ICFD 2022

CORK 3-5 MAY 2022

Book of Abstracts

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INFOGEST

Welcome Address

Dear Colleagues & Friends,

On behalf of the organising and scientific committees, we are delighted to welcome you to the **7th International Conference on Food Digestion (ICFD2022)** in the city of Cork, Ireland. Due to the COVID19 pandemic, this is the first time in 3 years that we have had the opportunity to come together for ICFD.

ICFD is a major event in the field of Food, Nutrition and Health. It is organized within the framework of the INFOGEST research network (www.cost-infogest.eu) whose objective is to "improve the health properties of food by sharing our knowledge on the digestive process". INFOGEST is a global network of approximately 580 research scientists (academic and food companies) from 150 institutions across 46 countries.

This conference will primarily focus on:

- Food structures & food digestion
- *in vitro*, *in vivo* and *in silico* digestion models
- Food digestion & bioactivity
- Food processing & bioaccessibility/bioavailability of health promoting compounds
- Effect of food on gut microbiota

The team from Teagasc look forward to welcoming you to Cork. Cork is the second largest city in the Republic of Ireland, founded 14 centuries ago on islands in an estuary, where the River Lee joins the world's second-largest natural harbour. The conference venue is the Maryborough Hotel. Nestled in the leafy Cork suburb of Douglas, the Maryborough is an 18th century house hotel located 15 minutes from Cork city centre. Built on 24 acres of scenic gardens it provides an excellent location for delegates visiting the Cork region.

The cultural night and conference dinner will be held at Ballymaloe, a historic Irish country house celebrated internationally as the home of Irish country cuisine. Set deep in a 300 acre farm, their menus celebrate the relationship from farm to table by sourcing food from their own farm and producers in the locality. We look forward to enjoying an excellent Irish dinner with everyone on Wednesday evening.

ICFD2022 is a conference not to be missed. We would like to thank you for joining us this week to experience our famous Irish warmth, welcome and culture.

Dr. Linda Giblin & Dr. André Brodkorb

Chair & co-Chair of Organising Committee, Teagasc

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& our session chairs & judges

Scientific Review Panel

Dr Didier Dupont
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Professor Gail Bornhorst
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Professor Tara Grauwet
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Dr Isidra Recio
CIAL, Spain

Conference Programme

Tuesday, 3rd May 2022	
Theme 1: Food structures and food digestion	
2:00-2:15	Introduction by organisers
2:15-2:20	Introduction by chair
2:20-3:00	**Keynote: From gifts of nature to tools for food digestion design *Tara Grauwet, Belgium***
3:00-3:20	Purpose-designed encapsulation systems that delay lipolysis and reduce food intake *Meinou Corstens, Netherlands*
3:20-3:40	Spatial-temporal mapping of the intra-gastric pH, pepsin concentration and proteolysis in pigs fed egg white gels *Steven Le Feunteun, France*
3:40-4:00	Small angle scattering techniques to study the nanostructural assembly of gastrointestinal digestion products *Marta Martinez-Sanz, Spain*
4:00-4:30	Coffee break (30 min)
4:30-4:50	A multi-center peptidomic investigation of simulated gastrointestinal food digestion *Reto Portmann, Switzerland*
4:50-5:10	Impact of age-related gastrointestinal alterations on in vitro macronutrients digestibility and calcium bioaccessibility of chia seeds (Salvia Hispanica L.) *Ever Hernández-Olivas, Spain*
5:10-5:30	Gastric digestion of whey protein gels: a randomized cross-over human trial with use of MRI *Ruoxuan Deng, Netherlands*
5:30-5:50	Magnetic Resonance Imaging for investigation of in vitro digestion of a bread and cheese meal *Maja Musse, France*
5:50-6:00	Closing session
6:00-8:00	Welcome reception

Wednesday, 4th March	
Theme 2: *in vitro*, *in vivo* and *in silico* digestion models	
8:30-8:35	Introduction by chair
8:35-9:15	**Keynote: Digestible Indispensable Amino Acid Score (DIAAS) for the evaluation of protein quality *Suzanne Hodgkinson, New Zealand***
9:15-9:35	Real ileal digestibility of faba bean protein in healthy humans *Claire Gaudichon, France*
9:35-9:55	Standardization of *in vitro* digestibility and DIAAS method based on the static INFOGEST protocol *Raquel Sousa, Switzerland*
9:55-10:15	Development of a gender-based *in vitro* digestion model and its application to study digestive proteolysis of animal and plant proteins *Carolina Lajterer, Israel*
10:15-11:00	Coffee Break and **Poster Session 1**
11:00-11:20	A novel *in vitro* model of healthy infant intestinal barrier with increased permeability *Alina Kondrashina, Ireland*
Theme 3: Food digestion and bioactivity	
11:20-11:25	Introduction by chair
11:25-12:05	**Keynote: How does your gut know what you've eaten*? Fiona Gribble, UK***
12:05-12:25	Peptides produced during gastrointestinal digestion of milk and egg proteins involved in enteroendocrine cell signalling *Isidra Recio, Spain*
12:25-12:45	Role of dietary proteins in the regulation of intestinal glucose metabolism *Camille Dugardin, France*
12:45-13:05	Study the fate of mycotoxins along gastrointestinal tract using a semi-dynamic digestion model followed by a static colonic fermentation *Maria Madalena Sobral, Portugal*
1:05-2:30	Lunch Break and **Poster Session 1**
2:30-2:50	The digestion of diacylglycerol isomers by digestive lipases and its impact on the metabolic pathways for TAG re-synthesis in enterocytes *Frederic Carriere, France*

Theme 4: Food processing and bioaccessibility/ bioavailability of health promoting compounds	
2:50-2:55	Introduction by chair
2:55-3:35	**Keynote: *in vitro* bioaccessibility of polyphenolic compounds: Some of the challenges standing in the way of engineering polyphenol-rich products *Avi Shpigelman, Israel***
3:35-3:55	The mechanical disintegration of apple reduces phenolic content but improves their bioaccessibility *Marilisa Alongi, Italy*
3:55-4:15	Investigation of the effect of different processing methods on the enzyme accessibility of bound phenolic acids in the wheat bran *Yesim Karademir, UK*
4:15-5:05	Coffee break and **Poster session 2**
5:05-5:25	Protein structure within infant milk formulas impact their in vitro dynamic digestion *Amélie Delgaire, France*
5:25-5:45	Development of meat models with fiber enrichment adapted to masticatory deficiency in elderly *Véronique Santé-Lhoutellier, France*
5:45-6:05	Proximate composition, microstructure, and protein and starch digestibility of seven accessions of Jack bean with different optimal cooking times *Fiametta Purwandari, Netherlands*
6:05-6:15	Closing session
6:45	Buses leave Maryborough Hotel for gala dinner venue
7:30	Cultural night at Ballymaloe House

Thursday, 5th May 2022	
Theme 5: Effect of food on gut microbiota	
9:00-9:05	Introduction by chair
9:05-9:40	**Keynote: Impact of diet on the gut microbiota at different life stages *Karen Scott, Scotland***
9:40-10:00	Glycomics and peptidomics to discover microbiota-modulating compounds in foods *Daniela Barile, USA*
10:00-10:20	Linking carbohydrate structure with function in the human gut microbiome using hybrid metagenome assemblies *Frederick Warren, UK*
10:20-11:05	Coffee break and **Poster session 2**
11:05-11:10	Introduction by chair
11:10-11:45	**Keynote: Dairy foods and their potential to impact the gut microbiome and health *Paul Cotter, Ireland***
11:45-12:05	Development of a mucin-associated in vitro model of the toddler gut microbiome adapted to infant specific diet and colonic environment *Elora Fournier, France*
12:05-12:25	INFOGEST Update *Didier Dupont, France*
12:25-1:00	Awards and closing session
1:00-2:00	Lunch break
2:00-3:30	INFOGEST working group meetings WG1, WG5 & WG6
3:30-5:00	INFOGEST working group meetings WG2, WG3 & WG4

Oral Presentations

Keynote Speaker: Professor Tara Grauwet, KU Leuven, Belgium

From gifts of nature to tools for food digestion design

Plant-based food sources (e.g. pulses, vegetables, fruits) are not just random delivery systems of nutrients (e.g. starch, protein, micronutrients). They are gifts of nature in which nutrients are ingeniously structured. Recent insight in the structural organization of nutrients in plant-based food systems, together with understanding on how food chain variables can affect food structural characteristics has paved to way to design of food with targeted *in vitro* digestion functionalities. Using this way of thinking, food technologists of the future have potential to develop food with more tailored nutrition, physiological, and health impact. In this presentation, the process-structure-digestion relation will be discussed for a range of pulses (e.g. bean, pea, chickpea). Both the impact of intrinsic factors (i.e. pulse type and resulting cell wall properties or starch-to-protein ratios) as well as extrinsic factors (i.e. thermal processing, mechanical disintegration) on this structure-digestion function will be shown. Through a simple switch in sequence between particular processing steps (mechanical disintegration versus thermal processing), it will be proven that pulse ingredients can be developed in which natural structural organization can be kept with perspectives for development of pulse-based foods with designed *in vitro* digestion kinetics up to *in vivo* satiety responses.

Biography

Tara Grauwet obtained her PhD in Bioscience Engineering from KU Leuven, Belgium, in 2010, with her work on proteins as indicator systems for temperature uniformity mapping in high pressure processing reactors. In 2011, she became a postdoctoral researcher establishing fingerprinting and profiling approaches to study changes of processed fruits and vegetables. In this context, she performed an intersectorial secondment at Unilever R&D, Vlaardingen, The Netherlands. In October 2014, she became an assistant professor at the Department of Microbial and Molecular Systems (M²S) of KU Leuven, Belgium. Spring 2020, she has been promoted to the rank of associate professor. Tara Grauwet and her team study food digestion as influenced by processing and structure using an engineering approach relying on *in vitro* and *in silico* modelling. Currently, as a PI, her team consists of 2 post-doc, 6 PhD students and 3 master students. The team is a subpart of the Laboratory of Food Technology, KU Leuven. Tara has co-authored more than 120 international peer reviewed publications and has a comparable number of active international conference contributions (h-factor=32). Tara and her team are proud owners of several international research awards.

Purpose-designed encapsulation systems that delay lipolysis and reduce food intake

Meinou Corstens[1], Claire Berton-Carabin[1,2], Freddy Troost[3], Ad Masclee[3], Karin Schroën[1]
[1]Wageningen University and Research, Department of Agrotechnology & Food Sciences, Laboratory of Food Process Engineering, Wageningen, The Netherlands., Wageningen, Netherlands, [2]INRAE, UR1268 BIA, 44316 Nantes, France, Nantes, France, [3]Maastricht University Medical Centre, Department of Internal Medicine, division of Gastroenterology-Hepatology, Maastricht, The Netherlands., Maastricht, Netherlands

Background: Dietary lipids and digestion products are strong inducers of satiety signals in the distal small intestine. To protect lipids against proximal digestion and enable distal (ileal) release, emulsified lipids can be encapsulated in calcium-alginate hydrogel beads. The objective of this study was to investigate the efficacy of emulsion-alginate beads in reducing *in vitro* gastrointestinal (GI) digestion, feelings of satiety and food intake.
Methods: The effectiveness of emulsion-alginate beads was tested during *in vitro* GI digestion and a randomized placebo-controlled trial with cross-over design with thirty-three healthy overweight volunteers. We compared a yogurt that contained either encapsulated emulsified lipids (active) or an equicaloric mixture of non-encapsulated nutrients with similar sensory properties (control) and monitored feelings of satiety, GI symptoms, and food intake during an ad libitum pasta meal.
Results: Lipolysis in emulsion-alginate beads can be controlled through variation of bead size and cross-link density of the hydrogel, resulting in a broad range of release profile. Food intake was significantly reduced with 51±20 kcal ($p=0.016$) after intake of the active yogurt (770±38 kcal) compared to the control (821±40 kcal).
Conclusions: The use of emulsion-alginate beads reveals important clues on satiety mechanisms, and is instrumental in the development of a food-based weight-management strategy

Spatial-temporal mapping of the intra-gastric pH, pepsin concentration and proteolysis in pigs fed egg white gels

Françoise Nau[1], **Steven Le Feunteun[1]**, Yann Le Gouar[1], Gwénaële Henry[1], Maryvonne Pasco[1], Catherine Guérin-Dubiard[1], Kéra Nyemb-Diop[1], Didier Dupont[1]
[1]STLO, INRAE, Institut Agro, Rennes, France

Background: This study describes a detailed spatial-temporal mapping of the intra-gastric ingress of gastric secretions in pigs fed with egg white gels (EWGs), with special regards to the changes in pH, pepsin concentration, and proteolysis over a 6h postprandial period.
Methods: Pigs were fed with three EWGs differing in their physicochemical properties. Measurements performed in 8 gastric locations included: pH, pepsin concentration using inhibition ELISA, and proteolysis using the OPA method. Plasmatic amino-acid concentration was also measured.
Results: Acidification started in the antrum before extending over the entire stomach to reach homogeneous pH values (pH 2 to 3). Pepsin distribution never became uniform. It also started to accumulate in the antrum but turned to be most abundant in the proximal stomach beyond ~ 4h. The digestion process of the more acidic and soft gel appeared slightly different, with a soon (60 min) though temporary increase in proteolysis, an earlier peak of plasmatic amino acids, and final pepsin concentrations 3 times higher than with the two other gels.
Conclusions: This study highlights the impact of physicochemical characteristics of protein gels on the gastric digestion progress, and provide meaningful *in vivo* data on the spatial-temporal relationships between gastric pH, pepsin and proteolysis.

Small angle scattering techniques to study the nanostructural assembly of gastrointestinal digestion products

Marta Martinez-Sanz[1], Cynthia Fontes-Candia[2], Laura Díaz-Piñero[1], Isidra Recio[1], Beatriz Miralles[1], Amparo López-Rubio[2]

[1]Institute of Food Science Research, Madrid, Spain, [2]Institute of Agrochemistry and Food Technology, Paterna, Spain

Investigating the relationship between structure and functionality is extremely important for a rational design of novel food products with improved nutritional and techno-functional properties. Although the study of the digestibility and bioaccessibility of different food products is an area of active research, which has rapidly grown due to the emergence of robust *in vitro* digestion protocols, very little is known with regards to the type of structural features formed by the assembly of the digestion products through intermolecular associations or by interaction with components present in the physiological medium. In this work, we report on the nanostructures formed upon gastrointestinal digestion of different types of food systems, such as hybrid protein-polysaccharide hydrogels and polysaccharide-based emulsion gels. The samples were subjected to *in vitro* digestions following the harmonized Infogest protocol and the digestion products were characterized by small angle X-ray scattering (SAXS). Our results evidence that the digestion products can interact with the bile salts present in the intestinal phase, leading to the formation of different structures, such as lamellae, micelles and vesicles. Interestingly, the composition and structural properties of the initial gel systems have a strong effect on the digestion mechanism and determine the structural assembly of the digestion products.

A multi-center peptidomic investigation of simulated gastrointestinal food digestion

Reto Portmann[1], Pablo Jimenez Barrios[2], Isidra Recio[2], Lychou Abbuehl[1], Beatriz Miralles[2], Sedef Nehir El[3], Valerie Braird-Bion[4], Julien Jardin[4], Didier Dupont[4], Barbara Deracinois[5], Thierry Sayd[6], Laetitia Theron[6], Angéline Duval[6], Christophe Chambon[7], Ivano De Noni[8], Milda Stuknyté[8], Daniela Barile[9], Yu-Ping Huang[9], Milena Corredig[10], Dr Rubén López-Nicolás[11], Dr Trine Dalsgaard[10], Lotti Egger[1]

[1]Agroscope, 3003 Bern, Switzerland, [2]Instituto de Investigación en Ciencias de la Alimentación, CIAL CSIC, Madrid, Spain, [3]Ege University, Food Engineering Department, Izmir, Turkey, [4]INRAE-Agrocampus Ouest, , France, [5]Univ Lille, UMRt BioEcoAgro 1158-INRAE, Lille, France , [6]INRAE-Centre de rech. Clermont-Auvergne-Rhône-Alpes, St Genes Champanelle, France, [7]INRAE, Plateforme D'exploration du Métabolisme Composante Protéomique (PFEMcp), Saint Genès-Champanelle, France, [8]Dip. Sci. per gli Alimenti, la Nutrizione e l'Ambiente – DeFENS, Milano, Italy, [9]Dep. of Food Sci. & Tech., RMI North, University of California Davis, Davis, USA, [10]Department of Food Science, Aarhus University, Aarhus , Denmark, [11]Dep. of Food Sci. and Hum. Nut. Univ. de Murcia, Murcia, Spain

Background: Mass spectrometry has become the technique of choice for the simultaneous assessment of a high variety of molecules in complex food matrices. For this reason, it is best suited for precisely monitoring the evolution of digestive processes *in vivo* and *in vitro*. However, due to the variety of equipment available in different laboratories as well as settings, statistical evaluations, and interpretations, it is difficult to predict a priori the ideal parameters for best results.

Methods: The present work addressed that gap by executing an inter-laboratory study with samples collected during *in vitro* digestion and presenting an overview of the state of the art mass spectrometry applications and analytical capabilities available for studying food digestion.

Results: Three representative high-protein foods, skim milk powder, chicken breast and tofu, were digested according to the INFOGEST protocol with sample collection at five time points during gastric and intestinal digestion. Nine laboratories analyzed all digesta with their in house equipment, conventional sample treatment procedure, data evaluation methods, and graphical representation tools.

Conclusions: The compiled results demonstrate a strong consensus among labs in terms of major protein degradation for the three foods matrices and present suitable methodical and statistical approaches for representing the analytical results.

Impact of age-related gastrointestinal alterations on *in vitro* macronutrients digestibility and calcium bioaccessibility of chia seeds (Salvia Hispanica L.)

Ever Hernandez-Olivas[1], Sara Muñoz-Pina[1], Ana Andrés[1], Ana Heredia[1]
[1]*Universitat Politècnica de València, Valencia, Spain*

Impaired gastrointestinal (GI) function characteristic of aging mainly impacts protein digestion. Chia seeds may be interesting for elderly people consumption, as they are rich in high biological value proteins, healthy lipids, fiber and micronutrients. Three *in vitro* models were used focused in the impact of oral, gastric and intestinal alterations appearing with ageing (E1: altered oral conditions, E2: altered oral and gastric conditions and E3: altered oral, gastric and intestinal conditions) on chia digestibility. Samples were also subjected to a standardized GI digestion as a control (C). Altered chewing (E1) and accumulative digestive disturbances (E3) decreased proteolysis, amino acids release and lipolysis ($p<0.05$). Glycolysis and calcium bioaccessibility diminished (40 and 24%, respectively) with a decrease of pancreatic enzymes and bile secretion (E3). Advantageously, age-related disorders did not affect the ratio of essential to non-essential amino acids in the digested samples as age-related disorders appeared. However, amino acids such as valine, leucine and isoleucine, of importance for sarcopenia prevention in elders, presented a reduction of 39, 49 and 44%, respectively with suboptimal GI conditions. Such data might be helpful for developing personalized nutrition strategies employing food and subject characteristics, including nutrient-rich plant foods to mitigate sarcopenia and osteoporosis in the elderly.

Gastric digestion of whey protein gels: a randomized cross-over human trial with use of MRI

Ruoxuan Deng[1], Monica Mars[1], Anja Janssen[1], Paul Smeets[1]
[1]*Wageningen University & Research, Wageningen, Netherlands*

Magnetic resonance techniques hold great potential to bridge the gap between *in vitro* and *in vivo* digestion research. Previously, we found that MRI parameters (T_2 and T_1) can monitor the changes in protein concentration and pH during semi-dynamic *in vitro* digestion. Here, we investigated the application of these MRI parameters in a human trial. 18 healthy male adults participated in a randomized cross-over trial with gels differing in hardness and protein content: Soft-LP, Hard-LP, Hard-HP. Before and after ingesting 200 g gel and 100 g water, MRI scans and subjective appetite ratings were taken until t=85 min after the start of ingestion. At t=100 min participants ate from an ad-libitum lunch. Gastric content volume and T_2 and T_1 of the gastric content were determined from the scans. The treatments showed effects on gastric emptying rate and T_2 and T_1 values: Hard-HP < Soft-HP < Soft-LP, although not all the time points are statistically significantly different. Gastric emptying rate did not affect satiety. In summary, High protein content is the main factor in delaying gastric emptying and high hardness is an additional factor. T_2 and T_1 measurements can provide extra information on the dilution and digestion taking place in the stomach.

Magnetic Resonance Imaging for investigation of *in vitro* digestion of a bread and cheese meal

Maja Musse[1], Steven Le Feunteun[2], Guylaine Collewet[1], Mattéi Ravilly[1], Stéphane Quellec[1], Sylvain Challois[1], Martine Morzel[2], Olivia Menard[2], Francoise Nau[2], Tiphaine Lucas[1]
[1]UR OPAALE, INRAE, Rennes, France, [2]UMR STLO, INRAE, Rennes, France

Background: MRI is a highly promising non-invasive approach for both *in vivo* and *in vitro* digestion researches as it can provide information on the status and amount of water and lipid protons throughout the enzymatic breakdown of food(s). These information can be used for spatially resolved measurements of multi-scale structural features and composition of simplified or complex food products. The present study aimed to evaluate MRI for monitoring the *in vitro* digestion of a complex meal.
Method: The meal consisted of bread and cheese (24% lipids, 33% proteins, and 43% carbohydrates) added with water, and using *in vivo* realistic boli particle size distributions (range: 0-50 mm). The erosion of large particles, the hydrolysis of nutrients, as well as the creaming of lipids were studied by MRI (1.5 T) using an adapted version of the semi-dynamic gastrointestinal INFOGEST protocol.
Results and Conclusions: Combining different MRI image modalities, it was possible to investigate separately several phases of the digesta, i. e. supernatant, large cheese and bread crust pieces, and the deposit of small fragments at the bottom of the vessel. Changes in their volume, NMR relaxation parameters and lipid amount were discussed together and related to variations in pH and composition.

