfovibrio and Lacrimispora. Notably, Bifidobacterium was positively correlated with acetic, propionic, and butyric acids, whereas Lacrimispora showed negative correlations with these metabolites. In conclusion, correlations between fig phenolic degradation, specific microbial enrichment, and SCFA production suggest a functional modulation of the gut environment. However, the extent of these metabolic benefits likely depends on inter-individual variability.

References:
1 Cui et al., (2019). Comprehensive Reviews in Food Science and Food Safety, 18(5), 1514-1532.
2 Curry et al., (2022). Nature Methods, 19, 845-853.
3Baenas, et al. (2020). LWT, 134, 110140.

Keywords

phenolic-derived metabolites, in vitro fermentation, gut microbiota, short-chain fatty acids, inter-individual vari

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Topic: Role of Gut Microbiota in Digestion

GUT-SPECIFIC CLOSTRIDIA CONVERT LINOLEIC ACID INTO POLYUNSATURATED FATTY ACIDS THAT ACT AS BUILDING BLOCKS FOR LIPID SYNTHESIS

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Abstract

During digestion, approximately 5-10% of dietary lipids reach the large intestine, where they interact with gut microbes. However, the interaction between lipids and the gut microbiota has remained largely unexplored. To address this gap, we investigated the interaction between linoleic acid (LA), a prevalent dietary polyunsaturated fatty acid (PUFA), and the human gut microbiota, focusing on how LA influences microbial composition and metabolism. We hypothesized that LA selects for several microbial species that cooperate to produce fatty acid units, which are then used to synthesize complex microbial lipids.

To test our hypothesis, we combined untargeted lipidomics, metagenomics, and isotopically labeled linoleic acid to study the microbial lipids resulting from interactions between gut microbiota and LA. First, we used static bioreactors and human feces to identify the gut microbes responsible for producing microbial lipids and their associated metabolic pathways and microbial lipids. Secondly, we employed a simulator of the human intestinal microbial ecosystem (SHIME) and human stools to validate our findings.

Our findings reveal that LA modulates microbial composition and its metabolic activities. By integrating isotope-tracking lipidomics and metagenomics, we identified that specific gut microbes actively metabolize LA, incorporating it into complex lipids and producing bioactive PUFAs, such as eicosapentaenoic acid. The administration of LA shifted the composition of the gut microbiota, with species within the Clostridia class becoming more prevalent. This shift occurred as LA activated its fatty acid metabolic pathways, generating a diverse array of microbial lipids, primarily fatty acids, phospholipids, and sphingolipids. Long-term in vitro feeding using SHIME corroborated these observations, demonstrating that bioactive long-chain PUFAs, such as linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid, were synthesized shortly after LA exposure. These findings were validated in vivo. In humans, the relative abundance of Clostridia significantly increased in the stools of participants who consumed a high proportion of unsaturated fat compared to saturated fat. In addition, a higher intake of unsaturated fats significantly increased linolenic acid and eicosapentaenoic acid levels in stools.

Given their potential impact on health and disease at both the intestinal and systemic levels, we envision targeting microbial lipids as markers of gut health.

Keywords

Dietary lipids, Gut microbiota, Microbial lipids, Lipidomics, Gut Health

Topic: Role of Gut Microbiota in Digestion

RIPENING-DRIVEN PROTEOLYSIS SHAPES COLONIC FERMENTATION PATTERNS OF A SEMI-HARD CHEESE AFTER SIMULATED DIGESTION

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Abstract

Cheese ripening is a dynamic process that defines flavour and texture development while promoting the gradual formation and transformation of peptides within the protein matrix. The degree of ripening determines the accumulation and nature of these proteolytic products, potentially influencing the fractions available for colonic fermentation. Although the biochemical changes in cheese during ripening have been extensively studied, the impact of these changes on gastrointestinal digestion and subsequent colonic fermentation remains incompletely understood.