Keynote Speaker: Dr Suzanne Hodgkinson, Massey University, New Zealand

Digestible Indispensable Amino Acid Score (DIAAS) for the evaluation of protein quality

Digestible Indispensable Amino Acid Score (DIAAS) has been recommended by recent FAO Expert Consultations as a superior method to evaluate protein quality than Protein Digestibility Corrected Amino Acid Score (PDCAAS). The advantages and current limitations for DIAAS uptake will be discussed. Ideally DIAAS would be determined with the help of human participants. However, the collection of digesta from the end of the small intestine (terminal ileum) is required to determine true ileal amino acid digestibility (TIAAD) required for the calculation of DIAAS. Thus an animal model is needed for routine analyses and the pig has been recommended as an appropriate animal model. Validation of the pig model was necessary. This validation work was carried out as part of Project PROTEOS; with a comparison of TIAAD values determined in the ileal cannulated pig with those determined in human ileostomates. The results from this validation work supporting the use of the pig as a model animal for determining TIAAD for calculation of DIAAS will be presented.

Biography

Dr Suzanne Hodgkinson completed her BSc in physiology and biochemistry followed by an MSc in digestive physiology and a PhD in physiology at Massey University. This was followed by a Post-doctoral Fellowship funded by what was then known as the New Zealand Dairy Board. She then spent fifteen years working as an academic in the Universidad Austral de Chile in Valdivia, Chile, where she set up research programmes in dog nutrition, pig and European wild boar production and nutrition. Returning to New Zealand in 2015, she is now leading the Nutrition Team of the Riddet Institute at Massey University. Dr Hodgkinson is involved with human studies as well as the use of animal models for nutritional studies. Along with commercial studies, Dr Hodgkinson continues to research methods to determine protein quality for human diets, and protein digestion and metabolism.

Real ileal digestibility of faba bean protein in healthy humans

Suvi Itkonen[1,2], Juliane Calvez[1], Martin Chapelais[1], Nadezda Khodorova[1], Gheorghe Airinei[1], Frederick Stoddard[3], Asko Simojoki[3], Anne-Maria Pajari[2], **Claire Gaudichon[1]**

[1]Université Paris Saclay-Agroparistech-INRAE, Paris, France, [2]Department of Food and Nutrition, University of Helsinki, Helsinki, Finland, [3]Department of Agricultural Sciences, University of Helsinki, , Finland

Background: A transition towards more plant-based diets, in particular containing legumes, requires a wider knowledge on plant protein bioavailability. Considering that faba beans are cultivated in different latitudes, we aimed to assess their ileal amino acid and nitrogen digestibility, the digestible indispensable amino acid score (DIAAS) and the net postprandial protein utilization (NPPU) in healthy volunteers.
Method: Participants were equipped with a naso-ileal tube. They ingested a test meal, which consisted of dehulled, mashed faba bean (20 g protein) that was intrinsically labelled with 15N. Ileal content, plasma and urine samples were collected regularly over an 8 h postprandial period.
Results: We aim to recruit eight healthy adult subjects. Preliminary results are given for 3 volunteers, (males, aged 25-56 years). Protein digestibility of faba bean was 68%, 83% and 86% (mean 79% ± 10%). Amino acid digestibility and deamination losses will be determined to calculate DIAAS and NPPU, respectively.
Conclusions: For the first time, we determined in humans the ileal nitrogen digestibility of faba bean ingested as whole grains. Preliminary results show that digestibility is modest, but could be higher than that predicted for other legumes using the indirect dual isotope method.

Standardization of *in vitro* digestibility and DIAAS method based on the static INFOGEST protocol

Lotti Egger[1], **Raquel Sousa[1]**, Isidra Recio[2], Lychou Abbühl[1], Cédric Brügger[1], Marta Santos Hernández[2], Adam Macierzanka[4], Patrycja Szumała[4], Addepalli Rama[5], Ana Isabel Mulet-Cabero[6], Natalia Perez-Moral[6], André Brodkorb[7], Conor Fitzpatrick[7], Anne Rieder[8], Carmit Shani Levi[9], Catarina Gonçales[10], Isabel Rodriguez Amado[10], Clarisse Nobre[11], Ana C. Pinheiro[11], Elena Arranz[12], Francesca Bot[12], James A. O'Mahony[12], Myriam Grundy[13], Igor Pacheco Cruz[14], Ailynne Sepúlveda-González[14], Irene Comi[15], László Abrankó[16], Judit Tormási[16], Felipe González[17], Patricia Ruas-Madiedo[17], Lorena Ruiz[17], Miguel Ângelo Faria[18], M. Madalena C. Sobral[18], Luis Miguel Jiménez-Munoz[19], Milena Corredig[19], Luis Jimenez[19], Muleya Molly[20], Natalia Rosa-Sibakov[21], Olivia Ménard[23], Vibeke Orlien[24], Ourania Gousetti[24], Iben LykkePetersen[24], Pasquale Ferranti[25], Raquel Olias[26], Cristina Delgado-Andrade[26], Ricardo Assunção[27], Ruben Lopez Nicolas[28], Sebnem Simsek[29], Sibel Karakaya[29], Sedef Nehir E[29], Serge Rezzi[30], Stéphane Canarelli[30], Valérie Petit[31], Yunus Emre Tuncil[32], Nathalie Hotrum[22], Kerensa Broersen[22], Dominique Heimo[3], Sébastien Dubois[3], Reto Portmann[1]

[1]Agroscope, Schwarzenburgstrasse, 161, 3003 Bern, Switzerland, 3003 Bern, Switzerland, [2]Instituto de Investigación en Ciencias de la Alimentación (CIAL, CSIC-UAM), Madrid, Spain, [3]Agroscope, Tioleyre 4, 1725 Posieux, Switzerland, 1725 Posieux, Switzerland, [4]Dept. of Coll. & Lipid Sc., Univ. of Techn., Gabriela Narutowicza 11/12, 80-233 Gdansk, Poland , 80-233 Gdansk, Poland, [5]CSIRO, 306 Carmody Rd, St Lucia QLD 4067, Australia, St Lucia QLD 4067, Australia, [6]Quadram Institute Bioscience, Norwich Research Park, Norwich, NR4 7UQ, Norwich, NR4 7UQ, UK, [7]Food Proteins and Colloids, Moorepark, Fermoy, Co. Cork P61, Ireland C996 Ireland C996, Cork, Ireland C996, [8]Nofima AS—Norwegian Inst. of Food, Fisheries and Aquaculture Res., PB 210,NO-1431 Ås, Norway , NO-1431 Ås, Norway, [9]Lab. of Food Chem. and Bioactives, Technion, Israel Inst. of Techn., Israel, Haifa 32000, Haifa 32000, Israel, [10]Internat. Iberian Nanotechnology Lab., Av. Mestre José Veiga 4715-330, Braga, Portugal, Braga, Portugal, [11]Centre of Biol. Engineering, Campus de Gualtar, University of Minho 4710-057, Braga, Portugal, Braga, Portugal, [12]School of Food and Nutritional Sciences, University College Cork, T12TP07 Cork, Ireland, Cork, Ireland, [13]Research Scientist, INRAE – UMR PEGASE 1348, 35590 Saint-Gilles, France, 35590 Saint-Gilles, France, [14]Nutribreeding Lab, INTA Universidad de Chile, El Libano 5524, Macul, 7830490 Santiago, Chile, 7830490 Santiago, Chile, [15]NMBU – KBM, Ch. Falsensvei 18, 1433 Ås, Norway, 1433 Ås, Norway, [16]MATE –Univ. of Agric. & Life Sci., 35-43 Villányi str., II/209, Budapest, Hungary 1118 Hungary 1118, Budapest, Hungary , [17]Instituto. de Prod. Lácteos (IPLA-CSIC), Paseo Rio Linares s/n, 33300 Villaviciosa, Asturias, Spain, 33300 Villaviciosa, Spain, [18]Dep. de Ciências Químicas, Univ. do Porto, R. JorgeViterbo Ferreira 228, 4050-131, Porto, Portugal, Porto, Portugal, [19]Luis Munoz, Department of Food Sci., Aarhus University, Agro Food Park 48, DK-8200 Aarhus N, DK-8200 Aarhus N, Denmark, [20]School of Bioscience, Univ. of Nottingham, Loughborough, Leicestershire LE12 5RD, UK, Leicestershire LE12 5RD, UK, [21]VTT Technical Res. Ctr. of Finland Ltd, Tietotie 2, P.O. Box 1000, FI-02044 VTT, Finland, , Finland, [22]Kernhemseweg 2, 6718 ZB EDE, The Netherlands, 6718 ZB EDE, The Netherlands, [23]UMR STLO INRAE, 65 rue de Saint Brieuc, 35042 Rennes Cedex – France , 35042 Rennes Cedex, France, [24]Univ. of Copenhagen, Dept. of Food Sci., DK-1958 Frederiksberg C, Denmark, DK-1958 Frederiksberg C, Denmark, [25]Dip. di Agraria, Università di Napoli, Parco Gussone, Ed. 84, Portici (NA) Italy, I-80055, Napoli, Italy, [26]Estación Experimental del Zaidín (EEZ-CSIC), Granada, Spain, Granada, Spain, [27]INSA Dept. de Alimentação e Nutrição, Av. Padre Cruz, 1649-016 Lisboa, Portugal, Cruz, Lisboa, Portugal, [28]Dpt. of Food Sci. & Hum Nut., Edf. Vitalis-PLEIADES, Campus Espinardo, 30100-Murcia, 30100-Murcia, Spain, [29]Department of Food Engineering, Faculty of Engineering, Ege University, 35100 Izmir, Turkey, 35100 Izmir, Turkey, [30]Institut Suisse des Vitamines, Route de la Corniche 1, 1066 Epalinges, Suisse, 1066 Epalinges, Switzerland, [31]Nestlé Res. Center, R. du Jorat 55, Vers-chez-les Blanc, 1000 Lausanne 26, Switzerland, 1000 Lausanne 26, Switzerland, [32]Food Engineering Department, Necmettin Erbakan University Konya, Turkey, Konya, Turkey

Background: The FAO recommends the digestible indispensable amino acid score (DIAAS) as the measure for protein quality, for which the true ileal digestibility needs to be assessed in humans or pigs. However, due to high costs and ethical concerns, the FAO strongly encourages as well the development of validated *in vitro* methods, which complement the *in vivo* experiments.
Method: Recently, an *in vitro* workflow, based on the validated static INFOGEST protocol, was developed and compared towards *in vivo* data. In parallel to the validation with *in vivo* data, the repeatability and reproducibility of the *in vitro* protocol were tested in an international ring trial (RT) with the aim to establish an international ISO standard method within the International Dairy Federation (IDF). Five different dairy products (skim milk powder, whole milk powder, whey protein isolate, yoghurt, and cheese) were analyzed in 32 different laboratories from 18 different countries, across 4 continents.
Results: *in vitro* protein digestibilities based on Nitrogen, free R-NH2, and total amino acids as well as DIAAS values were calculated and compared to *in vivo* data, where available.
Conclusion: The *in vitro* method is suited for quantification of digestibility and will be further implemented to other food matrices.

Development of a gender-based *in vitro* digestion model and its application to study digestive proteolysis of animal and plant proteins

Uri Lesmes[1], **Carolina Lajterer**, Carmit Shani Levi
[1]*Technion, Israel*

Clinical studies demonstrate gender differences in gastrointestinal physiology may delineate differences in the pharmacokinetics of some drugs. However, this notion has not been systematically explored in food research. This study aimed to underpin gender-based differences in GIT functions and apply them in a new gender specific *in vitro* digestion (IVD) model for studying digestive proteolysis.
First, we analyzed 40 different clinical trials to identify the physicochemical parameters that could enable recreating the unique GI conditions of healthy adult males and females. This enabled extraction of key parameters, e.g. salivary composition, gastric pH gradients, emptying rates and levels of enzymes and bile acids. Second, these parameters were programmed into BioXpert software to generate a dual bioreactor-based IVD model mimicking the gastro-intestine of a male or a female. Last, we applied this model to study the differential proteolysis of whey and soy proteins. SDS-PAGE and LC-MS proteomic analyses of bioaccessible peptides liberated from beta-lactoglobulin, alpha-lactalbumin and lactoferrin highlight differences in the breakdown patterns. Thus, this work introduces a new IVD model extending our ability to study gender-based differences in the digestive fate of foods alongside new evidence into the digestion of different protein sources in our diet.

A novel *in vitro* model of healthy infant intestinal barrier with increased permeability

Alina Kondrashina[1], Andre Brodkorb[1], Linda Giblin[1]
[1]*Teagasc Food Research Centre, Moorepark, Fermoy,, Ireland*

Currently cell-based intestinal barrier models represent the adult with fully developed monolayer integrity. However in infants, intercellular connections are not mature allowing absorption of large proteins. Robust models with higher permeability that represent the newborn are required to study nutrient and drug absorption at this life stage.
Differentiated 20 day Caco-2 monolayers were treated with sodium butyrate at various concentrations (0–250 mM) for 24 h. The resulting barriers were assessed by monitoring monolayer integrity, cytotoxicity, permeability and inflammatory response.
Caco-2 monolayers treated with 125 mM sodium butyrate created a healthy barrier with a stable transepithelial electrical resistance of 408±52 Ω×cm2. The ratio of lactulose to mannitol transport across this modified barrier increased 1.79-fold indicating higher permeability than untreated monolayers. The barrier demonstrated transport rates of 0.01-0.06% β-lactoglobulin that is similar to rates reported in newborns. Immunofluorescence revealed relocation of tight junction proteins, occludin and ZO-1, away from cell junctions. Cytokines, IL-6 and Tumour Necrosis Factor-α, modestly increased but levels did not suggest an inflammatory response.
We have created an *in vitro* leaky but healthy gut barrier resembling a newborn baby. For Infant formula manufactures, this *in vitro* model will allow testing of infant formula for promotion of gut maturity.

Keynote Speaker: Professor Fiona Gribble, University of Cambridge, UK

How does your gut know what you've eaten?

The gut epithelium is home to a population of sensory endocrine cells producing hormones that signal locally within the gut and distantly at tissues such as the brain and pancreas, sending messages about the quality and quantity of the food we eat. In the field of diabetes and obesity, the best studied enteroendocrine hormone is Glucagon-like peptide-1 (GLP-1), which has been exploited therapeutically for the treatment of type 2 diabetes and obesity through the development of GLP-1 receptor agonists and DPP4 inhibitors.
Our research is focussed on understanding the physiology of the enteroendocrine system, how it detects stimuli in the gut lumen, and its involvement in the control of metabolism and food intake. We use a variety of techniques to investigate chemosensory mechanisms in enteroendocrine cells, including live cell imaging, electrophysiology and transcriptomics, and have shown that electrogenic glucose uptake and activation of a range of G-protein coupled receptors can underlie sensing of different intestinal components in the post-prandial state. We aim that our research will identify new drugs for type 2 diabetes and obesity that act by targeting gut endocrine cells, thus mimicking the gut endocrine consequences of bariatric surgery.

Biography

Fiona Gribble is Professor of Endocrine Physiology at the University of Cambridge, Director of Postgraduate Education for the School of Clinical Medicine, and an Honorary Consultant at Addenbrooke's Hospital. She runs a joint research laboratory with Dr Frank Reimann based in the Institute of Metabolic Science, funded largely by the Wellcome Trust and MRC. The group focusses on identifying signalling pathways in the gut-brain-pancreatic axis that could be exploited to develop new drugs that modulate the gut hormone axis for the treatment of diabetes and obesity.

Peptides produced during gastrointestinal digestion of milk and egg proteins involved in enteroendocrine cell signalling

Santiaga-Maria Vivanco-Maroto[1], Marta Santos-Hernández[1], Beatriz Miralles[1], **Isidra Recio[1]**
[1]CSIC, Madrid, Spain

Enteroendocrine cells are capable of sensing luminal contents due to the existence of nutrient-specific receptors on their apical side. These cells secrete a variety of hormones, such as cholecystokinin (CCK) and glucagon like peptide-1 (GLP-1), involved in the control of digestion, food intake and glucose metabolism. We had identified peptides with MW > 500 Da, but not free amino acids, as main inducers of GLP-1 secretion in enteroendocrine STC-1 cells. However, little is known about the structural peptide characteristics required to induce gastrointestinal hormone secretion and the G-protein coupled receptors (GPCRs) involved in this intestinal signalling. Our aim was to evaluate milk- and egg white- peptides generated during gastrointestinal digestion as hormone inducers in STC-1 cells, and to evaluate the involvement of the calcium-sensing receptor (CaSR) and G-protein coupled receptor-93 (GPR93) as potential receptors concerned in the molecular signalling of these peptides. Our results show the key role of the amino acidic sequence on CCK and GLP-1 secretion, as the removal or substitution of one amino acid significantly modifies the secretagogue effect. The involvement of CaSR and GPR93 was demonstrated by the use of specific inhibitors but our results pointed to the involvement of additional receptors and/or transporters.

Role of dietary proteins in the regulation of intestinal glucose metabolism

Camille Dugardin[1], Léa Fleury[1], Véronique Touche[2], Farah Ahdach[1], Jean Lesage[3], Mathie Tenenbaum[1], Nadia Everaert[1,4], Olivier Briand[2], Sophie Lestavel[2], Rozenn Ravallec[1], Benoit Cudennec[1]

[1]Univ. Lille, Univ. Artois, Université de Liège, UMRT 1158 BioEcoAgro – Bénéfice santé d'hydrolysats de protéines et coproduits agro-alimentaires, Lille, France, [2]Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011- EGID, Lille, France, [3]Univ. Lille, Inserm, CHU Lille, U1286 - INFINITE, Lille, France, [4]Animal and Human Health Engineering, Department of Biosystems, KU Leuven, Heverlee, Belgium

Background: Several studies conducted in rodents and humans have demonstrated that high protein diets improve glucose homeostasis. Nevertheless, the mechanisms underlying this effect remain elusive. The aim of the present study is thus to investigate the role of dietary proteins in the acute regulation of intestinal glucose metabolism.
Method: To explore this hypothesis, several dietary proteins from various sources were selected and digested thanks to the INFOGEST static gastrointestinal digestion protocol. The digested proteins were thus tested for their ability to modulate DPP-IV activity but also intestinal glucose absorption using *in vitro* and *ex vivo* models. Wistar rats were then gavaged with these proteins and OGTT was performed. DPP-IV activity was also measured in gavaged rat's plasma.
Results: The digested proteins were able to decrease intestinal glucose absorption and inhibit DPP-IV activity *in vitro* and *ex vivo*. Moreover, acute ingestion of casein and fish gelatin led to improve glucose tolerance in rats without significant effect on insulin secretion. In parallel, protein ingestion also decreased DPP-IV activity in plasma.
Conclusions: These results strengthen the evidence that proteins and peptides generated by their digestion are key regulators of intestinal glucose homeostasis and highlight their role in intestinal glucose absorption.

Study the fate of mycotoxins along gastrointestinal tract using a semi-dynamic digestion model followed by a static colonic fermentation

Maria Madalena Sobral[1], Tiago Gonçalves[1], Zita Martins[1], Christine Bäuerl[2], Erika Cortés-Macías[2], Maria Carmen Collado[2], Isabel Ferreira[1]
[1]LAQV/REQUIMTE – Laboratory of Bromatology and Hidrology, Faculty of Pharmacy of University of Porto, Porto, Portugal, [2]IATA-CSIC —Institute of Agrochemistry and Food Science-National Research Council (IATA-CSIC) Agustín Escardino, 7, 46980 Paterna, Valencia, Spain

Background: Aflatoxin B1 (AFB1) and Ochratoxin A (OTA) are toxic mycotoxins commonly co-occurring in foods, including cereals and dairy products. So far, mycotoxins release from stomach to duodenum and the impact of the gradual acidification on their matrix-release has not been studied.
Methods: A snack meal (1 yogurt and 2 corn cookies) was prepared, artificially contaminated (AFB1, OTA, MIX), and digested using the INFOGEST semi-dynamic digestion model, followed by a static colonic fermentation phase. Mycotoxins were quantified by HPLC-FLD in all emptying. LDH and NO measurements were taken after exposing Caco-2 cells to bioaccessible mycotoxins. Microbiota shifts were assessed after colonic fermentation of non-bioaccessible fractions.
Results: The gastric and duodenal bioaccessibility of AFB1 and OTA differed in a single or co-exposure situation: AFB1 bioaccessibility increased (16%) while OTA's decreased (20%) in the MIX meal. The bioaccessible mycotoxins impacted both intestinal cells viability and NO production, and the non-bioaccessible ones shifted microbiota patterns at phylum and family levels.
Conclusions: A single exposure to AFB1 will significantly impact intestinal viability and probiotics growth, while OTA's will mostly trigger NO production; a co-exposure situation will impact both intestinal viability and NO production, but the impact on probiotics growth will be neglected.

The digestion of diacylglycerol isomers by digestive lipases and its impact on the metabolic pathways for TAG re-synthesis in enterocytes

Frederic Carriere[1], Jean-Claude Bakala-N'Goma[1], Leslie Couedëlo[2], Carole Vaysse[2], Marion Letisse[3], Veronique Pierre[3], Alain Geloen[3], Marie-Caroline Michalski[3], Michel Lagarde[3], Jean-David Leao[2]

[1]CNRS, Aix-marseille University, Marseille, France, [2]ITERG, Canejan, France, [3]University of Lyon, CarMeN laboratory, Villeurbanne, France

Specific activities of digestive lipases were measured using rapeseed oil triacylglycerols (TAG), purified 1,3-sn-diacylglycerols (DAG) and 1,2(2,3)-sn-DAG, and a DAGOIL containing 40 % w/w DAG. Gastric lipase was more active on 1,3-sn-DAG than on 1,2(2,3)-sn-DAG and TAG, whereas pancreatic lipase displayed a reverse selectivity with a higher activity on TAG than on DAG taken as initial substrates. However, in both cases, the highest activities were displayed on DAGOIL. Thus, DAG mixed with TAG is a better substrate for lipases than TAG. The intestinal absorption of the same acylglycerols was investigated in rats with mesenteric lymph duct cannulation. The levels of TAG synthesized in the intestine and total fatty acid concentration in lymph were not different when rats were fed identical amounts of TAG, 1,2(2,3)-sn-DAG, 1,3-sn-DAG or DAGOIL. Since the lipolysis of 1,3-sn-DAG leads to glycerol, the re-synthesis of TAG in the enterocytes can therefore entirely occur through the "glycerol-3-phosphate (G3P)" pathway, with the same efficiency as the 2-sn-MAG pathway predominantly involved in the intestinal absorption of TAG. Depending on their structure, 1,2(2,3)-sn-DAG versus 1,3-sn-DAG, DAG may control the pathway (2-sn-MAG or G3P) by which TAG are re-synthesized in the enterocytes and this may explain some metabolic effects of DAG.

Keynote Speaker: Associate Professor Avi Shpigelman, Technion, Israel

in vitro bioaccessibility of polyphenolic compounds: Some of the challenges standing in the way of engineering polyphenol-rich products

Polyphenols are secondary metabolites widely distributed in vegetables and fruits. In addition to sensorial aspects, they are known for their antioxidant, anti-inflammatory, and even direct action on cellular activities, therefore often perceived and considered as health-promoting compounds.
The diversity in the chemical structure of polyphenolic compounds also results in a range of stabilities and capacities to covalently and non-covalently interact with small and macromolecules. The interactions, in addition to the stability of the polyphenolic compounds, are also strongly affected by traditional and novel processing technologies and formulations, resulting in an array of effects and outcomes on bioaccessibility.
During the talk, building on the vast published literature regarding the bioavailability and bioaccessibility of polyphenols, we will discuss the gaps in our understanding of the impact of polyphenolic structure and food processing on bioaccessibility, and especially the way we study it. This will be presented by results from complex real foods and the most simplified models of purified compounds. Questions regarding the commonly used *in vitro* method with respect to levels of oxygen and bile used will also be presented focusing on possible under- and overestimation of the impact of processing and formulation on the potential health-promoting capacity of those components.

Biography

From 2014 Assoc. Prof. Shpigelman is the head of the Laboratory for Novel Food and Bioprocessing in the Faculty of Biotechnology and Food Engineering, Technion, Haifa, Israel. Avi completed all of his degrees in the Technion, followed by a post-doctoral stay in KU Leuven, Belgium. Avi's research focuses on the complex interplay between processing, the structure of food components, their behavior during the shelf life of the product, and finally their bioactivity in our body. The idea that drives the research is that the design of food processing should carefully balance all various aspects important to our health and well-being. Currently, the main areas of activity are novel protein sources and the effects of processing on the possible health-promoting capacity of foods, especially focusing on high pressure processing as an alternative non-thermal processing technology and polyphenolic compounds, focusing on their stability and bioaccessibility.

The mechanical disintegration of apple reduces phenolic content but improves their bioaccessibility

Marilisa Alongi[1], Lara Manzocco[1], Monica Anese[1], Andrea Gorassini[1], Giancarlo Verardo[1], Maria Cristina Nicoli[1]
[1]*University of Udine, Italy*

Background. Apples are widely consumed worldwide and the expression "an apple a day keeps the doctor away" probably contributes to their success. The health-promoting potential of apple is attributed to polyphenols. Although one third of apples is consumed as derivatives, little is known about the effect of processing on polyphenols and on their fate upon digestion. This study investigated the effect of apple disintegration on phenolic concentration and bioaccessibility.
Methods. Apple puree (P) and homogenate (H) were obtained upon high-speed (HSH) and high-pressure (HPH) homogenization and characterized for some physical properties. Whole apple (A), P, and H were *in vitro* digested. Polyphenols were identified and quantified in undigested and digested samples, and their bioaccessibility was computed.
Results. HSH induced cell detachment without affecting cell integrity, whereas HPH also damaged cells. Nevertheless, polyphenols decreased by less than 20% in both cases, regardless of the intensity of matrix disruption. Moreover, both HSH and HPH increased phenolic bioaccessibility.
Conclusion. Although HSH and HPH produced apple derivatives with different physical properties, they only caused a moderate decrease in phenolic content while even increasing their bioaccessibility. These derivatives may offer a more convenient way to consume apple while maintaining or even boosting its potential bioactivity

Investigation of the effect of different processing methods on the enzyme accessibility of bound phenolic acids in the wheat bran

Yesim Karademir[1], Alan Mackie[1]
[1]*University of Leeds, Leeds, United Kingdom*

Wheat contains substantial amounts of phenolic acids; particularly ferulic acid (FA) which is concentrated mainly in the bran (up to 14.56 mg/g). Because of low bioaccessibility, approximately 95% of this FA could reach to the colon where it may act as a natural antioxidant for epithelial cells after the action of bacterial enzymes (cinnamoyl esterase, xylanase and FA esterase). This study aimed to investigate the effect of different processing methods (particle size reduction, high pressure homogenization, microwave, ultrasound) on the enzyme accessibility of bound FA in a complex food matrix. The enzymatic release of phenolic acids after processing were analyzed by LC-MSMS. Modification in the cell wall structure was monitored by physical characterization analyses. The phenolic acid concentrations in the coarse wheat bran were 5581; 658; 121; 13 ug/g for ferulic; sinapic, coumaric and chlorogenic acid, respectively. The most efficient treatment to increase enzymatic release of phenolic acids was found to be high pressure homogenization (646 ug/g), followed by microwave (530 ug/g) and ultrasound (440 ug/g). No significant effect of different parameters (time, temperature, amplitude) was observed. These results highlight the benefit of using appropriate processing methods to increase enzymatic bioaccessibility of phenolic acids in the cereals by colonic microbiota.

Protein structure within infant milk formulas impact their *in vitro* dynamic digestion

Lucile Chauvet[1,2,3], Olivia Ménard[1], Yann Le Gouar[1], Julien Jardin[1], Marie Hennetier[4], Marieke Van Audenhaege[3], Thomas Croguennec[1], Didier Dupont[1], Marion Lemaire[3], **Amélie Deglaire[1]**
[1]UMR STLO, INRAE, Institut Agro-Rennes Angers, Rennes, France, [2]Institut NuMeCan, INRAE, INSERM, Université de Rennes, Saint Gilles, France, [3]SODIAAL International, Direction Recherche & Innovation, Rennes, France, [4]Université de Toulouse, Institut National Polytechnique de Toulouse – Ecole d'ingénieur de Purpan, Département Sciences Agronomique et Agroalimentaire, Toulouse, France

Infant formulas (IFs), the only adequate substitute to breastmilk, are complex matrices that require numerous ingredients and processing steps. The objective was to understand how protein structure within IFs modulates their digestive kinetics.
Four IFs (A/B/C/D), containing whey protein (WP) ingredients with different denaturation and glycation levels (A/B/C) and caseins with different structures (C/D), were subjected to infant *in vitro* dynamic digestion (DIDGI®). Digesta were regularly sampled to follow structural changes (A4F, microscopy), proteolysis (OPA, LC-MS-MS) and lipolysis (GC-MS). Data were analysed using repeated measures ANOVA.
Before digestion, lipoprotein structures were different among IFs. IF-A, characterized by higher glycated and denatured WP rates, tended to be more digested at 180min of intestinal phase than IF-C/D (degree of proteolysis +16% and lipolysis +5.2%). Peptides (protein-origin independent) appeared sooner into the gastric phase for IF-D (at 80 min vs. 180 min for IF-A/B), suggesting that the initial bigger lipoprotein structures in the matrix were less dense and more accessible to pepsin. Different bioactive peptides kinetics were also observed among IFs during digestion.
Overall, it highlights the importance of the structure of protein ingredients (WPs and caseins) selected for IFs. Further investigation will be conducted *in vivo* (mini-piglets) to complete these data.

Development of meat models with fiber enrichment adapted to masticatory deficiency in elderly

Marie-Agnès Peyron[1], Thierry Sayd[2], **Veronique Sante-Lhoutellier[2]**, Maire Agnés Peyron[1]
[1]Human Nutrition Unit, INRAE, Clermont-Ferrand, France, [2]QuaPA Unit, INRAE, Saint Genès Champanelle, France

Background: Designing foods for elderly requires enrichments to fit their nutritional needs. This work was designed to study the impact of deficient mastication of meat model foods, combined with aged digestion on nutrients bioaccessibility.
Method: Frankfurter sausages were prepared with fibers from manioc (MS) and psyllium (PS). *in vitro* food boluses were obtained with normal and deficient mastication (NM or DM) then digested in the digester (DIDGI) mimicking adult or elderly gastric conditions (Ad or E). Four oral and gastric combinations were: Ad-NM, Ad-DM, E-NM, E-DM. Boluses were characterized for physical and biochemical features. The kinetics of lipids, proteins and peptides appearance were assessed in digesta.
Results: After DM, bolus contained more large particles, PS being more disrupted, reducing peptides release in liquid phase than other sausages. E-DM conditions delayed nutrients gastric release, not reaching the threshold obtained during Ad-NM at the end of digestion. Nevertheless, psyllium enrichment tended to increase protein appearance in digesta for E-DM. Amount of peptides also increased in digesta in this group whatever the fiber type.
Conclusion: DM is clearly an aggravating factor of aged digestion in nutrient bioavailability. Designing enriched-foods fitting elderly nutritional needs requires careful consideration of oral capabilities to improve nutritional outcomes.

Proximate composition, microstructure, and protein and starch digestibility of seven accessions of Jack bean with different optimal cooking times

Fiametta Purwandari[1,2], Christien Westerbos[1], Keumwoo Lee[1], Vincenzo Fogliano[1], Edoardo Capuano[1]

[1]Wageningen University and Research, Wageningen, Netherlands, [2]Gadjah Mada University, Yogyakarta, Indonesia

Jack bean (Canavalia ensiformis) is an underutilized legume promising as an alternative protein source; however, Jack bean may develop a hard-to-cook (HTC) phenomenon, which limits its utilization and negatively impacts the environment. We investigated the impact of the degree of HTC (measured by the optimal cooking time) on proximate composition, microstructure, and digestibility of protein and starch in seven accessions of Jack bean. In addition, the effect of reduced cooking times on starch and protein digestibility was tested on one Jack bean accession. A two-phase *in vitro* digestion was performed according to the harmonized INFOGEST 2.0 protocol on isolated intact cotyledon cells. Cell microstructure was observed using CLSM after staining with calcofluor white and rhodamine B. Size, shape and microstructure of Jack bean cells were similar for all the accessions, with few big starch granules embedded in protein matrix. No correlation was found between protein and starch digestibility and the optimal cooking time/HTC degree. Therefore, it can be concluded that development of HTC did not influence protein and starch digestibility. However, reducing cooking time significantly affected starch digestibility but not protein digestibility. The present study contributes to our understanding of the effect of food processing on nutrients digestibility in legumes.

Keynote Speaker: Dr Karen Scott, University of Aberdeen, UK

Impact of diet on the gut microbiota at different life stages

Thanks to the many studies investigating the composition of the gut microbiota, we now have a clear understanding of the huge number of different bacterial species that reside in the human gut – and how they are different between every individual. These bacteria survive by degrading dietary substrates that reach the large intestine releasing microbial metabolites that can cross from the gut into the blood stream and circulate around the body. Consequently these microbial metabolites can exert effects, for better or worse, all round our body. The composition of our microbiota, and the types of foods we consume, combine to influence the types and quantities of these metabolites. Any changes introduced to the diet, whether consciously or subconsciously, can rapidly modify the composition and activity of the gut microbiota. In this talk we will explore the impact of dietary changes at different life stages (from babies to elderly individuals) on the gut microbiota, and the impact this has on the production of bacterial metabolites and the potential consequences for health.

Biography

Karen Scott is a Senior Research Fellow at the Rowett Institute, University of Aberdeen. She leads a research team investigating the (molecular) mechanisms by which key members of the gut microbiota interact with the diet and host, at different life-stages. The fermentation products of gut bacteria contribute to gut health, and are differentially expressed on different substrates, including prebiotics. *in vitro* bacterial growth studies utilising our large culture collection of gut anaerobes (in pure culture, mixed culture, fermentor systems, and also with human cells) and bioinformatic analyses illustrate niche-specific processes and bacterial interactions. Resident bacteria are also an important reservoir of transferable antimicrobial resistance genes, and other work investigates the evolution and spread of resistance from farm to fork.

Glycomics and peptidomics to discover microbiota-modulating compounds in foods

Daniela Barile[1], Bruna Paviani[1], Yu-Ping Huang[1], Gulustan Ozturk[1], Sierra Durham[1], Juliana Leite Nobrega De Moura Bell[1], David A. Mills[1], Helen Raybould[1]
[1]*University of California Davis, Davis, United States*

Background: Gut dysbiosis is a factor in various conditions linked to poor health from premature infants to adults. This presentation will discuss two mechanisms for gut microbiota modulation, via prebiotic oligosaccharides and anti-microbial peptides extracted from under-utilized food side-products.
Methods: We developed techniques for isolation of oligosaccharides and peptides using pH adjustment, enzymatic hydrolysis, fermentation, and filtration. Liquid chromatography coupled to mass spectrometry (nanoLC-QToF, LC-QQQ) allowed us to accurately and reproducibly measure glycans and peptides, thereby enabling scaling and processing decisions necessary while considering production of any novel commercial ingredient.
Results: Purified oligosaccharides and peptides from dairy streams were tested in a series of animal experiments. Oligosaccharides normalized the gut microbiota of obese mice fed high-fat diets, reversed gut permeability, and improved gut-brain signalling and appetite control. Oligosaccharides also successfully reduced inflammation and promoted microbiota-dependent lean mass gain in undernutrition models.
The peptide pool contained nearly 3000 sequences and demonstrated anti-microbial activity *in vitro* and *in vivo* (> 50% reduction in the uptake of enterohemorrhagic E. coli).
Conclusions: Novel "omics" combined with bio-guided processing enables achieving molecular-level understanding of microbiota-modulating compounds and guides the development of engineering methods to capture value from our food side-streams, improving sustainability the dairy enterprise.

Linking carbohydrate structure with function in the human gut microbiome using hybrid metagenome assemblies

Frederick Warren[1], Anuradha Ravi[1], Perla Troncoso-Rey[1], Jennifer Ahn-Jarvis[1], Andrew Page[1], Justin O'Grady[1]
[1]Quadram Institute, Norwich, United Kingdom

Complex carbohydrates that escape small intestinal digestion, are broken down in the large intestine by enzymes encoded by the gut microbiome. This is a symbiotic relationship between microbes and host, resulting in metabolic products that influence host health and are exploited by other microbes. However, the role of carbohydrate structure in directing microbiota community composition and the succession of carbohydrate-degrading microbes, is not fully understood.
In this study we evaluate species-level compositional variation within a single microbiome sample in response to six structurally distinct carbohydrates in a controlled model gut using hybrid Illumina and Oxford Nanopore metagenome assemblies. We identified 509 high-quality metagenome-assembled genomes (MAGs) belonging to ten bacterial classes and 28 bacterial families.
We found dynamic variations in the microbiome amongst carbohydrate treatments, over time. Recent advances in sequencing technology allowed us to identify significant unexplored diversity amongst starch degrading species in the human gut microbiota including CAZyme profiles for novel MAGs.

Keynote Speaker: Professor Paul Cotter, Teagasc, Ireland

Dairy foods and their potential to impact the gut microbiome and health

Dairy foods, both fermented and unfermented, have the potential to impact positively on the gut microbiota and, in turn, health. In addition to discussing this topic in general, some examples of research (*in vitro*, *ex vivo* and *in vivo*) in this area relating to (i) the relative impacts of whey versus casein, (ii) fermentates and (iii) milk kefir will be provided to highlight the potential in this continually emerging field.

Biography

Professor Paul Cotter is the Head of Food Biosciences at Teagasc and a Principal Investigator with the large Irish Research Centres, APC Microbiome Ireland, VistaMilk and Food for Health Ireland and CTO/co-founder of SeqBiome, a microbiome sequencing and bioinformatics service provider. He is a molecular microbiologist, with a particular focus on the microbiology of foods (especially fermented foods), the food chain and of humans, as well as probiotics and postbiotics. Prof Cotter is the author of >350 peer-reviewed papers, was included in the Clarivate list of highly cited researchers for 2018-2021 and is the Field Chief Editor of Frontiers in Microbiology.

Development of a mucin-associated *in vitro* model of the toddler gut microbiome adapted to infant specific diet and colonic environment

Elora Fournier[1], Sylvain Denis[1], Claude Durif[1], Muriel Mercier-Bonin[2], Prof Lucie Etienne-Mesmin[1], Stéphanie Blanquet-Diot[1]
[1]University Clermont Auvergne, MEDIS, Clermont-Ferrand, France, [2]INRAE, TOXALIM, Toulouse, France

Early life is a critical period where gut ecosystem and functions are establishing with significant impact on health. For regulatory, time and cost reasons, *in vitro* gut models are a relevant alternative to *in vivo* assays in preclinical food or pharma studies.
An exhaustive literature review was conducted to adapt the Mucosal Artificial Colon (M-ARCOL) to specific 6 months – 3 years infant physicochemical (pH, transit time and nutritional composition of ileal effluents) and microbial parameters. Fecal samples from 5 toddlers were used to inoculate the toddler M-ARCOL. Gut microbiota structure (lumen and mucus-associated microbiota) and functions (gas and short chain fatty acids -SCFAs-) were monitored and compared to toddler *in vivo* data for validation.
In accordance with *in vivo* data, toddler microbiota produces in average 99 mM SCFAs / day with 57% Acetate / 24% Propionate / 17% Butyrate profiles. 16S metabarcoding shows donor- and microenvironment- (lumen versus mucus) dependent profiles. Bacterial populations known to be more abundant during infancy are predominant *in vitro* (e.g. Akkermansiaceae, Enterobacteriaceae). Low alpha-diversity indexes are measured in accordance to toddler data. This new toddler colon model provides a powerful platform for gut microbiome mechanistic studies related to pre/probiotic, nutrient or pediatric drug evaluation.

Poster Presentations

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2	Pepsin activity as a function of pH and digestion time under static *in vitro* conditions	*Léa Salelles et al.*
3	Dry-heated plant proteins with reducing sugars and their *in vitro* infant digestion	*Jiaying Tang et al.*
4	Goat skim milk: Heating induced different protein denature degrees influence the infant digestion	*Qing Ren et al.*
5	Alpha-lactalbumin particulates for controlled delivery: Impact of dietary fibers on stability, digestibility, and gastro-intestinal release of capsaicin	*Alon Romano et al.*
6	Study of the protein quality and digestibility of innovative meat analogues products	*Tullia Tedeschi et al.*
7	The reformulation of milk with high melting point lipids and its effect on in-vitro gastrointestinal digestion.	*Conor Fitzpatrick et al.*
8	Structural changes induced by high temperature and basic pH hinder the digestibility of whey proteins	*Francesca Accardo et al.*
9	Role of cell wall integrity of wheat durum in modulating the starch digestibility during the bread processing	*Marianna Tagliasco et al.*
10	Effect of heating on clot formation and milk protein digestion during *in vitro* infant gastric digestion	*Julie Miltenburg et al.*
11	Influence of cooked wheat pasta on lipid digestion of sour cream and vegetable oil based analogue	*Judit Tormási et al.*
12	Protein digestibility of cooked wheat pasta affected by co-consumption with fatty cream toppings	*Judit Tormási et al.*
13	Formulating cellulose nanocrystal Pickering emulsions and their impact on lipid digestion	*Lin Zhang et al.*
14	Understanding the secondary structure changes of plant-based proteins under elderly in-vitro oral conditions	*Ingrid Contardo et al.*
15	Development of polysaccharide-casein gel-like structures resistant to *in vitro* gastric digestion	*Marta Martinez-Sanz et al.*
16	Infant *in vitro* semi-dynamic digestion of conventional heat-treated vs. membrane filtered infant formula with an increased native whey protein content	*Yihong Chen et al.*
17	Effect of microstructure on the *in vitro* digestion of egg white protein gels	*Alisha Kar et al.*
18	Impact of particle size and water content on particle breakdown and starch digestibility of chickpea-based snacks during *in vitro* digestion	*Weiyi Sun et al.*
19	Application of the INFOGEST 2.0 protocol to the *in vitro* digestion of concentrated emulsions: a case study on mayonnaise-like products	*Paula Kiyomi Okuro et al.*
20	Development of a prebiotic strawberry preparation for the dairy industry	*Daniela A. Gonçalves et al.*

21	Cereals and legumes co-ingestion under infant digestive conditions: starch digestibility	*Marianela Rodriguez et al.*
22	Proteolysis from different dietary sources: a comparative *in vitro* study under altered gastrointestinal conditions of senior population	*Sara Munoz Pina et al.*
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24	Impact of high protein drinks formulation on *in vitro* dynamic digestion and absorption of macronutrient in the elderly	*Stefano Nebbia et al*
25	Impact of GOS/FOS and fat origin on the macronutrients kinetics of digestion and the calcium bioaccessibility of growing-up milks	*Manon Bonnnot et al*
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28	Comparison of the *in vitro* digestibility, PDCAAS, and functionality of pea proteins from different providers.	*Angeline Riquelme et al.*
29	*In vitro* starch and protein digestion kinetics of cooked Bambara groundnuts depend on processing intensity and hardness sorting	*Shannon Gwala et al.*
30	Standardized ileal digestibility of amino acids and nitrogen in human milk and infant formula – an *in vivo* study	*Elise Charton et al.*
31	Quantifying gastric coagulation of milk proteins in humans using MRI	*Elise Van Eijnatten et al.*
32	Can nitrite-free recipes of cured meat products prevent the formation of nitroso compounds?	*Noémie Petit et al.*
33	Consequences of oral deficiencies on intestinal bioaccessibility of nutrients in elderly	*Susana Ribes et al.*
34	Impact of the addition of vegetable lecithin in oil on lipolysis rate under simulated physiological conditions	*Marion Letisse et al.*
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40	The effect of beta-glucans on yogurt disintegration during *in vitro* digestion	*Aleksandra Florczuk et al.*

41	Retardation of starch hydrolysis using psyllium mucilage hydrogels	*Lucija Strkalj et al.*
42	New insights into infant gastric digestion according to age for breastfed and formula-fed: towards a validated *in vitro* model.	*Gaetan Drouin et al.*
43	The effect of beta-glucans on yogurt texture and protein digestibility	*Marek Aljewicz et al.*
44	*In vitro* digestion of an experimental infant formula containing both intact and hydrolyzed milk proteins.	*Tim Lambers et al.*
45	How processing of plant-based shakes can impact lipid and protein microstructural organisation and digestive functionality	*Jessica Guevara et al.*
46	The interference of dietary fibre with lipolysis depends on the method of administration of β-glucans with different solubilisation processes	*Kathrin Haider et al.*
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54	Effect of low acyl gellan gum on white rice starch hydrolysis and glycaemic index: a static in-vitro digestion study	*Norah Alshammari et al.*
55	*In vitro* DIAAS of sustainable proteins using the INFOGEST digestion protocol	*Renata Ariens et al.*
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57	Development and validation of an *in vitro* colonic model of gut microbiota dysbiosis associated to obesity	*Ophélie Uriot et al.*
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61	Development, validation and application of an *in vitro* DIAAS method based on the INFOGEST protocol	*Raquel Sousa et al.*
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127	Cr content and bioaccessibility in enteral nutrition formulas: Influence of protein and fat content and contribution to Dietary Reference intake	*Javier Martínez-Castro et al.*
128	Effect of processing of infant milk formula on growth performance, protein digestibility and gut barrier physiology *in vivo*	*Cathal Dold et al.*
129	Iron, zinc and carotenoids bioaccessibility in biofortified and non-biofortified foods based on millet, cowpea and orange fleshed sweet potatoes	*Mbeugué Thiam et al.*
130	Effect of feeding and pulsed electric field on lipid and protein hydrolysis of seabass fillets subjected to *in vitro* digestion	*Giorgia Antonelli et al.*
131	Impact of *in vitro* digestion on the phytochemical profile of *Salicornia ramosissima* and *Sarcocornia fruticose*	*Juliana Oliveira et al.*
132	The effect of popping, a sustainable form of processing, on antinutritional factors in pulses	*Joseph Godrich et al.*
133	Quinoa peptides as DPP-IV inhibitors: an *in silico* approach	*Maria Lilibeth Manzanilla Valdez et al.*
134	Iron, zinc and β-carotene bioaccessibility in biofortified and non-biofortified foods based on millet, cowpea and orange fleshed sweet potatoes	*Mbeugué Thiam et al.*
135	Effect of Zn and Se biofortification on macro and microelement *in vitro* bioaccessibility in fresh wheatgrass juice	*Andrijana Rebekić et al.*
136	Bioaccessibility and cellular uptake of free carotenoids and esters from orange peel: comparison between conventional and ionic liquid mediated extractions	*Daniella Murador et al.*

1

Variation of *in vitro* digestibility of commercially available pea protein powder dispersions

Luis Jiménez-Munoz[1], Michelle Torp Nielsen[1], Laura Román[1], Milena Corredig[1]
[1]Aarhus University, Aarhus, Denmark

With raising consumer demand of plant-derived proteins, there has been an increased interest in pea ingredients. This work compared the digestion behavior of three different commercial pea protein ingredients, two protein isolates and one less refined: protein concentrate. It was hypothesized that differences in processing history and composition would affect their solubility and their breakdown during *in vitro* simulated gastrointestinal digestion. The concentrate showed greater solubility and smaller particle size than the isolates. When heat-treated, the release of free amino groups decreased for the isolates, but increased for the concentrate. Analysis by liquid chromatography showed differences in peptide distribution before digestion: the concentrate had higher presence of high molecular weight proteins. SDS-PAGE showed presence of albumin fraction PA-1 in the concentrate but not in the isolates. Moreover, the gel showed that the breakdown under gastric conditions was different between the untreated and heated concentrate. Individual free amino acids detected by LC-TQMS in the intestinal digestion indicated a significantly higher release of methionine in the concentrates than the isolates. The results demonstrate the impact of processing conditions on techno-functional, nutritional and digestive profile of diverse pea protein ingredients.