In this study, semi-hard cheeses produced in independent dairies and aged for 2, 6, 12, and 18 months were subjected to static INFOGEST digestion. Then, the residual fraction was subjected to *in vitro* colonic fermentation with a pooled human faecal microbiota. Microbial composition was analysed by metagenomics, and short- and branched-chain fatty acids were quantified. All cheese digests significantly altered the microbial community compared to the control. An increase in *Bifidobacterium* was observed, suggesting a bifidogenic effect of the matrix, particularly in less-ripened cheeses (2-6 months). In contrast, longer-aged samples (12-18 months) were associated with less diverse communities and a relative enrichment of *Enterobacteriaceae*, likely reflecting differences in ripening progression. Notably, *Akkermansia*, widely regarded as a marker of intestinal ecological balance, showed a consistent increase compared with the control, further supporting the modulatory potential of cheese digestion residues. Short-chain fatty acid-producing genera were also enriched. Acetate remained the predominant metabolite (40-45 mM), whereas butyrate concentrations were consistently higher in cheese-fermented samples (approximately 22 mM) than in the control (around 14 mM). Branched-chain fatty acid production was moderately enhanced compared with the control, reflecting the matrix's protein-rich nature. Along with the increase in short-chain fatty acids, these levels are consistent with balanced proteolytic fermentation rather than an excessive metabolic shift.

These findings indicate that the ripening stage is not merely a determinant of sensory quality, but a technological parameter capable of steering colonic microbial ecology and metabolic output.

Topic: Role of Gut Microbiota in Digestion

CAN SUSTAINABLE AND INNOVATIVE POLYSACCHARIDES ALSO ENHANCE PREBIOTIC POTENTIAL?

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Abstract

Polysaccharides (PS) are widely used to improve food quality, texture, mouthfeel, and flavour by acting as thickeners, stabilisers, texturisers, and gelling agents. Beyond these technological roles, dietary PS that escape digestion in the upper gastrointestinal tract can reach the colon and be selectively utilised by host microorganisms, a core criterion of the modern prebiotic concept (Gibson et al., 2017). Resistant starch (RS) and chitosan-derived oligosaccharides (COS) are two structurally distinct, yet conceptually aligned, microbiota-accessible carbohydrates with potential to modulate microbial ecology and fermentation outputs.

In this presentation, we propose two pilot studies to evaluate sustainable and innovative strategies to enhance the prebiotic properties of foods. i) Type 4 resistant starch (RS4) was modified using plasma-activated water (PAW), annealing (ANN), or their combined treatment (PAW+ANN), with untreated RS4 as a control. PAW (acidic pH and reactive species) and ANN (increased gelatinisation temperatures and narrowed gelatinisation range) were applied to induce structural changes potentially affecting starch accessibility and fermentability (Gebremical et al., 2024). Our results indicate that treated samples, especially PAW+ANN, can positively modulate the intestinal microbiome. These observations are consistent with reports that RS fermentation favours acetate/butyrate production and shifts taxa such as Ruminococcus and Lachnospiraceae (Klostermann et al., 2024).

ii) In the second pilot study, we assessed commercial chitosan (CHIC), a sustainable chitosan obtained from seafood waste (CHIW), and hydroxypropyl methylcellulose (HPMC), using fructooligosaccharides (FOS) as a prebiotic positive control. CHIC and CHIW were associated with enrichment of beneficial taxa (ruminococci, lactobacilli, and bifidobacteria) and increased production of key SCFAs, notably butyrate and propionate. COS effects depend on physicochemical features (e.g., degree of deacetylation and molecular weight) and have been linked to selective stimulation of beneficial taxa, SCFA modulation, and barrier-supporting functions (Edo et al., 2025).

Together, these findings support RS and COS as complementary prebiotic polysaccharides capable of promoting beneficial saccharolytic communities and SCFA profiles, particularly butyrate, while, in some settings, reducing signatures of undesirable proteolytic/pathobiont taxa. Overall, these findings highlight a promising route to combine environmentally friendly processing with sustainable biopolymers to design functional foods with enhanced prebiotic potential.

Topic: Role of Gut Microbiota in Digestion

ARTISANAL COLONIAL CHEESE-DERIVED LAB SHOW ENHANCED INFOGEST SURVIVAL AFTER FREEZE-DRYING IN AN INULIN–RICE PROTEIN MATRIX