2

Pepsin activity as a function of pH and digestion time under static *in vitro* conditions

Léa Salelles[1], Juliane Floury[1], **Steven Le Feunteun[1]**
[1]STLO, INRAE, Institut Agro, Rennes, France

Background: This study aimed at investigating the activity of porcine pepsin on egg white proteins (EWP) and casein micelles (CA) over a broad range of pH (from 1 to 7) for short (3 min) and long (2 h) digestion times.
Methods: Static *in vitro* gastric digestions were conducted at different pH on both substrates. Degrees of hydrolysis (DH) were determined using the OPA and pH-stat methods.
Results: At short time, different pH activity profiles were obtained for both substrates. Remarkably, the DH of CA after 2 h was constant from pH 1 to pH 5, and only reduced by half at pH 6. This demonstrates that pepsin can hydrolyse caseins from the very beginning of gastric digestion. The shape of the kinetics over 2 h also appeared rather characteristic of the substrate and largely independent on pH. These hydrolysis profiles could be accurately fitted by a power law, an empirical model that also proved very well adapted to other data of ours.
Conclusions: Our findings suggest that pepsin activity under weakly acidic conditions (pH ≥ 4) should not always be neglected, in particular for milk caseins, and that pepsin reaction kinetics are proportional to the power of the digestion time.

3

Dry-heated plant proteins with reducing sugars and their *in vitro* infant digestion

Jiaying Tang[1], Harry Wichers[1], Kasper Hettinga[1]
[1]*Wageningen University and Research, Wageningen, Netherlands*

Introduction: Dry heating of plant proteins happens during production of plant-based infant formula. This may lead to glycation and hence altered digestion. Therefore, the aim of this study was to investigate the glycation level of plant proteins with reducing sugars after dry heating, and its effect on infant digestibility.
Method: Isolated soy and pea proteins were mixed with glucose in weight ratio 1:4. The mixtures were dry-heated at 60 ˚C for 6 and 48 h at a relative humidity of 60%. Surface hydrophobicity, particle size distribution, and glycation were studied, as well as the *in vitro* infant digestion.
Results: Soy and pea proteins that were dry-heated with glucose for 0 h to 48 h showed increased glycation and aggregation and decreased surface hydrophobicity. During gastric digestion, degree of hydrolysis decreased with increased dry-heating duration; and increased when digestion progressed. However, samples dry-heated for 48 h showed the strongest resistance to gastric digestion. When intestinal digestion progressed, the degree of hydrolysis almost remained unchanged.
Conclusion: Dry heating increased glycation and aggregation of protein-glucose mixtures and decreased the gastric digestion. Resistance to digestion was shown in the gastric phase, but not in the intestinal phase.

4

Goat skim milk: heating induced different protein denature degrees influence the infant digestion

Qing Ren[1], Harry Wichers[1], Kasper Hettinga[1]
[1]*Wageningen University, Wageningen, Netherlands*

The effects of different protein denaturation degrees on digestibility of goat skim milk were studied using an infant *in vitro* digestion model. Most previous goat milk digestion studies ignored the gastric clot composition and evaluate the digestion only based on the supernatant. In this study, however, the digestion rate was analyzed in both the supernatant and the gastric clot. After digestion, protein concentration, composition, hydrolysis, and peptidomics of the supernatant part were studied, whereas the protein composition of the gastric clots were identified by SDS-PAGE gel and LC-MS/MS. The results indicated that, compared to mild temperature heated samples (≤80°C), samples heated at 85°C showed more extensive clot formation with higher digestion rate, but resulted in a larger amount of undigested whey proteins due to severe aggregation. Mild temperature heating reduced whey protein digestion in the supernatant, while casein showed no big difference in digestion with different heat treatments. Looking at the peptidome, β-casein is the major source of bioactive peptides formed during digestion. The 65°C heated samples showed higher bioactive peptides intensity compared to the other groups. Overall, this study showed different heating temperatures induce different protein denaturation degrees which affect their digestion.

5

Alpha-lactalbumin particulates for controlled delivery: Impact of dietary fibers on stability, digestibility, and gastro-intestinal release of capsaicin

Alon Romano[1], Yizhaq Engelberg[1], Carmit Shani-Levi[1], **Uri Lesmes[1]**
[1]*Technion – Israel Institute of Technology, Haifa, Israel*

Consumer demand for functional foods rich in proteins, fiber and beneficial compounds, such as capsaicin (CAP), is constantly growing. This study hypothesized that the electrostatic biopolymer interactions between bovine alpha-lactalbumin (ALA), and ionic polysaccharides, namely alginate (ALG) or chitosan (CHI), can be harnessed to form fine particulates with improved stability, attenuated susceptibility to digestive proteolysis and controlled release of CAP. Quantitative analyses show that the addition of dietary fiber increases the encapsulation efficiency of CAP as well as enhance the particulates physical and pH stability (affirmed by various light scattering techniques). Shelf-life assessment (for 30 days) shows that the biopolymer complexation helps retain 50% of the entrapped CAP and limits protein aggregation. Semi-dynamic *in vitro* digestions highlight ALA-ALG particles attenuate ALA digestive proteolysis and CAP release under oral and gastric conditions. Further, LC-MS/MS proteomic analysis of gastric aspirates reveals polysaccharide addition does not have a marked effect on levels of bioaccessible bioactive peptides. Moreover, predictive PeptideRank bioinformatic tool helps identify potential release of possible novel bioactive peptides whose bioactivity requires further investigation. Thus, this study adds another tier to the existing body of evidence supporting protein-polysaccharide complexation as a possible avenue to develop edible delivery systems.

6

Study of the protein quality and digestibility of innovative meat analogues products

Tullia Tedeschi[1], Sara Cutroneo[1], Nicoletta Pellegrini[2], Stefano Sforza[1]
[1]Food and Drug Department, University of Parma, Italy, PARMA, Italia, [2]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy

Background: Meat analogues are plant-derived protein products that are usually processed to resemble meat flavour, texture and appearance. These products are usually perceived by consumers as healthier than meat, even though little is known about their real nutritional quality especially regarding the protein quality and digestibility. Thus, this research work is focused on the evaluation of the actual protein quality and digestibility of a selection of these new products belonged to the burger category in comparison with beef burgers.
Methods: Protein quality was evaluated by amino acids determination and the effect of the cooking process was also investigated. The INFOGEST gastro-intestinal *in vitro* static digestion procedure was applied on both the burger typologies. Digestibility was evaluated in terms of total protein solubilized and degree of hydrolysis. Peptidomic analysis of the different digestates was also approached.
Results: All burgers showed a good protein integrity and quality. In general, the essential amino acid pattern is comparable with the requirements for adults and children. The investigation of the protein digestibility showed a similar digestibility of plant-based burgers compared to meat ones in terms of degree of hydrolysis.
Conclusions: In the light of these interesting results, the study will continue by comparing other meat analogues categories.

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The reformulation of milk with high melting point lipids and its effect on *in vitro* gastrointestinal digestion.

Conor Fitzpatrick[1, 2, 3], Daniela Freitas[1], Dr Seamus O'Mahony[2], Dr André Brodkorb[1, 3], Dr Sean Hogan[1, 3]
[1]Teagasc Food Research Centre, Cork, Ireland, [2]UCC, Cork, Ireland, [3]Vistamilk, Cork, Ireland

Background: Bovine milk digestion has been shown to be affected by its lipid profile, which can be altered due to stage in lactation, feeding strategy and breed of cow. As well as this, the lipid profile of infant formula is based on vegetable oil, showing that the lipid profile of milk products can vary significantly. This study aims to examine the effects of changes to the lipid profile of bovine milk on digestion.
Method: The melting points of several lipids were analysed using diffusion scanning calorimetry. Based on these results, Milk Protein Concentrate was reformulated to create emulsions containing different ratios of high and low melting point lipids. These emulsions were digested using the INFOGEST semi-dynamic *in vitro* digestion model. Samples were taken during digestion, and digesta will be analysed for changes in proteins, lipids and rheology.
Results: Visual and microstructural observation of digesta revealed significant differences in the digestive behaviour. SDS-PAGE, soluble nitrogen and OPA assays showed the effect on protein digestions. Changes in the released fatty acid profile of the digesta correlated with the melting behaviour of the lipids.

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Structural changes induced by high temperature and basic pH hinder the digestibility of whey proteins

Francesca Accardo[1], Giulia Leni[1], Tullia Tedeschi[1], Barbara Prandi[1], Stefano Sforza[1]
[1]*Department of Food and Drug, University Of Parma, Parma, Italy*

Background: The nutritional value of dietary proteins is an essential parameter related to the quality of their constituents, the amino acids, as well as to their digestibility. During protein extraction, acid or basic environments combined with heat are commonly applied to increase the extraction yield. Through these processing regimens, proteins may undergo modifications which might hamper digestibility.
Method: Whey proteins, chosen as a model, were treated for 3 hours at five different pH values (2, 7, 9, 11, 13) and three temperature conditions (30, 60, 90°C). The main structural and chemical modifications (protein aggregation, hydrolysis, insolubilization, amino acid degradation and racemization) were evaluated in detail. *In vitro* static gastrointestinal digestion was performed for better understanding the impact of these changes on protein digestibility. The degree of protein hydrolysis and the released peptides were measured by applying different analytical techniques including LC/MS.
Results: Results showed a higher propensity for molecular modifications due to basic pH, favoured when combined with heat. Increased modifications caused a decrease in both the number of identified peptides and the degree of protein hydrolysis.
Conclusions: Basic processing could compromise protein digestion, integrity and nutritional quality as a consequence of modifications.

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Role of cell wall integrity of wheat durum in modulating the starch digestibility during the bread processing

Marianna Tagliasco[1], Maria Tecuanhuey Lopez[2], Reynard Reynard[2], Rachel Zuliani[2], Edoardo Capuano[2], Nicoletta Pellegrini[1, 2]
[1]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy, [2]Food Quality and Design Group, Wageningen University and Research, Wageningen, The Netherlands

In this study, we investigated the effect of increasing particle size, and therefore of a higher fraction of intact cells, in decreasing the starch digestibility of durum wheat flour, as well as of dough and bread obtained therefrom. Flours of small (<200µm), medium (>1000 µm <1800 µm), and large (>1800 µm) particle size were selected. Confocal laser scanning microscopy showed that the integrity of the cells wall was retained during the whole of bread processing for the medium and large particle sizes, whereas the cell walls were mostly damaged in the small particle size. The *in vitro* starch digestibility (Englyst's method) of flour was significantly affected by its particle size, the starch digestibility of small particle flour being two times higher than the large one. In the dough, no differences in starch digestibility were found between the three particle sizes. In bread, instead, a modest decrease of starch digestibility for the bread produced with large particle flour was observed, mainly due to the denser structure of the crumb. In conclusion, a higher particle size (and thus a higher fraction of intact cells) could limit starch digestibility in flour but not in bread.

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Effect of heating on clot formation and milk protein digestion during *in vitro* infant gastric digestion

Julie Miltenburg[1], Shanna Bastiaan-Net[2], Tamara Hoppenbrouwers[1, 2], Harry Wichers[2], Kasper Hettinga[1]

[1]Food Quality & Design, Wageningen University & Research, Wageningen, Netherlands, [2]Food & Biobased Research, Wageningen University & Research, Wageningen, Netherlands

Background: Heating of milk proteins can influence their gastric clot formation and protein hydrolysis. Usually only the soluble proteins are analyzed, leading to an incomplete picture of protein digestion. Therefore, we aimed to study the effect of heating on infant gastric digestion of milk proteins by analyzing both the soluble digesta and the insoluble clot.
Methods: Unheated and heated skim milk (80°C, 30 min) was digested by use of *in vitro* infant gastric digestion. The protein compositions of both the soluble digesta and clots were measured to determine protein hydrolysis. The clot structure was analyzed by confocal laser scanning microscopy.
Results: A tight clot with small pores was formed from unheated milk, whereas a looser clot with larger pores was formed from heated milk. Intact caseins were absent in soluble digesta, but were present in the clots along with intact whey proteins and peptides. Caseins were digested more slowly in the clots from heated milk than in those from unheated milk.
Conclusions: Heating leads to a looser gastric clot structure and a slower casein digestion. Intact caseins were only detected in the clots, showing that analyzing both soluble digesta and insoluble clots is needed to study gastric milk protein digestion.

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Influence of cooked wheat pasta on lipid digestion of sour cream and vegetable oil based analogue

Judit Tormási[1], **László Abrankó[1]**
[1]Hungarian University of Agriculture and Life Science (MATE), Budapest, Hungary

Digestibility of sour cream (SC) and palm oil (PO) based sour cream analogue (SCA) (both 20% lipid content) was evaluated with special focus on the co-digestion of these products with cooked pasta (CP) using the Infogest protocol. Lipolysis was measured by quantifying free fatty acids (FFA) in the small intestinal digesta by GC-FID. Total lipolysis (sum of FFAs) was higher in SCA (62%) than SC (52%), which might be explained by the added PO in SCA, which is more accessible for lipases than membrane shielded endogenous milk fat droplets of SC. FA-specific evaluation showed a significant relative decrease of MUFAs and PUFAs in the SCA digesta. This can be explained by the delayed lipolysis of sn-2 FAs that in PO are typically the unsaturated ones. When SC or SCA were co-digested with CP (1SC(A):4CP), cca. 30% increase in total lipolysis was observed for both SC and SCA and specific underrepresentation of unsaturated FAs in SCA digesta disappeared. It is hypothesised that the addition of CP helps in emulsification and removal of primarily formed saturated FFAs and allows the continuation and completion of the lipolysis. Eventually this results in similar representation of MUFAs/PUFAs in the digesta as in the product.

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Protein digestibility of cooked wheat pasta affected by co-consumption with fatty cream toppings

Judit Tormási[1], László Abrankó[1]
[1]*Hungarian University of Agriculture and Life Sciences, Budapest, Hungary*

Protein digestibility of a cooked dried pasta (CP) meal amended with 20% sour cream (SC) or vegetable oil based sour cream analogue (SCA) was studied using the Infogest protocol. Protein bioaccessibility was assessed by measuring the OPA active peptide ratio found in the precipitated small intestinal digesta. Low (49%) bioaccessibility was found for CP, which increased by 19% with the addition of SCA. On the contrary, co-digestion with SC resulted in 28% decrease. Since the key difference between SC and SCA is the type of fat they contain, co-digestion experiments of CP were performed with either milk fat (MF) or palm oil (PO). MF alone did not show any change, whereas PO slightly further increased protein digestibility. Conclusively, it is hypothesised that PO or MF affects differently the formation of coagulated milk protein gel structure. This coagulum shields pasta protein network, thus makes access harder for proteases to pasta proteins. Presence of PO, presumably forming smaller droplets than MF droplets, may result in looser milk protein coagulum compared to that of formed with MF. This difference in the tightness of milk protein coagulum may explain the better wheat protein accessibility observed with SCA than with SC.

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Formulating cellulose nanocrystal Pickering emulsions and their impact on lipid digestion

Lin Zhang[1], Balazs Bajka[1], Cecile Dreiss[1]
[1]King's College London, London, United Kingdom

Background: Understanding the process of lipid digestion and absorption and using effective ways to control it is paramount for the development of food formulation strategies to address the obesity crisis.
Objectives: To develop and characterise Pickering emulsions stabilised by cellulose nanocrystals (CNCs) or combined with other polysaccharides, including methyl cellulose (MC) and chitosan (CS), to regulate lipid digestion, using simulated *in vitro* digestion.
Methods: CNCs alone, or in combination with MC or CS were used to generate Pickering emulsions. CNCs were characterised by atomic force microscopy (AFM), dynamic light scattering (DLS), and emulsions by laser diffraction and fluorescence microscopy. The pH-stat method was used to measure free fatty acids (FFA) release.
Results: CNCs were rod-like (length = 81.9±0.3 nm) and had a negative Zeta-potential (around -53 mV). All formulations generated stable emulsions, however emulsions utilising CNCs alone demonstrated significant instability in the simulated intestinal environment. The combination of CNC with MC or CS resulted in greater stability and reduced FFA release *in vitro*.
Conclusions: CNCs can be used to stabilise Pickering emulsions alone. Utilising CNCs, in combination with polysaccharides with different physico-chemical properties, has the potential to generate novel food emulsion systems for improved regulation of lipid digestion.

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Understanding the secondary structure changes of plant-based proteins under elderly *in vitro* oral conditions

Ingrid Contardo[1, 2], Javier Enrione[1, 2]
[1]*Biopolymer Research & Engineering Laboratory (BiopREL), School of Nutrition and Dietetics, Faculty of Medicine, Universidad de los Andes, Chile., Santiago, Chile,* [2]*Centro de Investigación e Innovación Biomédica (CiiB), Universidad de los Andes, Chile., Santiago, Chile*

Aging impairs the digestion process, generating nutritional needs that include protein-rich foods. In addition, more attention from consumers is being given to plant-based protein isolates due to environmental concerns. The conformation status of proteins plays a key role in resistance to proteolysis at gastrointestinal level. Salivary alterations during aging could modify the secondary structure of proteins, thus affect the subsequent gastrointestinal digestibility. However, studies looking at conformational changes of the plant-based proteins under elderly oral digestion are scarce. Accordingly, this work investigates the effect of oral digestion under elderly conditions on the secondary structure of chickpea and quinoa proteins. The samples were exposed to an *in vitro* digestion setting (INFOGEST) simulating elderly oral conditions. Protein isolates and their digests were characterized by hydrophobicity, Z potential (by Dynamic Light Scattering) and secondary structure (by Raman spectroscopy and Circular Dichroism). The elderly oral conditions did not generate significant changes ($p>0.05$) on the net surface charge and the hydrophobicity of studied proteins. The β-sheet configuration increased (~35%) compared to undigested samples, and α-helix decreased to ~25%. An increase in β-sheet structures at the oral level could subsequently impact on a lower plant-based protein digestibility at the gastrointestinal level in elderly population.

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Development of polysaccharide-casein gel-like structures resistant to *in vitro* gastric digestion

Cynthia Fontes-Candia[1], **Marta Martinez-Sanz[2]**, Isidra Recio[2], Beatriz Miralles[2], Amparo López-Rubio[1]
[1]Institute of Agrochemistry and Food Technology, IATA-CSIC, Valencia, Spain, [2]Institute of Food Science Research, CIAL-CSIC-UAM, Madrid, Spain

Controlling protein digestion is a promising strategy to modulate hormonal responses involved in satiety and appetite regulation. In this context, polysaccharide-casein gel-like structures have been developed and subjected to *in vitro* gastrointestinal digestions to evaluate their potential for delaying casein hydrolysis. The effect of the polysaccharide type (agar vs. κ-carrageenan), the polysaccharide:casein ratio and the physical state of the structures (hydrogels vs. aerogels) on the delayed digestion capacity was investigated. The digestion products were characterized in terms of microstructure, molecular weight distribution and peptide profile. Our results suggest that the gel-like structures exerted a protective effect against the degradation of casein upon digestion, being this effect dependent on the type and concentration of polysaccharide and the physical state of the structures. In general, the hydrogels showed a greater protective effect than the aerogels, due to a limited diffusion of the protein towards the liquid medium. Moreover, a higher polysaccharide:protein ratio produced stronger gel networks providing greater protection. Agar-based and κ-carrageenan hydrogels with 25% polysaccharide and agar-based aerogels with 75% polysaccharide were determined as the most optimum formulations, since they were able to preserve intact casein after the gastric phase while promoting the release of larger peptides during the intestinal phase.