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Abstract

Artisanal Colonial cheese (ACC; queijo colonial artesanal, QCA) from Southern Brazil harbors autochthonous lactic acid bacteria (LAB) adapted to stressful processing conditions, representing a valuable source of technologically resilient cultures for future plant-based fermentations. In this study, LAB were isolated from 30 ACC samples (≈20 d ripening; Rio Grande do Sul, Santa Catarina, and Paraná; n=10/state) and ten selected isolates (Lactobacillus plantarum PR103, RS290, SC043, SC421, SC451; Lactobacillus paracasei PR165, RS191, RS267, SC033; and Lactobacillus brevis SC044) were evaluated as free cells or after freeze-drying in a plant-derived protective matrix (inulin:rice protein isolate, 1:1 w/w). Cell suspensions (~9 log CFU mL−1) were frozen (−80 °C) and lyophilized for 24 h (15 Pa; −55 °C) to aw 0.20–0.25, and powders were stored at 4 °C. Survival was assessed using the standardized INFOGEST in vitro gastrointestinal digestion protocol, applying a two-stage simulation (gastric: pH 3.0, pepsin 2000 U mL−1, 2 h; intestinal: pH 7.0, bile salts 10 mM, pancreatin with trypsin activity 100 U mL−1, 2 h; 37 °C) followed by MRS plate counts (log CFU g−1). Reductions are reported as Δlog (initial minus phase count). The gastric phase caused the main losses: free cells showed Δlog 0.26–1.76 (mean 1.30), while lyophilized cells showed Δlog 0.01–1.26 (mean 0.78). After the intestinal phase, cumulative reductions increased to Δlog 0.67–2.49 (mean 1.89) for free cells and Δlog 0.02–1.48 (mean 0.95) for lyophilized cells, confirming a consistent protective effect of the inulin–rice protein matrix. The most resilient profiles were observed for L. plantarum RS290 (Δlog 0.01 gastric; 0.02 intestinal, lyophilized) and L. paracasei PR165 (Δlog 0.56 gastric; 0.09 intestinal, lyophilized). To support downstream application in plant-based fermentations, cytocompatibility was examined by exposing intermediate-differentiated Caco-2 monolayers to digested cells (2 h) and measuring MTT reduction; cell viability remained predominantly ≥70%, with no significant overall effects of treatment or strain (two-way ANOVA, $p>0.05$), except a strain-specific difference for L. plantarum SC451. Overall, ACC-derived LAB, particularly when freeze-dried with plant-derived protectants, retained high post-digestion viability and showed suitable epithelial compatibility, supporting their selection as resilient cultures for future plant-based beverage fermentation and probiotic delivery.

Keywords

Artisanal Colonial cheese; autochthonous lactic acid bacteria, plant-based fermentation, freeze drying

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Topic: Role of Gut Microbiota in Digestion

IN VITRO COLONIC FERMENTATION AND MICROBIAL TRANSFORMATION OF WILD AND COMMERCIAL AUSTRALIAN YAMS: A METABOLOMICS AND METAGENOMICS APPROACH

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Abstract

Australian yams hold cultural significance for First Nations peoples and migrant communities, yet their gut microbiome-modulating effects remain unexplored. This study integrated metabolomics with metagenomic profiling to investigate how yam origin (wild vs commercial) and processing (boiling vs roasting) influence colonic fermentation outcomes. Wild yams exhibited enrichment in condensed tannins and procyanidins, whilst leucine-rich dipeptides distinguished varieties metabolically. Commercial boiled yams generated the highest butyrate (65.3 mM) through an increase in Bifidobacterium (27%) and Bacillota (39%). Wild boiled yams produced the highest propionate (55.3 mM) via Bacteroidota (51%), dominated by Prevotellaceae. Alpha diversity analysis revealed that commercial boiled fermentation induced the greatest diversity reduction. Beta diversity explained 73.3% of compositional variance, demonstrating that processing method and fermentation time were dominant drivers of microbial community. Correlation analysis identified Megasphaera-butyrate (R^2 = 0.95), Megasphaera-valerate (R^2 = 0.96), and Segatella-propionate (R^2 = 0.74) relationships, validating syntrophic SCFA production. These findings establish Australian yams as functional foods with tunable prebiotic properties.

Keywords

wild and commercial Yam, thermal processing, in vitro digestion, metabolomics, gut microbiota

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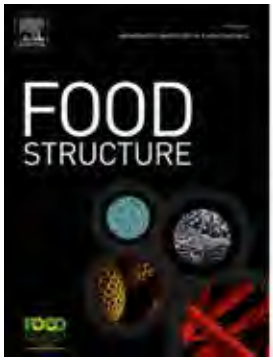

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