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Infant *in vitro* semi-dynamic digestion of conventional heat-treated vs. membrane filtered infant formula with an increased native whey protein content

Yihong Chen[1, 2], Michael Callanan[2], Linda Giblin[1], André Brodkorb[1]
[1]Teagasc Food Research Centre, Fermoy, Ireland, [2]Munster Technological University, Cork, Ireland

Background: Using membrane filtration technology to produce infant milk formula (IMF) has been found to preserve the native structure of whey proteins. This study aims to determine how IMF processing influences protein digestibility.
Methods: Membrane filtration and split-stream technology was applied to IMF (MEM-IMF) processing, whereby only the casein fraction was exposed to high temperature (HT) treatment. A control IMF was also produced by a standard HT treatment (HT-IMF). Both IMFs were compared by using a semi-dynamic infant *in vitro* digestion method. Confocal laser scanning microscopy, SDS-PAGE, HPLC, and degree of hydrolysis assay were used to monitor digestive behaviour.
Results: MEM-IMF released more water-soluble whey protein in the serum of reconstituted IMF. During gastric digestion MEM-IMF formed smaller protein gels compared to HT-IMF. These factors increased surface area, which improved enzyme accessibility, thereby accelerating digestion, releasing free amino groups, and disintegrating curds more quickly.
Conclusion: The presence of native whey protein in the IMF altered the gastric digestive kinetics by changing the gastric coagulations and formation of aggregates, potentially accelerating the rate of gastric emptying *in vivo*.

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Effect of microstructure on the *in vitro* digestion of egg white protein gels

Alisha Kar[1], Maria Garcia Guerrero[1], Riley Graham[1], Alex Olenskyj[1], Gail Bornhorst[1]
[1]*University of California-Davis, Davis, United States*

Egg white protein has been widely accepted as a functional food and egg white gelation is crucial to food structure. With a growing propensity towards optimizing functional food design, it is imperative to evaluate the impact of food structure on breakdown and nutrient release during digestion.
Egg white gels (11.26% w/v) prepared at pH 3, 5 and 7.5 were subjected to static *in vitro* oral digestion (30 seconds) and *in vitro* gastric digestion for 15, 30, 60, 120, 180 and 240 minutes (37 °C, 100 rpm) with adjusted or unregulated pH. Texture, protein hydrolysis, acid and moisture uptake were measured during digestion.
Differences in the initial pH impacted gel hardness and gel microstructure. Gel hardness was highest at pH 5 (8.10 N at pH 5 vs. avg 3.62 N at pH 3.5 and 7.5). Differences in gel microstructure significantly impacted protein hydrolysis, acid and moisture uptake ($p<0.05$). The acid uptake was highest at pH 7.5 (14.6% higher than pH 3) while the moisture uptake was highest at pH 3 (0.7% higher than pH 5 and pH 7.5).
Microstructure of egg white gels significantly influenced mass transport of moisture and acid, affecting the nutrient release and associated bio-functional properties.

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Impact of particle size and water content on particle breakdown and starch digestibility of chickpea-based snacks during *in vitro* digestion

Weiyi Sun[1], Giustino Tribuzi[1], Gail Bornhorst[1]
[1]*University of California-Davis, Davis, United States*

Background: Chickpea is an agriculturally-important legume that is an excellent source of protein, fiber, and minerals. Therefore, developing chickpea-based snacks could provide consumers with snack products rich in protein.
Methods: Chickpea puree (high moisture content) and cracker (low moisture content) were each produced with large (7 mm sieve; coarse) or small (2 mm sieve; fine) particle size. All treatments underwent static *in vitro* oral digestion, dynamic gastric digestion in the Human Gastric Simulator (HGS), and static *in vitro* small intestinal digestion. The pH, moisture content, gastric emptying, particle size, and starch and protein hydrolysis were measured.
Results: The emptying rate from the HGS was significantly ($p<0.05$) higher for fine puree compared to the other treatments. The reducing sugars and free amino groups released from fine puree were higher than coarse puree, and fine cracker was higher than coarse cracker due to the influence of initial particle size. For example, after 360 min total *in vitro* digestion, the starch hydrolysis of the fine cracker (48.1±3.2%) was higher than the coarse cracker (36.3±5.8%). Overall, crackers had higher protein and starch hydrolysis than puree.
Conclusion: The study showed that the smaller initial particle size and drying would increase the rate of breakdown and starch and protein digestibility.

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Application of the INFOGEST 2.0 protocol to the *in vitro* digestion of concentrated emulsions: a case study on mayonnaise-like products

Paula Kiyomi Okuro[1,2], Alice Kermarrec[1], Michèle Viau[1], Hanitra Rabesona[1], Rosiane L. Cunha[2], Claire Berton-Carabin[1,3], Anne Meynier[1]

[1]INRAE, UR BIA, F-44316, Nantes, France, [2]Department of Food Engineering, School of Food Engineering, University of Campinas, 13083-862 , Campinas, Brazil, [3]Laboratory of Food Process Engineering, Wageningen University & Research, 6700 AA, Wageningen, The Netherlands

Concentrated and high internal phase emulsions have been used as carriers for lipids or bioactive compounds, but their fate during *in vitro* digestion is scarcely reported. In that respect, some fundamentals are (i) gastric lipase catalyzes approximately 10-25% of the total triacylglycerol hydrolysis throughout the gastrointestinal tract in healthy adults; (ii) the high lipid content can affect the gastric emptying rate; (iii) catalytic pre-digestion by gastric lipase can have a relevant role on pancreatic lipase action; and (iv) lipids are considerable stimulants of pancreatic enzyme secretion by the body. Herein we propose to evaluate the *in vitro* digestion of high-lipid emulsions in an approach that considers the addition of gastric lipase and that optimizes the addition of enzymes and bile, as well as the initial dilution of the sample in the simulated oral phase. Two types of commercial mayonnaise-like products are studied, which vary by their oil type (sunflower or rapeseed) and content (50 or 75 wt.%, approximately), and by their formulation (conventional mayonnaise or vegan alternative). This work reports on the effects of these experimental conditions on the rate and extent of lipolysis, and constitutes an important step towards dynamic *in vitro* digestion studies of such systems.

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Development of a prebiotic strawberry preparation for the dairy industry

Daniela A. Gonçalves[1, 2], Vitor D. Alves[3], José A. Teixeira[1, 2], **Clarisse Nobre[1, 2]**
[1]Centre of Biological Enginnering - University of Minho, Braga, Portugal, [2]LABBELS –Associate Laboratory, Braga, Guimarães, Portugal, [3]Frulact, Ingredientes para a industria alimentar, Maia, Portugal

Background: Food industry has been pressed to develop products with reduced sugar and caloric value, with the challenge of keeping rheological and sensory characteristics. Herein we developed a functional strawberry preparation for the dairy industry, by in-situ enzymatic conversion of sucrose into prebiotic fructo-oligosaccharides (FOS). Methodology: Two enzymatic complexes (Pectinex®Ultra SP-L and Viscozyme®L) were applied in the preparation. Operational parameters were optimized to maximize FOS yield: temperature, pH, enzyme:substrate ratio (E/S). Rheological, physicochemical and functional properties (INFOGEST gastrointestinal digestion protocol) were evaluated. Results: At optimal conditions (60 °C, pH 5.0), Pectinex produced 265±3 g/L FOS, yielding 0.581±0.006 g(FOS)/g(initial.GF) after 7 h reaction (E/S:1/40); and Viscozyme produced 295±1 g/L FOS, yielding 0.664±0.004 g(FOS)/g(initial.GF) after 5 h (E/S:1/30), both resulting in preparations with 50% (w/w) FOS. The caloric value was reduced 24%, including 80% sucrose reduction. Differences in colour, water activity and ºBrix were not relevant, while consistency and viscosity decreased ≃70% and pH increased from 4.4 to 4.7. FOS showed resistance to gastrointestinal digestion; only kestose was slightly hydrolysed at intestinal phase. Conclusions: A prebiotic strawberry preparation was successfully produced at lab scale, by in-situ enzymatic conversion of caloric into functional sugars. Next, the process will be scaled-up at industrial level.

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Cereals and legumes co-ingestion under infant digestive conditions: starch digestibility

Marianela Rodriguez[1], Alberto León[3], Mariela Bustos[2], **Sonia de Pascual-Teresa[1]**
[1]Ictan-csic, Spain, [2]Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Cordoba, Argentina, [3]Instituto de Ciencia y Tecnología de los Alimentos Córdoba, CONICET-UNC, Cordoba, Argentina

In recent years, much research has focused on the effect of co-ingestion of different foods on digestion, in a more realistic way of assessing nutrients digestion. The aim of present research was to study the effect of legumes and cereals co-ingestion on starch digestibility, using infant digestive conditions. INFOGEST *in vitro* digestion method was performed on durum wheat (DW), brown rice (BR) and white maize (WM) co-ingested with lentil, chickpea and pea, with modifications to mimic infant digestion conditions. Cereals co-ingested with lentils showed an increased extent (40-85%) of starch hydrolysis. The addition of pea to cereals did not affect starch digestibility. The addition of chickpea presented a different response depending on the cereal added, i.e. no effect was observed in starch digestion when mixed with WM or BR, but WM decreased the kinetic constant 70%, while BR increased it by25%. Although, the co-ingestion of DW and chickpea showed around 30% reduction on both starch hydrolysis parameters. Results agree with other authors that stated that soluble fibers incorporated with legumes could affect the kinetic of digestion. Co-ingestion of cereals and legumes, which is so important for infants, affects starch digestibility depending on the intrinsic characteristics of both cereals and legumes.

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Proteolysis from different dietary sources: a comparative *in vitro* study under altered gastrointestinal conditions of senior population

Sara Munoz Pina[1], Ever Hernández Olivas[1], Ana Andrés[1], Ana Heredia[1]
[1]*Universitat Politècnica De València, Research Institute Of Food Engineering For Development, Valencia, Spain*

A protein intake between 1.0-1.2 g/kg body weight/day is recommended for the elderly to avoid sarcopenia and/or immune weakening. Hence, high-quality protein foods of both animal and vegetable origin might be included in the diet by elders. However, protein's nutritional value can be hindered by the deterioration of gastrointestinal (GI) function that accompanies aging. Thus, this study aims to assess and compare the protein digestibility of different meats, fish, eggs, cereals, legumes, and dairy products by using three *in vitro* digestion models mimicking the elderly altered conditions (E1: altered oral conditions, E2: altered oral and gastric conditions, and E3: altered oral, gastric, and intestinal conditions). Results showed that proteolysis is affected by simulating reduced chewing cycles (E1) even in foods like cheese and fish; cumulative digestive disturbances (E3) decreased ($p<0.05$) proteolysis up to 40% in beef, hard-boiled egg, chickpeas and aged cheese. Besides, amino acids such as leucine resulting from proteolysis are significantly reduced in all *in vitro* digested foods. However, the essential amino acid/non-essential amino acid ratio was also analyzed, where promising data showed a considerable increase even under the worst GI scenario (E3). Therefore, this overview could support dietary recommendations for elders to improve their diet and health.

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Impact of milk specie and structure during *in vitro* gastrointestinal digestion of cow and sheep milk products

Tanguy Saviard[1], Stefano Nebbia[2], Olivia Menard[2], Jordane Ossemond[2], Gwenaële Henry[2], Raphaël Chacon[1], Steven Le Feunteun[2], Françoise Nau[2], Didier Dupont[2], Linda Le Roux[1]
[1]Sill, Plouvien, France, [2]STLO, INRAE, Institut Agro, Rennes, France

The traditional dairy industry, mainly based on cow milk (CM), is becoming increasingly diversified with milk from other species. Sheep milk (SM) is known to differ from CM in nutritional composition, physicochemical properties and structure which may lead to different digestion behaviors.
This work aimed to investigate the impact of the milk specie and structure on the digestion of CM and SM products. Using an *in vitro* static gastrointestinal digestion model, milks and yogurts from CM and SM were compared on their kinetics of proteolysis and lipolysis, fatty acids, amino acids and calcium releases as well as their structure.
Different structural behaviors were found between milks and yogurts during the gastric phase with SM showing the biggest particles. Higher proteolysis and amino acid release were found for CM compared to SM with higher proteolysis found for milks compared to yogurts for both species. The kinetic of lipolysis was specific for each products, but finally, SM products showed higher lipolysis and fatty acid release than CM ones. SM products released more calcium than CM ones with no differences between milks and yogurts.
In conclusion, milk specie had more impacts on the digestion behavior compared to milk structure between SM and CM.

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Impact of high protein drinks formulation on *in vitro* dynamic digestion and absorption of macronutrient in the elderly

Stefano Nebbia[1], Olivia Menard[1], Marie-Françoise Cochet[1], Gwénaële Henry[1], Nathalie Daniel[1], Cian Moloney[2], Michelle Collins[2], Francois Morgan[2], Didier Dupont[1]
[1]STLO, INRAE, Institut Agro, Rennes, France, [2]Glanbia Ireland, Ballyragget, Ireland

Due to the lower efficiency of the elderly digestion system, new formulations are needed in order to increase the bioaccessibility and bioavalability of macronutrients.
The aim of the work was to evaluate the effect of the nature of the proteins (liquid vs spray dried milk proteins) and lipids (vegetable oil vs bovine milk fat) ingredients on the macronutrient digestion of three experimental elderly drinks. An *in vitro* dynamic digestion model was set up using the elderly's gastrointestinal parameters. The intestinal absorption of the free amino acids (AAs) was investigated with a co-culture system of enterocytes (Caco-2) and goblet (HT29-MTX) cells.
The spray dried treatment of protein ingredient did not affect the kinetics of macronutrients digestion. In opposite, the addition of bovine milk fat had an effect on the intestinal digestion of protein with a slightly smaller release of free AAs but did not affect the kinetics lipid digestion. Although differences were found in the AAs bioaccessibility at the end of the intestinal phase, samples had the same AAs bioavailability.
Milk fat with a higher level of phospholipids and a lower ω6/ω3 PUFA ratio, can be a good alternative to the use of the vegetable fat in drinks for elderly people.

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Impact of GOS/FOS and fat origin on the macronutrients kinetics of digestion and the calcium bioaccessibility of growing-up milks

Manon Bonnnot[1], Cian Moloney[2], Cian Hickey[2], Eric Baucher[1], Olivia Ménard[1], Michelle Collins[2], Francois Morgan[2], Didier Dupont[1], **Stefano Nebbia[1]**
[1]STLO, INRAE, Institut Agro, Rennes, France, [2]Glanbia Ireland, Ballyragget, Ireland

Growing-up milks (GUM) are increasingly introduced into the diets of 1-3 years children in order to compensate the nutritional deficiencies which may occur in the transition phase of infant nutrition.
In order to improve the GUM formulations, the aim of this work was to evaluate the impact of the GOS/FOS and the nature of the fat (vegetable oil (VO) vs milk bovine fat (MBF)) on the lipid and protein kinetics of digestion and on the minerals' bioaccessibility of experimental GUM after *in vitro* static digestion.
The GOS/FOS did not impact the overall degree of proteolysis and lypolysis. In opposite, faster intestinal protein digestion together with higher release of free fatty acids in gastric phase was found in the VO-GUM compared to the MBF-GUM. In accordance to the nature of the fat origin, different fatty acid profiles were found between samples. Higher calcium bioaccessibility was found in the MBF-GUM correlated to their lower palmitic fatty acid release. In addition, GOS/FOS may improve the bioaccessibility of calcium.
In conclusion, the combined effect of MBF and GOS/FOS is the optimal ingredient association in order to have the best lipid profile and the highest calcium bioaccesibility.

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Effect of *in vitro* simulated gastrointestinal digestion on the morphology of amyloid-like fibrils of hen egg ovalbumin and lysozyme

Trui Luyckx[1], Charlotte Grootaert[1], Margarita Monge-Morera[2], Jan Delcour[2], Joëlle Housmans[3, 4], Frederic Rousseau[3, 4], Joost Schymkowitz[3, 4], Sebastien Carpentier[5], John Van Camp[1]
[1]Laboratory of Food Chemistry and Human Nutrition, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium, [2]Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre, KU Leuven, Leuven, Belgium, [3]Switch Laboratory, VIB-KU Leuven Center for Brain & Disease Research, Leuven, Belgium, [4]Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium, [5]BIOMED facility for Systems Biology based Mass spectrometry, KU Leuven, Leuven, Belgium

Background: The use of amyloid-like protein fibrils (ALFs) in food formulations may be very promising in terms of improving techno-functional properties, but may raise some concerns in terms of food safety. A first step in the assessment of bioavailability is digestion.
Method: *In vitro* gastrointestinal digestion was performed according to the INFOGEST method. Evaluation of ALF breakdown throughout the gastrointestinal digestion included transmission electron microscopy (TEM) imaging, Thioflavin T (ThT) fluorescence, free amine group quantification and LC-MS analysis.
Results: After gastric and small intestinal digestion of ovalbumin ALFs, ThT levels decreased to 75.3 ± 9.5% and 6.4 ± 1.6% of the initial value, respectively. For lysozyme, fluorescence levels corresponded to 1.7 ± 8.8% (gastric) and 31.3 ± 5.8% (small intestinal) of the initial ThT value. These results suggest significant enzymatic degradation of β sheet rich protein regions under gastric and small intestinal conditions. Complementary TEM imaging shows remaining fibril structures after small intestinal digestion, albeit of smaller length.
Conclusions: It was observed that ALFs from ovalbumin and lysozyme are efficiently digested by both gastric and pancreatic enzymes. Nevertheless, fibrillar morphology was still present after gastrointestinal digestion. Therefore, additional research should be considered to evaluate potential health impact of ALF consumption.

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Modulation of extra virgin olive oil digestibility through oleogelation

Francesco Ciuffarin[1], Sonia Calligaris[1], **Marilisa Alongi[1]**, Maria Cristina Nicoli[1]
[1]University of Udine, Udine, Italia

Background. Extra virgin olive oil (EVOO) represents a key player in the Mediterranean diet for its health-promoting capacity. Although its use as a functional ingredient would be particularly interesting, the direct addition of EVOO to food is challenging due to its liquid state. EVOO conversion into a solid-like material through by oleogelation could enlarge its possible applications.
Methods. EVOO was gelled by adding 10% (w/w) of saturated monoglycerides (MG), rice bran waxes (RW), sunflower waxes (SW) or a β-sitosterol/γ-oryzanol mixture (PS). Oleogels were characterized for their structure and subjected to static *in vitro* digestion. The fatty acid release and destructuring behavior were assessed.
Results. The resulting oleogels differed for rheological properties and firmness due to the differences in gel network structure. PS oleogel was the firmest sample followed by SW, RW and MG ones. During *in vitro* digestion, the fatty acid release was significantly lower for all oleogels compared to unstructured oil. The different network provided by the four oleogelators not only influenced FA release, but also the intestinal micellar size.
Conclusion. Acquired results could open new horizons for EVOO application through oleogelation not only to obtain novel EVOO-based fat replacers but also to better deliver the EVOO health functionalities.

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Comparison of the *in vitro* digestibility, PDCAAS, and functionality of pea proteins from different providers.

Angeline Riquelme[1], Sofía Estrugo[1], Javiera Mujica[1], Mariana Guimaraes[1], Ana Batista Gonzalez[1], Rodrigo A. Contreras[1]
[1]The Not Company, Santiago de Chile, Chile

Background: Pea proteins are widely used as ingredients in the plant-based industry to develop plant-based milks, ice creams, and meats. However, there is a lack of consistency regarding pea proteins' physicochemical characteristics and functionality from different providers. This study aimed to assess the *in vitro* digestibility, PDCAAS, and the foaming capacity of different pea proteins in the market.
Methods: Protein digestibility was evaluated using the INFOGEST *in vitro* protocol and followed through electrophoresis in polyacrylamide gel (SDS-PAGE) and the release of amino acids using the ophtaldehide (OPA) reagent. The PDCAAS was determined *in vitro* using a commercial kit. The foaming capacity of the proteins was evaluated by visual foam density and visual foam droplet size.
Results: We found differences in the digestibility of the different proteins evaluated, as well as in the PDCAAS. The differences in the PDCAAS values were mainly due to differences in the amino acid profiles of the proteins, and the proteins also had different foaming capacities.
Conclusions: This study shows that pea proteins from different providers might have different nutritional and functional characteristics. Our results have implications in product development since proteins with the best functionality would not necessarily provide the best nutrition.

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In vitro starch and protein digestion kinetics of cooked Bambara groundnuts depend on processing intensity and hardness sorting

Shannon Gwala[1, 2], Andrea Pallares Pallares[1], Katharina Pälchen[1], Marc Hendrickx[1], Tara Grauwet[1]
[1]KU Leuven, Leuven, Belgium, [2]Teagasc, Fermoy, Ireland

When pulse seeds from a single batch are cooked, considerable variability of hardness values in the population is usually observed. Sorting the seeds into hardness categories could reduce the observed diversity. We investigated the effect of processing intensity whether or not combined with sorting into hardness categories on *in vitro* starch and protein digestion kinetics of Bambara groundnuts. The estimated lag phase describing the initial phase of starch digestion was not significantly different despite processing intensity or hardness category, implying that cell wall barrier properties for these samples were not majorly different. However, the kinetic parameters of starch digestion were higher for the low hardness compared to the high hardness category. Kinetic evaluation of digested soluble protein showed that low hardness samples were digested faster than high hardness samples. Faster protein hydrolysis in the low hardness samples was accompanied by faster starch digestion, indicating the possible role of the protein matrix barrier. Individual cells of comparable hardness obtained from two different processing times had similar starch and protein digestion kinetics. Our work demonstrated that, beyond cooking time, hardness is a suitable food design attribute that can be used to modulate starch and protein digestion kinetics of pulse cotyledon cells.

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Standardized ileal digestibility of amino acids and nitrogen in human milk and infant formula- an *in vivo* study

Elise Charton[1,2], Gwénaële Henry[1], Armelle Cahu[2], Yann Le Gouar[1], Paul Moughan[4], Carlos Montoya[4,5], Amandine Bellanger[3], Didier Dupont[1], Isabelle le Huërou-Luron[2], Amélie Deglaire[1]
[1]UMR STLO , INRAE, Institut Agro-Rennes Angers, Rennes, France, [2]Institut NuMeCan, INRAE, INSERM, Univ Rennes, Saint-Gilles, France, [3]CHU Rennes, Département Pédiatrie et Néonatalogie, Rennes, France, [4]Riddet Institute, Massey University, Palmerston North, New-Zealand, [5]Smart Foods Innovation Centre of Excellence, AgResearch Limited, Palmerston North, New-Zealand

Human milk (HM) and infant formula (IF) protein digestibility has been rarely studied *in vivo* with no value for tryptophan. The study aimed to measure their standardised ileal digestibility (SID), i.e. after endogenous Nitrogen loss correction, using Yucatan mini-piglets as infant model.
Nineteen days-old piglets received HM (n=7), IF (n=8) or protein-free (n=6; endogenous Nitrogen loss determination) diets with Cobalt-EDTA (indigestible marker). Diets were hourly administrated during 6h before euthanasia and digesta collection. SID of Nitrogen and amino acids (AAs) were determined. Differences between groups were assessed using an ANOVA.
AAs and Nitrogen contents were similar between HM and IF diets, except lower lysine content in HM (-32%). Endogenous losses corrected digestibility values of 6 to 14%. SID of total Nitrogen was significantly lower for HM (91.3±1.2% vs. 98.0±0.9%), but SID of AA Nitrogen was not significantly different (97.5±0.5%). HM and IF had similar SID ($p>0.05$) for most AAs, including tryptophan (96.2±0.5%, $p=0.07$), unlike that a few AAs (Lys, Phe, Thr, Ala, Pro), slightly but significantly differing.
Overall, this indicates that a larger proportion of non-protein Nitrogen is transferred to the microbiota with HM, which is likely of physiological relevance, although not yet considered in IF production.

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Quantifying gastric coagulation of milk proteins in humans using MRI

Elise Van Eijnatten[1], Wolf Rombouts[2], Linette Pellis[2], Guido Camps[1], Paul Smeets[1]
[1]Wageningen University and Research, Wageningen, The Netherlands, [2]Ausnutria B.V., Zwolle, The Netherlands

Background: Methods to quantify gastric coagulation in humans are currently lacking. We explored the suitability of image texture metrics for quantifying gastric coagulation of milk proteins on magnetic resonance imaging (MRI) images.
Method: Eighteen overnight fasted men (26±8.1 years, BMI 23±1.6 kg/m2) consumed a 300-mL drink with 30g caprine or bovine casein (similar nutrient composition) in a single-blind randomized cross-over study. Gastric MRI scans were performed at baseline and every ten minutes up to 60 minutes postprandial. Four image texture metrics were calculated on the stomach contents: homogeneity, contrast, coarseness and busyness.
Results: Coagulation was clearly visible on the MRI images from 30 minutes onwards for both treatments and increased over time. This was in line with mixed model analyses of the image texture measures over time (all $p<0.001$). There were no differences in image texture between the treatments, except that 'contrast' was lower for cow casein ($p=0.036$).
Conclusion: Image texture measures are a promising approach to objectively quantify coagulation; they were in line with visual assessment of increasing coagulation over time. Further validation of the coagulation patterns and clot characteristics that could underlie differences in MRI image texture measures by combining *in vitro* and *in vivo* work is warranted.

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Can nitrite-free recipes of cured meat products prevent the formation of nitroso compounds?

Elena Keuleyan[1], Sylvie Blinet[1], Laurent Aubry[1], Aurélie Promeyrat[2], Gilles Nassy[2], Véronique Santé-Lhoutellier[1], **Noémie Petit[1]**
[1]INRAE, Qualité des Produits Animaux, Saint Genès-Champanelle, France, [2]IFIP – Institut du Porc, Le Rheu Cedex, France

Background: The presence of nitrite in cured meat products can lead to the formation of nitroso-compounds (NOCs), some of which have been linked to a higher risk of developing colon cancer. With possible new regulations to limit or suppress additives, industries have looked for alternatives. The objective is to study the formation mechanism of NOCs in cured meat products prepared with nitrite-free recipes (PROSUR®, yeast, vegetable-stock), 0 (NC) and 120ppm (PC) NO2.
Methods: The formation of NOCs, oxidation products, and nitrosylheme was quantified in all products before and during digestion. Samples were subjected to simulated *in vitro* dynamic digestion (DIDGI©), and analyses were carried out at different time points throughout the digestion.
Results: Nitrosylheme was lower than 15% without added nitrite (NC) and yeast formulation but more lipid oxidation products were found. In the intestinal phase of digestion, lipid oxidation increased for all samples regardless of the formulation. Nitrosamines were higher at the beginning of gastric digestion for PC and vegetable-stock samples, but decreased during digestion for all samples.
Conclusions: Nitrite-free recipes may reduce NOCs formation in cured meat products and require more investigation.

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Consequences of oral deficiencies on intestinal bioaccessibility of nutrients in elderly

Susana Ribes[1, 2], Mélany Genot[1], Pau Talens[2], Véronique Santé-Lhoutellier[1], Marie Agnès Peyron[3,] Susana Ribes[1, 2]
[1]INRAE, QuaPA UR 370, F-63122 Saint Genès Champanelle, France, [2]DTA, Universitat Politècnica de València, Camino de Vera s/n, 46022 , Valencia, Spain, [3]Université Clermont Auvergne, INRAE, UNH, F-63000 Clermont-Ferrand, France

Background: Food transformation starts during mastication, which combined with salivation, reduces particle size and form swallowable boluses. In elderly, oral functions are modified by changes in muscular force or saliva production, among others, providing an inadequate food fragmentation potentially impacting on oral and gastrointestinal digestions. This work aimed to evaluate the consequences of oral deficiencies on glucose release and protein digestibility of bread.
Method: *In vitro* boluses were prepared with AM2 masticator apparatus using normal mastication (NM) and deficient mastication (DM) programming. NM was simulated using *in vivo* data and DM was obtained by modifying NM programming, combining force and saliva alterations. Static *in vitro* digestions, simulating the elderly physiological conditions, were performed. Boluses particle size and glucose release, protein digestibility, and FTIR analyses during oral and gastrointestinal digestions were conducted.
Results: DM boluses showed larger particles than NM boluses, affecting nutrients release and digestion. Indeed, digesta from DM boluses exhibited lower protein hydrolysis degree and glucose release. FTIR results revealed greater β-sheets at the expense of other conformations in protein's secondary structure; to be linked to biochemical results.
Conclusion: This work demonstrates the impact of oral deficiencies on nutrients bioaccessibility and stresses the importance of designing foods for elderly.

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Impact of the addition of vegetable lecithin in oil on lipolysis rate under simulated physiological conditions

Marion Letisse[1], Sabine Danthine[2], Emile Meynard[1], Annie Durand[1], Chloe Robert[1], Carole Vaysse[3], Marie-Caroline Michalski[1]
[1]Carmen Laboratory, Lyon, France, [2]Université de Liège - GxABT, Gembloux, Belgium, [3]ITERG, Canejan, France

Background: Lecithins have seen increased usage over the past years in the food industry as an emulsifier for processed foods to improve and preserve their texture. According to recent studies, lecithins have shown evidence of enhancing lipid metabolism *in vivo* and *in vitro* due to their physicochemical properties with suggested mechanisms related to impacts on digestion.
Method: We studied gastric and pancreatic lipolysis *in vitro* using the static human digestion pH-stat model adapted from Minekus et al. on palm-containing oil mixtures enriched or not with 10% lecithin from soy or rapeseed. Samples lipid classes were analyzed during digestion by HPTLC. The importance of the lipid composition with or without lecithin on physicochemical properties of the different oil mixtures tested was identified by Differential Scanning Calorimetry.
Results: First results obtained during pancreatic digestion phase suggest that addition of different vegetable lecithins impact lipolysis kinetics and specifically regarding intermediate monoacylglycerol generation. The thermal properties were also impacted by the incorporation of lecithins in the formulations, especially with 10% of soy lecithin.
Conclusion: Lecithin addition in oil mixtures may impact both thermal properties and *in vitro* lipolysis.

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Novel biodegradable systems to be used for prolonging food shelf-life

Seyedeh Fatemeh Mirpoor[1], Michela Famiglietti[1], Loredana Mariniello[1], Valeria Giosafatto[1]
[1]Department of Chemical Sciences, University Of Naples Federico II, Complesso Universitario Di Monte Sant'Angelo, Naples, Italy

Petroleum-based plastics are considered a major threat of dramatic environmental pollution and bioplastics seem an attractive eco-friendly alternative. By-products of cardoon (Cynara cardunculus) oilseed following oil extraction, known as seed oil cakes (SOCs), are a rich source of proteins and they are promising candidates to act as raw material for the production of protein-based bioplastics. Cardoon proteins (CPs) at different concentrations (200-400 mg) at pH 12 revealed the ability to give rise by casting to greenish films, in the presence of the minimum concentration of 30% (w/w of proteins) of glycerol, used as a plasticizer. Furthermore, the matrix of the films was strengthened by means of microbial transglutaminase (mTGase). SDS-PAGE analysis revealed that CPs act as both acyl donor and acceptor substrates of the enzyme. Following mTGase treatment, an improvement of film properties, namely mechanical and barrier properties as well as hydrophobicity, was observed. CP-based films were used for wrapping peanuts and they show to be able to prevent lipid oxidation and rancidity, thus, prolonging their shelf life. Preliminary digestibility studies showed that these films are digested in the gastric environment leading to the possibility to eat the protecting material together with the wrapped food.

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In vitro digestion of argan protein-amylose-based films

Michela Famiglietti[1], Seyedeh Fatemeh Mirpoor[1], Valeria Giosafatto[1], Loredana Mariniello[1]
[1]Department Of Chemical Sciences, University Of Naples Federico II, Complesso Universitario Di Monte Sant'Angelo, Naples, Italy

Oilseed processing of argan crops produces a large amount of waste which can be recovered as by-products. In our laboratories we have already demonstrated that argan proteins, extracted from oilcake, act as microbial transglutaminase (mTG, E.C.2.3.2.13) substrate and they are a good and sustainable option to produce novel edible films.
The aim of this work was to verify the digestibility of blended films made up by argan proteins and amylose, obtained in barley by RNA interference technology, in presence and in absence of mTG to develop a matrix with different possible applications in food and pharmaceutical industries. We verified that both films, prepared respectively with unmodified and mTG-modified argan proteins, are completely digested during oral and gastric digestion. In fact, amylose is digested by amylase in simulated oral digestion process and the remaining proteins are digested by simulated gastric digestion because of the presence of pepsin. These results lay the foundations for next studies about these novel edible films.

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Comparison of dynamic *in vitro* digestion of human milk vs standard infant formula to better understand their digestive kinetics

Elise Charton[1,2], Olivia Menard[1], Marie-Françoise Cochet[1], Jordane Ossemond[1], Gwénaële Henry[1], Amandine Bellanger[2,3], Didier Dupont[1], Isabelle Le Huërou-Luron[2], Amélie Deglaire[1]
[1]UMR STLO , INRAE, Institut Agro-Rennes Angers, Rennes, France, [2]Institut NuMeCan, INRAE, INSERM, Univ Rennes, Saint-Gilles, France, [3]CHU Rennes, Département Pédiatrie et Néonatalogie, Rennes, France

Human milk (HM) and Infant formula (IF) are assumed to present different digestion kinetics, while rarely directly compared. The study aimed to address this question using a dynamic digestion model (DIDGI®) at the infant stage.
Fresh HM (pool from 50 mothers) and standard IF with similar total nitrogen content were digested in triplicate. Digesta were sampled regularly in gastric and intestinal phase from 0 to 180 min to evaluate structural changes (confocal microscopy and laser light scattering), proteolysis (SDS-PAGE and densitometry, OPA), and nitrogen digestibility (µ-Kjeldahl). Differences between groups were assessed using a two-way repeated measures ANOVA.
The microstructure of the digesta differed between HM and IF along the gastric digestion. Proteolysis of the common HM and IF proteins, caseins and α-lactalbumin, was significantly slower for HM than for IF. The intestinal degree of proteolysis was lower for HM than IF, at least during the first two hours. Total N digestibility was lower with HM, such as observed *in vivo*. Peptidomic and lipolysis data will complete the dataset.
Despite nutritional similarity, this study highlights the influence of the matrix on the digestion kinetics and gives some further understanding to the global value of digestibility, such as determined *in vivo*.

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The effect of industrial processes on the digestion of milk protein matrices in rats

Juliane Calvez[1], Nathalie Atallah[1, 2, 3], Claire Gaudichon[1], Barbara Deracinois[3], Rozenn Ravallec[3], Christophe Flahaut[3], Nadezda Khodorova[1], Martin Chapelais[1], Audrey Boulier[2], Alain Baniel[2], Benoit Cudennec[3]

[1]UMR PNCA, AgroParisTech, INRAE, Université Paris-Saclay, Paris, France, [2]Ingredia S.A, Arras, France, [3]Univ Lille INRAE–ICV, Lille, France

The aim of this study was to analyze the impact of industrial processes on the way milk proteins are digested and metabolized.
For this purpose, rats consumed a meal containing one of four 15N-labelled milk matrices: native micellar caseins (casein), caseins low in calcium (casein-lowCa), modified concentrations of 37% whey and 63% caseins (casein-whey) and pure whey (whey). Rats were euthanazied 6h after meal ingestion. Digestive contents were collected to determine orocaecal digestibility and analyze the peptidomes. Blood and organs were collected to evaluate protein synthesis and dietary nitrogen incorporation.
Nitrogen orocaecal digestibility was slightly lower for the casein-whey matrice (93.5±1.0%) compared to the other matrices (94.9±1.0% for casein, 95.5±0.9% for casein-lowCa, 95.2±0.9% for whey). Higher dietary nitrogen incorporation was observed in plasma proteins, jejunum, liver and muscle after whey ingestion, but no difference on protein synthesis was found. Some gastro-intestinal resistant peptides were identified, and differences were observed according to the four milk matrices.
The different processes applied to obtain milk matrices seem to impact the way proteins are degraded during digestion and postprandially incorporated to body, without decreasing protein quality as observed with the high digestibility values.

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Effect of fat and protein content on the bioavailability of blackberry polyphenols in model sports nutrition beverages containing dairy-blackberry blends

Tessa Marianne van de Langerijt[1], Shane V. Crowley[1], James A. O'Mahony[1], Yvonne C. O'Callaghan[1], Nora M. O'Brien[1], Katerina Tzima[2], Dilip K. Rai[2]
[1]School of Food and Nutritional Sciences, University College Cork, Cork, Ireland, [2]Department of Food Biosciences, Teagasc Food Research Centre, Ashtown, Ireland

Background: Products that promote muscle synthesis and recovery are a key focus in sports nutrition and include dairy-fruit blends (DFB) that combine high quality sources of protein and polyphenols. However, the effect of fat and protein content in these blends on the bioaccessibility and bioavailability of such polyphenols requires further investigation.
Method: This study aimed to determine if ingredient interactions in DFB made of full-fat, semi-skimmed, skimmed, and high-protein milk in combination with blackberry polyphenols (BBP) influence this functionality. The DFB were digested *in vitro*, after which their bioavailability was analysed with a Caco-2 cell model.
Results: The percentage of total BBP in the serum phase of the DFB decreased from 65.2 to 54.2% with increasing fat concentration. The bioaccessibility of anthocyanins after *in vitro* digestion increased from 11.4% in the control (water), to 31.5-48.2% in DFB. The effect of DFB on the bioavailability of individual polyphenols in a Caco-2 cell model varied markedly for individual polyphenols. For example, milk decreased the bioavailability of cyanidin-3-O-glucoside but slightly increased the bioavailability of rutin.
Conclusions: DFB reduced deterioration of certain polyphenols during *in vitro* digestion, but had only a limited effect on the bioavailability of these components.

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The effect of beta-glucans on yogurt disintegration during *in vitro* digestion

Aleksandra Florczuk[1], Adam Macierzanka[2], Marek Aljewicz[1]
[1]University of Warmia And Mazury, Olsztyn, Polska, 2Gdansk University of Technology, Poland, Gdansk, Poland

Background: Beta-glucans have been increasingly used as structure-building components in dairy products. However, the structural behavior of such foods in the gastrointestinal tract is unknown, which presents limitation in understanding the health benefits they can provide. This study aimed to effect of various β-glucans on structural changes of yogurt during *in vitro* gastrointestinal digestion.
Methods: Yogurts containing 0.25% or 1% (w/v) β-glucan (oat β-glucan or curdlan) were digested using the INFOGEST *in vitro* static digestion model. The digesta microstructure was analyzed by confocal laser scanning microscopy, and the digestion progress by electrophoresis SDS-PAGE.
Results: The structure of the added β-glucan modulate the progress of protein digestion. The yogurts containing β-glucans showed longer breakdown the protein-polysaccharide-fat structure's times, and the 1% addition of β-glucan to yoghurt caused the slowest degradation of the structure. The yoghurts with oat β-glucan disintegrated more rapidly during digestion, while the curdlan-modified yoghurts were characterized by the formation of larger complexes that disintegrated slower.
Conclusion: Structurally different β-glucans contributed to the aggregates formation that varied insolubility during the digestion. Therefore, the methodological approach described here can be a powerful tool in designing foods for modulating bioaccessibility of nutrients carry out nutritional and health needs of different populations.

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Retardation of starch hydrolysis using psyllium mucilage hydrogels

Lucija Strkalj[1], Gleb Yakubov[1]
[1]*Division Food, Nutrition and Dietetics, University Of Nottingham, Nottingham, United Kingdom*

Background: High glycaemic index of gelatinised starches causes a rapid rise in blood sugar, which is one of the key dietary factors associated with the development of type 2 diabetes. Addition of dietary fibre is known to slow down starch hydrolysis and smooth out glucose uptake. Complex xylans from Plantago ovata seed mucilage (psyllium/isabgol/ispaghula) have unique gelling behaviour with tuneable barrier properties and desirable nutritional effects on starch hydrolysis. In this work, we have developed and characterised composite hydrogels fabricated using gelatinised potato starch and a hot water soluble fraction of psyllium mucilage.
Methods: Potato starch-psyllium hydrogels were characterised using small amplitude oscillatory shear rheometry and stress relaxation experiments. Starch hydrolysis was performed using porcine pancreatic α-amylase (37 °C, 2 hours).
Results: Developed composites show characteristics of weak physical gels with a degree of phase separation between starch- and xylan-rich domains. The presence of psyllium resulted in the inhibition of starch hydrolysis due to competitive binding of α-amylase and lowering enzyme mobility inside gel matrix.
Conclusions: Psyllium shows great promise as a matrix for encapsulation of gelatinised starch. Interactions between starch, mucilage and α-amylase are found to provide a complex set of inhibitory functions to enable fine-tuning of starch hydrolysis.

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New insights into infant gastric digestion according to age for breastfed and formula-fed: towards a validated *in vitro* model.

Gaetan Drouin[1], Alina Kondrashina[1,2], André Brodkorb[1]
[1]Protein Biochemistry Laboratory - Food Chemistry Department - Teagasc, Fermoy, Ireland, [2]H&H, Fermoy, Ireland

Early infant development is an important stage of life that evolves quickly over the months when digestion takes center stage. However, only few *in vivo* data are available on infant digestion mainly due to ethical considerations for accessing digestive samples.
The gastric digestion of 48 pre-term and full-term infants, breastfed or infant milk formula fed with a nasogastric tube, have been studied over the time of digestion (fasting, 30, 60, 90min). The digestive parameters (enzyme activities, pH, gastric emptying), microstructure of the meals and gastric aspirates (CLSM, Mastersizer), protein breakdown (SDS-PAGE, OPA, SEC-HPLC, amino acid release) and lipid breakdown (HT-GC-FID, SPE-GC-FID) have been measured.
Breastfed infants had a significant higher lipase activity due to the presence of BSSL, higher particle size (10 fold), different microstructure behaviour, higher proteolysis, and differential triglyceride digestibility. Over the six first month of life, the pH decreased, pepsin activity increased as well as protein breakdown, while lipid breakdown remained more similar due to the gastric lipase early maturity. These differences raised the importance of digestion during this period.
These new digestive parameters data would be useful to set up a validated gastric static *in vitro* digestion model based on *in vivo* nutrient breakdown data.

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The effect of beta-glucans on yogurt texture and protein digestibility

Marek Aljewicz[1], Aleksandra Florczuk[1]
[1]Department of Dairy Science and Quality Management, Faculty of Food Science, University Of Warmia and Mazury, Olsztyn, Poland

Background: By changing the texture of dairy products, polysaccharides can modify the digestion rate and bioavailability of protein. Based on these observations, it can be hypothesized that by modifying the structure and rheological properties of yogurt, β-glucans influence the digestion rate of milk proteins.
Methods: The experimental material consisted of yogurts made with the addition of 0.25% and 1% of purified beta-glucan preparations (oat beta-glucan and curdlan). The process of yogurt digestion was carried out according to the INFOGEST protocol using the *in vitro* static digestion model. Protein digestibility was measured using the o-phthaldialdehyde and HPLC SEC method. Yogurt texture was analyzed by the back extrusion test.
Results: Beta-glucans significantly affected firmness, consistency index and milk protein digestibility in yogurt. Protein digestibility decreased with increasing concentrations of polysaccharides. Such a correlation was observed after both gastric and intestinal digestion. Protein digestibility was higher in yogurt with oat beta-glucan than in yogurt with curdlan.
Conclusion: Despite their proven health-promoting effects, beta-glucans may significantly reduce the digestibility and nutritional value of milk proteins due to changes in the product's structure and substrate availability to enzymes.

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In vitro digestion of an experimental infant formula containing both intact and hydrolyzed milk proteins.

Tim Lambers[1], Jeroen Wissing[1], Joris Roggekamp[1]
[1]*FrieslandCampina, Amersfoort, The Netherlands*

Background: Compared to their intact counterparts, protein hydrolysates have several advantages for digestion, mainly related to altered digestion kinetics, effects on coagulation and/or gastric emptying that may impact digestion related complications in infants. Intact milk proteins, however, provide benefits beyond their nutritional value. Here we've explored *in vitro* digestion of an experimental infant formula containing both intact and hydrolyzed milk proteins.
Method: Semi-dynamic gastric digestion was simulated for infant conditions and protein digestion was analyzed by size-exclusion chromatography and by measuring available amino groups over time. Protein coagulation was evaluated by visual inspection and wet-weight measurements of the coagulate retained on a 1 mm mesh filter.
Results: Relative to an intact milk protein formula, the experimental formula displayed a higher initial protein digestion during simulated gastric digestion as illustrated by a larger proportion of smaller peptides and higher level of available amino groups during digestion. Gastric protein coagulation was not affected by the hydrolysate addition.
Conclusion: Formula containing both intact and hydrolyzed milk proteins display altered gastric digestion kinetics *in vitro*. Further clinical studies should demonstrate if these observations result in overall changed protein digestion kinetics and/or impact functional gastrointestinal disorders similarly as has been demonstrated for full hydrolysate formula.

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How processing of plant-based shakes can impact lipid and protein microstructural organisation and digestive functionality

Jessica Guevara[1], Sarah Verkempinck[1], Lisbeth Hernandez[1], Marcos Infantes[1], Marc Hendrickx[1], Ann Van Loey[1], Tara Grauwet[1]
[1]KU Leuven, Department of Microbial and Molecular Systems (M2S), Laboratory of Food Technology and Leuven Food Science and Nutrition Research Centre (LFoRCe), Kasteelpark Arenberg 23, PB 2457, 3001 Leuven, Belgium, Leuven, Belgium

Plant-based shakes are emulsified systems consisting of components isolated from their natural source. Different techniques can be applied to prepare these shakes including a simple mixing step potentially followed by high pressure homogenisation (HPH). These techniques may affect the macronutrients microstructural organisation which might, in turn, impact digestive functionality. In this work, shakes were prepared (5% oil, 6% protein, 1% lecithin, 88% water) (w/w) using two techniques, only mixing versus mixing followed by HPH, as well as two sequences, processing all ingredients together versus split-stream processing of particular ingredients. Shakes only mixed consisted of large, undissolved, irregular particles (1-100 µm). Eventually, this type of microstructural organisation resulted in a relatively low lipid and protein digestion after 2h of gastric digestion (9% and <1%, respectively). In contrast, shakes that were subjected to HPH displayed small, highly dissolved, homogeneous particles (<10 µm). As a result, lipids and proteins were digested to a significantly higher extent in the stomach (40% and 10%, respectively). The small intestinal digestion kinetics indicated a significant impact of proteins on lipolysis but no significant effect of lipids on proteolysis. These results highlighted the relevance of food processing on macronutrient microstructure and further gastrointestinal functionality.

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The interference of dietary fibre with lipolysis depends on the method of administration of β-glucans with different solubilisation processes

Kathrin Haider[1], Frederick Warren[1], David Hayashi[2], David Neville[3], Peter Wilde[1]
[1]Quadram Institute Bioscience, Norwich, United Kingdom, [2]Mondelēz International LLC, Chicago, United States, [3]Mondelēz Global Science Centre, Reading, United Kingdom

Background: Human studies show that sufficient dietary fibre (DF) intake helps maintain a healthy body weight, irrespective of calorie restriction. A potential mechanism is reduced lipid digestion, which is hypothesised to depend on the DF composition, structure and physico-chemical properties.
Methods: Barley and oat bran fibre (intact and extracts), with different physico-chemical properties including β-glucan (BG) content and molecular size were digested together with an O/W emulsion *in vitro* under simulated gastric conditions followed by duodenal conditions in a pH-stat where bovine bile extract was added. Additionally, lipase activity assay in absence of bile salts was assayed. Viscosity and other physico-chemical properties were measured.
Results: Barley bran and its extracted BG appear more potent in delaying lipolysis than oat bran and its BG extracts, despite our recent results showing oat bran retains more bile salts. A viscosity effect was observed at the higher oat bran concentrations. The lipase activity was reduced by both barley and oat bran in absence of bile salts.
Conclusion: Cereal DF has the potential to slow down lipid digestion via increased viscosity, bile salt and/or lipase interaction. Presence of insoluble DF seems to play a role in the solubilisation and consequential effects of the soluble fibre.

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What are the mechanisms of gastrointestinal lipases adsorption onto heterogenous biomimetic vegetal membranes?

Jeanne Kergomard[1,2], Frédéric Carrière[3], Gérard Lambeau[4], Gilles Paboeuf[1], Nathalie Barouh[5], Pierre Villeneuve[5], **Claire Bourlieu-lacanal[2]**, Véronique Vié[1]
[1]IPR Institute of Physics, UMR UR1 CNRS 6251, Rennes 1 University, Rennes, France, [2]INRAE/UM/Institut Agro Montpellier UMR 1208 IATE, Montpellier, France, [3]Aix-Marseille Université, CNRS, UMR7281 Bioénergétique et Ingénierie des Protéines, Marseille, France, [4]Université Côte d'Azur, Centre National de la Recherche Scientifique, Institut de Pharmacologie Moléculaire et Cellulaire, UMR7275 Valbonne Sophia Antipolis, Sophia Antipolis, France, [5]CIRAD, UMR QUALISUD, F34398 Montpellier, France, [6]Qualisud, Univ Montpellier, Avignon Université, CIRAD, Institut Agro, Université de La Réunion, Montpellier, France

Gastrointestinal lipases are crucial for lipid hydrolysis and must get adsorbed onto the substrate interface prior lipolysis. Such adsorption had not been deeply investigated on biomimetic vegetal membranes. Our objective was thus to characterize such adsorption and lipolysis using complementary biophysical tools (tensiometry, ellipsometry, atomic force microscopy) and *in vitro* digestion.
Heterogenous monolayers based on galactolipids, phospholipids, and phytosterols were used. Four lipases were studied: i) gastric lipase (GL), ii) pancreatic lipase 2 (PLRP2), (iii) pancreatic triacylglycerol lipase and its cofactor, colipase (PTL/coPTL), and (iv) pancreatic secreted phospholipase A2 (sPLA2-IB).
A strong surfactant property of GL and its preferential adsorption onto expanded lipid phase and at the phase boundary were observed, in line with previous results on milk fat globule membrane. With PLRP2, changes in surface pressure indicated a lipolytic activity. Such variation was not observed upon PTL/coPTL adsorption, and may be related to the absence of activity of PTL on polar lipids. The injection of sPLA2-IB did not indicate a clear trend of lipolysis on the lipid film but changed the morphology of condensed domains.
This study is a step forward to understand the interactions of gastrointestinal lipases with plant lipid membranes, an overlooked aspect of lipid digestion.

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Mechanisms of interesterified fat digestibility in a muffin matrix

Louise J Salt[1], **Giuseppina Cockram[1,4]**, Mary L Parker[1], Mahamoud Hussein[1], Charlotte E Mills[2,3], Robert Gray[2], Sarah E Berry[2], Peter J Wilde[1]
[1]Food Innovation and Health Programme, Quadram Institute Bioscience, Norwich, United Kingdom, [2]Department of Nutritional Sciences, King's College , London, United Kingdom, [3]Department of Food and Nutritional Sciences, University of Reading, Reading, United Kingdom, [4]Department of Chemical, Biological, Pharmaceutical and Environmental Science, University of Messina, Messina, Italy

Background: Trans-fats can be replaced by interesterified (IE) fats in foods to reduce the impact of trans-fats on health. However, little is known about their metabolism. Here we report on the mechanisms involved with IE fat digestion kinetics using same IE and non-IE fats.
Methods: Muffins baked using an IE fat [80% palm stearin/20% palm kernel oil], non-IE fat [same fatty acid composition as IE fat] and rapeseed oil (RO) were digested under simulated conditions using a dynamic gastric model (DGM).
Results: Interesterified fat and non-IE fat were largely solid in the gastric phase and strongly associated with the crumb. This led to significant phase separation and a delay in gastric emptying of fat. Whereas RO was visible as liquid droplets and most droplets separated from the crumb and were emptied more consistently from the stomach No significant difference in lipolysis rates was observed between IE/non-IE fats but the rate for RO fat was slower (due to long chain PUFAs).
Conclusions: Interesterification did not affect digestibility, but creaming of the hard fat in the stomach caused delayed gastric emptying. The rate and extent of lipolysis was determined by the amount of fat available and the structure of the fat.

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Impact of Cas9-mediated gene mutations in the starch branching enzymes on potato starch structural characteristics and *in vitro* digestion behaviour

Hannah Harris[1], Alison M. Smith[2], Frederick J. Warren[1]
[1]Food Innovation and Health, Quadram institute Biosciences, Norwich, United Kingdom, [2]Molecules from Nature, John Innes Centre, Norwich, United Kingdom

Introduction: Starch branching enzymes (SBE) are a key requirement for the production of amylopectin in potato starch. There are two isoforms of SBE; SBE1 and SBE2. Cas9-mediated gene mutations of SBE1 and SBE2 have been demonstrated to alter the amylopectin fine structure as well as granule morphology.
Method: We performed analysis on 4 different lines of Cas9 mutated starch and a WT control. These were; 4-44 (complete SBE1/SBE2 mutation), 212-36, 227-25 (partial SBE1/ SBE2 mutation) and 230-51 (SBE2 only mutation). Digestibility of these starches was measured using an *in vitro* model of digestion, in different states.
Results: The raw starches were poorly digested for all the lines. Freshly gelatinised starch showed very similar digestion behaviour between the WT and mutant starches. Differences were observed after 18 hours of retrogradation which were magnified after 7 days of retrogradation where two groups were identified. WT and 227-25 were 53.9% and 59.6% digestible, while 4-44, 230-51, and 212-36 were 44.7%, 46.7%, and 44.4% digestible, respectively.
Discussion: Surprisingly mutations in SBE did not alter digestion of freshly gelatinised starch. However, significant reductions in digestibility of some mutant lines relative to WT were observed following retrogradation were observed. This suggests that Cas-9 modification may be a novel route to obtain reductions in starch digestibility in potato.

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Towards innovative lentil ingredients with different degrees of nutrient bioencapsulation: consequences for *in vitro* starch and protein digestion

Dorine Duijsens[1, 2], Tara Grauwet[1, 2]
[1]Lab of Food Technology, KU Leuven, [2]Lab of Food Technology, KU Leuven

Pulses are put forward as part of healthy and sustainable (more plant-based) diets. Pulse nutrients are bioencapsulated inside cotyledon cell walls, surviving upon cooking followed by mechanical disintegration. This barrier attenuates amylolysis, contributing to the low glycemic index of cooked pulses. While consumption remains low in most western countries, incorporating pulse ingredients into staple foods could increase their consumption without requiring drastic dietary habit changes.
Traditionally, pulse flours are produced by milling raw seeds, completely disrupting cellular barriers. In this recently published work, the *in vitro* starch and protein digestibility was evaluated for lentil ingredients with different degrees of nutrient bioencapsulation. Next to isolated cotyledon cell (ICC) powders, innovative whole precooked lentil (WL) powders, high in protein and fiber, were introduced. Though the abundance of individual cells was lower in WL compared to ICC, both ingredients showed significant attenuation of starch and protein hydrolysis compared to a raw-milled lentil flour. The applied cooking time influenced nutrient digestibility by affecting (i) the yield of ICC and the contribution of individual cells in WL, and (ii) the susceptibility of the cell to digestive enzyme action. The developed ingredients show important potential for application in healthy low glycemic foods rich in pulse protein.

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Impact of the extent of thermal denaturation and protein concentration on the digestibility and bioactive properties of milk protein concentrate

Mohammadreza Khalesi[1], Dick FitzGerald[1]
[1]*University of Limerick, Limerick, Ireland*

Background: Protein denaturation and the protein concentration may impact the digestibility of milk protein concentrate (MPC).
Method: Two MPC samples, i.e., low heat-denatured MPC (MPCS1) and high heat-denatured MPC (MPCS2) were subjected to the INFOGEST in-vitro digestion protocol. Digestion was assessed using two different substrate concentrations, i.e., 2% (w/v, L-MPC85) and 20% (w/v, H-MPC85). Sampling was carried out at the end of the gastric (GD) and the intestinal digestion (GID) phases. Samples were compared in terms of their digestion properties and *in vitro* biological activities.
Results: The degree of hydrolysis (DH) of the GID samples was higher compared to the GD digested samples. Moreover, the DHs of L-MPC were higher than the H-MPC digested samples. Molecular mass distribution analysis showed a lower extent of gastric digestion for L-MPCS2 compared to L-MPCS1 and a lower extent of gastric digestion for H-MPCS1 compared to H-MPCS2. The extent of digestion for all GID samples was similar. The highest dipeptidyl peptidase IV inhibitory activity and the highest antioxidant activities were observed for MPC samples following GID treatment.
Conclusions: The extent of whey protein thermal denaturation and the initial substrate concentration specifically impacted the *in vitro* digestibility of the MPC85 specifically at the gastric phase.

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Establishing a multireactor semi-dynamic *in vitro* system to determine more physiologically relevant digestion kinetics

Sarah Verkempinck[1], Dorine Duijsens[1], Jessica Guevara-Zambrano[1], Daphne Michels[1], Tara Grauwet[1]
[1]KU Leuven, Laboratory of Food Technology, Leuven, Belgium

Semi-dynamic digestion models aim to fill the gap between static and dynamic *in vitro* models implementing particular dynamic conditions to specific phases of the gastrointestinal tract. However, few systems are available allowing independent sampling throughout digestion to determine digestion kinetics. Therefore, we introduce an automated system (BioXplorer100, H.E.LGroup) to simulate semi-dynamic digestion conditions simultaneously in multiple reactors. Reproducibility was shown for two foods: a liquid Ensure® drink, and a solid lentil sample. When comparing static and semi-dynamic digestion kinetics, a different digestion pattern was observed. In the static case, a fast hydrolysis rate was observed until a plateau was reached. Oppositely, for the semi-dynamic case, a delayed start of the hydrolysis process was noticed. In the gastric phase, this was explained by the decreasing pH and large pH-dependency of pepsin activity. In the small intestine, the lag phase was shorter, yet clearly present. We related this to the gradual enzyme (and bile salt) secretion, which had to find their substrate before hydrolysis could start. Generally, we showed that the BioXplorer100 could be used to mimic semi-dynamic digestion conditions and determine more physiological relevant digestion kinetics. Next, mathematical modelling should be considered to take new steps towards predictive in silico models.

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Development of a realistic *in vitro* digestion model (RGM) coupled UV-VIS-SWNIR fibre optics spectroscopy

Daniel Madalena[1], Ana Pinheiro[2], Óscar Ramos[2], António Vicente[1]
[1]CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal, [2]CBQF - Centre of Biotechnology and Fine Chemistry, Catholic University, Porto, Portugal

Background: The development of realistic gastric models unlocked the possibility of studying important digestion phenomena occurring during the digestion of food (e.g., retropulsion). Understanding the dynamics of food digestion in real-time, without sample manipulation, is still a challenge, but brings a huge potential in providing important insights regarding the dynamic process of food digestion (e.g., real time nutrient release kinetics)
This study presents a realistic 3D printed *in vitro* gastric model coupled with ultraviolet-visible-short-wave-near-infrared (UV-VIS-SWNIR) spectroscope that can be used for real time quantification of nutrients/bioactive compounds.
Methods: The INFOGEST semi-dynamic *in vitro* protocol was used to simulate the digestion of rice (model food). The spectroscope was calibrated for glucose analysis, and the spectra were pre-processed and both chemometric and machine learning techniques were used for glucose quantification using the correlation coefficient as assessment metric.
Results: The machine learning algorithms showed to be more accurate at predicting glucose release during the *in vitro* gastric digestion.
Conclusions: The gastric compartment development techniques provide the opportunity to develop a potential standard dynamic *in vitro* gastric model. Furthermore, it was possible to accurately measure and quantify glucose release during the *in vitro* digestion process, in real time, using UV-VIS-SWNIR fibre optics spectroscopic.

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Effect of low acyl gellan gum on white rice starch hydrolysis and glycaemic index: a static *in vitro* digestion study

Norah Alshammari[1], Syahrizal Muttakin[2], Ourania Gouseti[3], Serafim Bakalis[3], Alison Lovegrove[4], Guruprasad P. Aithal[1], Moira A Taylor[5], Luca Marciani[1]

[1]Nottingham Digestive Diseases Centre And National Institute For Health Research (NIHR) Nottingham Biomedical Research, Nottingham, United Kingdom, [2]Indonesian Ministry of Agriculture, Jakarta, Indonesi, [3]Food Analytics and Biotechnology, University of Copenhagen, , Copenhagen, Denmark, [4]Rothamsted Research, , UK, [5]School of Life Sciences, University of Nottingham, UK and National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, Nottingham University Hospitals NHS Trust and University of Nottingham, Nottingham, UK

Background: Addition of hydrocolloids when cooking rice in water may reduce starch hydrolysis. This study investigated the effect of adding different doses of low acyl gellan gum (LAGG) on white rice during cooking.

Method: A static *in vitro* digestion model (Brodkorb 2019) was used to determine starch hydrolysis. Four different samples were evaluated: jasmine rice control (A) or cooked with 1% (B), 2% (C) or 3% (w/rice w) LAGG (D). Glucose release was determined using a sugar reduction assay (PAHBAH). The colour was compared with the maltose standard curve at consecutive time points for 2 hours. The estimated glycaemic index was calculated (Goni 1997).

Results: The amount of LAGG added to the water during cooking affected the starch hydrolysis. Starch hydrolysis was 84%, 62%, 50% and 40% for samples A, B, C and D respectively. The addition of LAGG to jasmine rice reduced the estimated glycaemic index value by 8%, 20% and 24% respectively for samples B, C and D compared with the control.

Conclusions: Addition of LAGG during cooking reduced starch digestion and estimated glycaemic index of white jasmine rice with a dose-response. LAGG might be an effective way to reduce glycaemic response following white rice consumption in humans.

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In vitro DIAAS of sustainable proteins using the INFOGEST digestion protocol

Renata Ariens[1], Dianne van de Berg-Somhorst[1], Karim El Bachrioui[1], Anouk Boudewijn[1], Ron van den Dool[1], Aard de Jong[1], Harry Wichers[1, 2], Jurriaan Mes[1], Shanna Bastiaan-Net[1]
[1]Wageningen Food & Biobased Research; Wageningen University and Research, Wageningen, Netherlands, [2]Laboratory of Food Chemistry; Wageningen University and Research, Wageningen, Netherlands

Background: Using the INFOGEST static digestion model we evaluated several sustainable proteins for their digestibility and quality. The protein concentrates were based on bovine plasma, corn, lesser mealworm, *Fusarium*, pea, potato and yeast while whey and an empty control digest were used as a reference.
Methods: The static INFOGEST digestion protocol was expanded with a filtering step using a 5 kDa cross-flow filter to separate the undigested peptides (retentate), from the smaller peptides and amino acids (filtrate). Based on the obtained data we determined a nitrogen conversion factor for these sources, degree of hydrolysis, true ileal digestibility, *in vitro* digestible indispensable amino acid score (IVDIAAS) and total absorbable essential amino acids.
Results: Whey, blood plasma and yeast have a high IVDIAAS (90.6-85.8%) followed by corn, potato, pea (55.0-73.8%), where corn had the lowest IVDIAAS due to lower amounts of lysine. The IVDIAAS from whey and corn correlated well with the available *in vivo* DIAAS from literature.
Discussion: Although further optimisation and validation of our IVDIAAS method with *in vivo* data is required, we expect that the method is a useful tool to evaluate protein quality and bio-accessibility and could complement or replace animal based models in the future.

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A novel biorelevant *in vitro* dynamic digestion simulator reproducing the biomechanics of the gastrointestinal tract

Ines Greco[1], Olivia Menard[1], Jiajun Feng[1], Steven Le-Feunteun[1], Romain Jeantet[1], Didier Dupont[1]
[1]INRAE STLO UMR 1253, Rennes, France

Background: The biomechanics of the gastrointestinal tract strongly influence food disintegration and digestion *in vivo*. Most currently available *in vitro* dynamic digestion models lack, however, the ability of mimicking the oro-gastrointestinal morphology and contractions, possibly overlooking the effect of mechanical forces in the digestive process.
Methods: We investigated the operation of a biomechanically-relevant digestion simulator equipped with a silicon real-size model of the human stomach (dynamic *in vitro* human stomach system, DHS-IV) using dairy food matrices (liquid, semisolid and solid). The system mimics morphology, anatomical structures and biochemical environments of oesophagus and gastrointestinal tract present *in vivo* in adult humans. A set of precisely controlled rollers mimics the dynamic aspects and peristaltic contractions.
Results: We reproduced the gastric emptying curves and gastric digestion conditions for different food matrices observed *in vivo*, recreating human chewing and ingestion rates. We describe here the key structure and functioning of the digestor, the operational parameters that influence mostly the gastric emptying rate, and the adjustments that have shown to be necessary to control gastric emptying and digestion parameters.
Conclusions: The biomechanically-relevant *in vitro* digestion system (DHS-IV) can provide an effective approach for studying the structural and physicochemical changes during digestion in the stomach.

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Development and validation of an *in vitro* colonic model of gut microbiota dysbiosis associated to obesity

Ophélie Uriot[1], Charlotte Deschamps[1], Morgane Brun[1], Mélanie Pouget[2], Lucie Etienne-Mesmin[1], Monique Alric[1], Cyril Chaudemanche[3], Yves Boirie [2,4] Stéphanie Blanquet-Diot[1]
[1]UMR 454 MEDIS, Université Clermont Auvergne, Clermont-Ferrand, France, [2]Service de Nutrition Clinique, Centre Hospitalo-Universitaire, Clermont-Ferrand, France, [3]General Mills France, Boulogne-Billancourt, France, 4UMR 1019 INRAe, Unité de Nutrition Humaine, Université Clermont Auvergne, Clermont-Ferrand, France

Background: Obesity is a high prevalent multifactorial disease strongly associated with nutritional disorders and gut microbiota perturbations. To date, there is no relevant *in vitro* model reproducing the nutritional, physicochemical and microbial parameters of the obese human colon.
Methods: An intensive literature review was performed to adapt the Mucosal Artificial Colon (M-ARCOL) model to the specific colonic environment of obese patients (pH, retention time and composition of ileal effluents). Stools from 9 donors (4 healthy and 5 obese) were used to inoculate two bioreactors ran in parallel, set-up to reproduce either healthy or obese parameters.
Results: Shifs in microbiota activity and composition were observed with obese parameters, in accordance with *in vivo* data. Less methane but more short chain fatty acids and associated energy were produced. A decrease in obesity-associated marker populations (Archaea, Akkermanciaceae, Rikenellaceae and Christensenellaceae) was also observed in lumen and mucus-associated microbiota, together with a lower bacterial diversity. Interestingly, when applying healthy parameters on obese stools opposite trends were obtained demonstrating gut microbiota resilience.
Conclusion: Obese M-ARCOL model can be used as an alternative to *in vivo* animal assays in preclinical trials to perform mechanistic studies and evaluate nutritional strategies aiming to restore gut microbiota eubiosis.

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Protein profile and simulated digestive behavior under infant consensus conditions of breast milk from overweight and normal weight mothers

Beatriz Miralles[1], Silvia Sánchez-Hernández[2], Pablo Jiménez-Barrios[1], Laëtitia Théron[3], Manuel Olalla-Herrera[2], Isidra Recio[1]

[1]Cial (CSIC-UAM), Madrid, Spain, [2]Department of Nutrition and Bromatology, University of Granada, Granada, Spain, [3]INRAE UR370 Qualité des Produits Animaux, F-63122 Saint Genès-Champanelle, France

Human milk proteins have shown to vary in concentration and distribution through lactation. While some regulatory components, such as hormones, show association with the mothers' body mass index, BMI, there is limited information on the possible influence of this condition on the whole protein distribution. The objective of this work was to evaluate the protein profile to identify differences in protein expression. Furthermore, individual samples were subjected to an *in vitro* digestion model that takes into account the specificities of full-term newborns and the digestion products have been compared with data available from the digestive contents in infants after intake of human milk. The 2D plot representation of the MALDI spectra taking the signals with highest variance and the application of predictive models resulted in very favorable recognition levels according to the donor BMI. The newborn digestion model resulted in a degradation rate of the most abundant human milk proteins, α-lactalbumin and lactoferrin, similar to the observed *in vivo*. Although the peptide fingerprint from digests did not discriminate the samples by the donor BMI, in the data altogether, resistant protein's identity and a large number of specific sequences closely resembled those previously found in newborn digests.

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The complexity of human bile and how to replace it for *in vitro* digestion studies

Dorota Dulko[1], Ilona Kłosowska-Chomiczewska[1], Teresa Del Castillo-Santaella[2], Julia Maldonado-Valderrama[2], **Adam Macierzanka[1]**
[1]Gdansk University of Technology, Gdansk, Poland, [2]University of Granada, Spain

Animal bile extracts have widely been used in *in vitro* digestion models as they are considered to reflect the complexity of bile salts (BS) in human bile (HB). Little attention has been given to the role of other biliary surfactants (e.g., phospholipids, PLs).
We collected HB samples from 76 individuals and analysed their BS/PL profiles/concentrations. The interfacial behaviour of HB during *in vitro* small intestinal lipolysis was studies with a pendant drop film balance equipped with a subphase multi-exchange device. We found that the evolution in the oil-water interfacial tension during the lipolysis with HB could be replicated by substituting the HB with simple mixtures of individual BS and phosphatidylcholine (PC).
We also used bovine milk β-lactoglobulin (βLg) to assess the BS impact on intestinal proteolysis. HPLC analysis showed the protein digestibility increased dramatically upon increasing BS concentration (0-10 mM). The effect was consistent regardless of whether individual BS or real HB were applied, but the exact impact of HB could only be closely replicated if individual BS were used in mixtures with PC during the digestion.
This is the first time when simple BS/PC mixtures have been validated as tuneable, human-relevant substitutes of difficult-to-obtain HB for *in vitro* proteolysis and lipolysis studies.

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MRI: a useful tool to link *in vitro* and *in vivo* (human) digestion

Ruoxuan Deng[1], Paul Smeets[1], Monica Mars[1], Anja Janssen[1]
[1]*Wageningen University & Research, Wageningen, The Netherlands*

Validation of *in vitro* digestion models remains a big challenge due to their inherent simplifications, such as the absence of feedback mechanisms. Here, we posit that Magnetic Resonance Imaging (MRI), a non-invasive versatile technique, can be used to monitor both *in vitro* and *in vivo* digestion.
Gastric digestion of protein gels was monitored by MRI in a semi-dynamic *in vitro* gastric model (MR-GAS) and a human trial (n=18). MRI measurements included anatomical images and longitudinal (T_1) and transverse (T_2) relaxation times of gastric chyme. In the MR-GAS, protein hydrolysis and pH change were assessed in the digesta. The *in vivo* anatomical MRI scans were used to determine the solid and liquid gastric contents as well as gastric secretion and emptying rates.
T_1 and T_2 results from MR-GAS with gastric dynamics from the literature were different from *in vivo* data. After applying the mean *in vivo* dynamics in MR-GAS, the digestion rate of protein gels were comparable with the human data. Therewith the observed changes in protein hydrolysis and pH in the MR-GAS model was useful to interpret *in vivo* T_1 and T_2 data.
In conclusion, MRI can contribute to informing digestion models and linking *in vitro* and *in vivo* research.

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Development, validation and application of an *in vitro* DIAAS method based on the INFOGEST protocol

Raquel Sousa[1, 2], Isidra Recio[2], Dominique Heimo[3], Sébastien Dubois[3], Paul Moughan[4], Suzanne Hodgkinson[4], Reto Portmann[1], Lotti Egger[1]
[1]Agroscope, Bern, Switzerland, [2]Instituto de Investigación en Ciencias de la Alimentación (CIAL, CSIC-UAM), Madrid, Spain, [3]Agroscope, Posieux, Switzerland, [4]Riddet Institute, Massey University, Palmerston North, New Zealand

Background: Protein remains the only macronutrient that requires human or animal-based bioassays to evaluate its nutritional quality. In line with the prevailing trend to minimize animal testing, there is a demand for the use of validated *in vitro* digestion methods.
Method: The INFOGEST *in vitro* digestion static protocol shows promise to be a robust and reproducible screening tool for assessing food digestibility and *in vitro* DIAAS values.
Results: After optimization, *in vitro* digestibility values were obtained for different foods (black beans, pigeon peas, bran and peanuts) and ingredients (zein, whey protein, and collagen). In addition, to illustrate how food processing affects digestibility, we have determined the *in vitro* DIAAS values of two highly transformed veggie burgers, containing extruded ingredients and compared the results with a meat burger, before and after grilling, respectively. Beef burger was better digested than veggie burgers, yielding DIAAS values. Texturizing processes did not significantly affect protein digestibility, DIAAS values and grilling only led to a slight decrease in pea-faba burgers but not in soy and meat burgers.
Conclusions: *In vitro* digestibilities based on the INFOGEST protocol showed a high correlation with *in vivo* results obtained within the PROTEOS project.

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Effect of whey to casein ratio in goat infant milk formulation on protein digestibility, amino acid bioavailability and health biomarkers

Alina Kondrashina[1], Linda Giblin[2], Jonathan Lane[1]
[1]H&H Group, H&H Research, Global Research and Technology Centre, Moorepark,, Ireland, [2]Teagasc Food Research Centre, Moorepark, Fermoy, Ireland

Ratio of whey:casein is an important parameter in infant milk formulation (IMF) affecting its digestibility. In first stage IMF this ratio is often 60:40, closely resembling human milk, however, a 20:80 ratio is used for "hungrier" babies. In this study, we compared goat IMFs with whey:casein ratios of 62:38 (HWC) and 20:80 (LWC) to human milk for protein digestion and health promoting indicators.
IMFs and human milk were digested with an infant adapted Infogest digestion protocol. Digestibility of protein was assessed by degree of hydrolysis, peptide size distribution and amino acid analyses. An intestinal barrier model (epithelial Caco-2 and goblet HT-29MTX cells) was used to study bioavailability of nutrients and functional effects. Immunomodulation by goat IMFs was assessed via quantified secretion of interleukins from THP-1 macrophages.
After digestion HWC had significantly higher degree of hydrolysis and amino acids release compared to LWC and human milk ($P<0.05$). LWC, HWC and human milk had similar immunomodulatory properties and intestinal barrier integrity measured by transepithelial electrical resistance, resulting in similar total amounts of bioavailable amino acids ($P>0.05$).
Altogether, HWP demonstrated higher digestibility of protein compared to LWP. Interestingly, bioavailability of amino acids and functional effects were similar for LWC, HWC and human milk.

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The effect of phospholipids on the small intestinal lipolysis.

Adam Macierzanka[1], Julia Maldonado-Valderrama[2], Teresa del Castillo-Santaella[2], **Katarzyna Klosowska[1]**
[1]Gdansk University of Technology, Gdansk, Poland, [2]University of Granada, Granada, Spain

Background: The small intestinal lipolysis takes place at the oil/water interface, hence the rate of this reaction can be influenced by physiological surfactants released with bile. There are two major surface-active components of bile – bile salts (BS) and phospholipids (PL). The role of PL in the lipolysis has not been extensively studied so far.
Method: The interplay of the two biosurfactants was studied at the interface during the lipolysis, and the specific effect of PL on the digestion of food emulsion was assessed using the in-vitro static model of human digestion.
The examination of interfacial phenomena occurring during the intestinal lipolysis, at various concentrations of PL and BS, has been performed with a subphase multi-exchange device - the OCTOPUS. The in-vitro static digestion of food emulsion was investigated using a pH-stat method.
Results: Our results provide insights into how the fluctuations in the BS/PL ratio influence the lipolysis efficiency. This brings about a fundamental view of the role of PL in the interfacial aspects of intestinal lipolysis.
Conclusions: The BS/PL ratio can vary in the human small intestine for various physiological and pathological reasons, therefore PL may play an important role in the intestinal lipid digestion.

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The protein digestibility of NUTRALYS® pea protein is high regardless of the methodology used

Caroline Perreau[1], Florence Guillin[1,2], Juliane Calvez[2], Claire Gaudichon[2], Delphine Genete[1], Florence Ringard[1], Quentin Bailleul[1], Catherine Lefranc-Millot[1], Laetitia Guerin-Deremaux[1]
[1]Roquette, Lestrem, France, [2]UMR PNCA, AgroParisTech, INRAE, Université Paris-Saclay, Paris, France

Demand for alternative protein sources with a high quality is soaring, supported by sustainability and positive health effect reasons. Evaluation of protein digestibility can be achieved using different methodologies: clinical, *in vivo* or *in vitro* studies. The current study aims at evaluating the digestibility of NUTRALYS® pea protein and casein with these different methodologies.
Four different methodologies were used to evaluate protein digestibility. A human trial was conducted on 15 healthy volunteers. An *in vivo* study was conducted on 10 rats per group. Concerning the *in vitro* experiments, digestibility of both tested ingredients was assessed with two different analyzes after an enzymatic digestion.
NUTRALYS® pea protein and casein demonstrated a true ileal amino acid digestibility of 94%±3 and 97%±1 respectively with the human trial, a true fecal nitrogen digestibility of 97%±2 and 98%±1 respectively with the *in vivo* experiment, an amino acid digestibility of 100% for both with the first *in vitro* methodology and a protein digestibility of 94%±1 and 95% respectively with the second *in vitro* methodology.
The two evaluated proteins displayed a high protein digestibility whatever the four methodologies used. These results show that the plant-based protein NUTRALYS® can be digested at a level comparable to milk protein.

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Porcine jejunal brush border membrane vesicles: structural and functional proteomic characterization

Gianluca Picariello[1], **Gianfranco Mamone[1]**
[1]National Research Council (CNR), Avellino, Italia

Background: Despite the physiological importance of the hydrolases from the intestinal brush border membrane (BBM), a step simulating the intestinal digestion has not been included yet in the harmonized protocols of *in vitro* digestion, due to the commercial unavailability of these enzymes and lack of consensus for the conditions of use. The proper utilize of BBM requires a detailed investigation of their enzymatic composition.
Experimental: Specimens of intestinal jejunum were obtained from adult pigs. BBM vesicles were purified optimizing previously described methods and assayed for aminopeptidase N and dipeptidyl peptidase IV activity. Shotgun proteomics was carried out with a nanoflow UHPLC-Q Exactive Orbitrap. Protein identification, biostatistics, and relative quantification (iBAQ) were performed using MaxQuant. Protein functional classification was performed with KEGGS and GO PANTHER.
Results: Overall, 1586 proteins were identified. The predominant enzyme fraction (> 190 gene products) was represented by hydrolases, including peptidases, lipases, and glycosidases. Aminopeptidase N represented > 50% of the peptidase abundance. Notably, BBM also contains a complex array of protease inhibitors that may modulate the activity of hydrolases.
Conclusions: Considering the similarity with the human counterpart, intestinal porcine BBM are suited for simulating the human small intestinal digestion.

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Why does chymotrypsin hydrolyse only 35% of specific peptide bonds?

Gijs Vreeke[1], Peter A. Wierenga[1]
[1]*Wageningen University, Wageningen, The Netherlands*

Background: Chymotrypsin is used in biochemistry textbooks to explain protease specificity, with a considered specificity for aromatic and hydrophobic amino acid residues. However, the experimental DHmax turns out to be only ~35% of the theoretical DHmax calculated with this specificity. To investigate this, we need to follow peptide formation by this broadly specific enzyme.
Method: UPLC-PDA-MS was used to quantify peptide concentrations during chymotrypsin hydrolysis of α-Lactalbumin, β-Lactoglobulin and β-casein. These were used to determine the specificity, preference and selectivity of chymotrypsin.
Results: Chymotrypsin showed a preference for phenylalanine, tyrosine, tryptophan, methionine and leucine (73% of the cleavage sites) but was able to hydrolyse peptide bonds after almost all other amino acids (27%). For the peptide bonds within the specificity, ~40 % of the cleavage sites were not hydrolysed. For 73 % of the cleavage sites of phenylalanine, tyrosine, tryptophan and methionine that were not hydrolysed, hindrance could be explained by a proline present in the P3, P1' or P2' position. For leucine, only 34 % of the non-hydrolysed cleavage sites had a proline in these positions.
Conclusion: The automated method allowed characterisation of hydrolysates from a-specific proteases. Hindrance by proline did not explain all missed cleavages within the specificity.

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Lactoferrin protein complexes of relevance in infant nutrition

David Goulding[1,2], Jonathan O'Regan[1,2], Lionel Bovetto[3], Karine Vidal[4], Nora M O'Brien[1], James A O'Mahony[1]

[1]University College Cork, Cork, Ireland, [2]Nestlé Development Centre Nutrition, Askeaton, Ireland, [3]Nestlé Institute of Material Sciences, Nestlé Research, Lausanne, Switzerland, [4]Nestlé Institute of Health Sciences, Nestlé Research, Lausanne, Switzerland

Background: Lactoferrin is an ~80 kDa, heat sensitive, iron-binding glycoprotein found in most mammalian milks. Lactoferrin is a multi-functional protein, with bioactivities including immune-modulation, anti-inflammatory and anti-microbial effects. The primary industrial application of lactoferrin is as an ingredient in infant nutrition, as it is the second most abundant whey protein in human milk. Lactoferrin has strong potential to interact with other proteins, attributable in part to its highly basic isoelectric point (~pH 9). This can enable it to interact with and form protein complexes, influencing digestion and bioactivity.
Method: Different types of protein complexes containing bovine lactoferrin were formed by manipulation of environmental conditions. The complexes were then subjected to static infant gastrointestinal digestion simulation, inspired by the INFOGEST protocol, prior to analysis using a cell culture model of the intestinal epithelia. Un-complexed controls were used throughout.
Results: The digestion and bioactivity of lactoferrin was altered depending on whether lactoferrin was complexed or not. Bioactivity of lactoferrin gastrointestinal digesta was improved when pre-complexed with select anionic proteins.
Conclusions: This study shows that the form in which lactoferrin is presented can determine how it is digested in gastrointestinal simulations and how well it delivers its bioactive functions.

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PDMS-based tools to mimic human digestion

Catarina Goncalves[1], Patrícia Rodrigues[1], Miguel Xavier[1], Victor Calero[1], Lorenzo Pastrana[1]
[1]*International Iberian Nanotechnology Laboratory (INL), Braga, Portugal*

The safety assessment of novel foods requires appropriate methodologies to mimic the human digestion process. Microfluidics offers the opportunity to develop relevant tools, such as organ-chip devices capable of high throughput and significant reductions in sample and reagent consumption and waste generation. Polydimethylsiloxane (PDMS) remains the first-choice material for the fabrication of microfluidic-based devices. Despite its numerous advantages, the hydrophobicity and high porosity of PDMS can lead to the absorption of small hydrophobic molecules that might interfere with the interpretation of experimental outcomes.
In this work, we investigated the relationship between the absorption of small hydrophobic compounds by PDMS and different factors such as solute/solvent pairings, solute concentration, and residence time. The absorption was evaluated by assessing the fluorescence intensity after flowing through the PDMS channel. Moreover, we tested several surface and bulk modifications of PDMS found in the literature and demonstrated that none of the tested modifications, though leading to increased hydrophilicity as determined by water contact angle measurements, effectively reduced the absorption of a test hydrophobic molecule. Thus, we conclude that when the absorption of small molecules by PDMS is deemed to be problematic, alternative materials should be considered for device fabrication.

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Bioaccessibility of the Fe, Mg and Zn from wheat grass in the human body: Caco-2 cellular model for intestinal absorption

Katarina Mišković Špoljarić[1], Sanja Grubišić[2], Marija Kristić[2], Miroslav Lisjak[2], Andrijana Rebekić[2]

[1]Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Josipa Huttlera 4, Osijek, Croatia, [2]Faculty of Agrobiotechnical Science Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, Osijek, Croatia

Background: Wheatgrass is health supplement rich with vitamins, antioxidants, minerals, and proteins. Permeability data obtained by Caco-2 intestinal model correlate with human *in vivo* absorption mimicking transport through enterocyte membrane. The aim of this study is to evaluate bioaccessibility of minerals (Fe, Mg, Zn) indispensable for cells activity.
Methods: The strain of wheat grass (Divana, Croatia) was evaluated in the form of fresh juice, digested juice, and powder before and after biofortification. Samples were collected every 15 minutes. Concentration in time dependant manner were estimated by AAS.
Results: Results of absorption for analysed elements indicate different rate of absorption. After 45 minutes portion of absorbed elements are as follows: Fe (86.7 – 97.97 %), Mg (68.8 – 83.8 %) and Zn (65.7 – 81.53 %). The highest rate of absorption for Fe and Zn is observed after 15 minutes while absorption of magnesium is reached after 30 minutes.
Conclusion: Results indicate passive model of transport for analysed minerals with the highs rate of absorption in the first 30 minutes indicating medium speed of absorption. Iron is better absorbed from biofortificated sample while magnesium and zinc are more effective absorbed from native sample.

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Gastrointestinal *in vitro* digestion and immunoregulatory effect of pomegranate oil extracted by clean technologies in Caco-2 cells

Celia Bañares[1], Noelia Carballeda-Sangliao[1], Joaquin Garcia-Cordero[1], Assamae Chabni[2], **Sonia de Pascual-Teresa[1]**, Carlos Fernando Torres[2]
[1]ICTAN-CSIC, Madrid, Spain, [2]CIAL-UAM-CSIC, Madrid, Spain

Pomegranate seeds contain up to 20% oil with a high content of punicic acid (85%), which is responsible of several bioactive activities in human health. In this work, pomegranate oils extracted by pressing (PP) and supercritical CO_2 (PSCF), have been studied in a static gastrointestinal *in vitro* digestion model to evaluate their digestibility and bioaccessibility. The micellar phases obtained were evaluated by an *in vitro* model of intestinal inflammation and Caco-2 cells exposed to the inflammatory mediator LPS. Inflammatory response was assessed by measuring the production of IL-6, IL-8 and TNF-α, and by evaluating the monolayer permeability. The results obtained indicate that PP extraction provides the highest amount of micellar phase (ca. 93%) with free fatty acids and monoacylglycerols as major components. The micellar phase obtained under PSFC extraction is ca. 82% with the same species composition. PP extracted oil shows an anti-inflammatory response, reducing the production of IL-8, IL-6 and TNF-α, and increasing TEER values in comparison with LPS stimulated cells. In the case of PSCF extraction, the reducing response of inflammation happens only for IL-8. The present work demonstrates the good digestibility, bioaccessibility and immunoregulatory response of both pomegranate oils, especially that obtained by PP extraction.

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Study on the bioaccessibility and bioactivity of health promoting compounds from purple corn after *in vitro* digestion

Marianela Rodriguez[2], Mariela Bustos[3], Luisina Monsierra[2], Pablo Mansilla[3], **Sonia de Pascual-Teresa[1]**

[1]Ictan-csic, Madrid, Spain, [2]Facultad de Ciencias Agropecuarias, UNC, Cordoba, Argentina, [3]Instituto de Ciencia y Tecnologia de los Alimentos Córdoba (ICyTAC), Conicet - UNC, Cordoba, Argentina

Background: Corn is one of the main cultivated crops worldwide. Additionally, purple corn has a high content of phenolic compounds, including anthocyanins, which have been related to a high antioxidant activity. During digestion, antioxidants compounds suffer different reactions that might modify their structure, affecting their absorption and bioactivity. The aim of this study was to stablish the bioaccessibility and bioactivity of antioxidants compounds from purple corn. Methods: The INFOGEST simulated *in vitro* digestion model was used (Brodkorb et al, 2019). Final digestion samples were used to treat caco-2 cells in the trans-well model (Hubatsch, et al 2007). Total polyphenol content (TPs Folin-Ciocalteu and Fast-blue), HPLC-DAD/MS-MS and antioxidant activity (by FRAP and DPPH) were evaluated before and after digestion. Results: HPLC-DAD/MS-MS analysis of purple corn extracts showed the presence of three main anthocyanins, cyanidin-3-(6''malonylglucoside), cyanidin-3-glucoside, and cyanidin-3-(3”,6”,dimalonylglucoside). The *in vitro* digestion process caused a reduction on TPs and in individual anthocyanins. The antioxidant activity was affected by the digestion process. At the end of digestion, the three main anthocyanins were still present in a lower concentration. Conclusion: Corn anthocyanins are bioaccessible in the INFOGEST model. However, their bioavailability is largely affected and the amounts that cross the intestinal barrier are neglible.

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Caco-2 cell response induced by peptides generated after digestion of heat treated egg white proteins

Elena Molina[1], Noelia Jurado-Chivato[1], Mónica Llorente-Sainz[1], Rosina López-Fandiño[1], Sara Benedé[1]
[1]Instituto de Investigación en Ciencias de la Alimentación (CIAL, CSIC-UAM), Madrid, Spain

Background. Heat treatment of food proteins may induce structural modifications, which will have a strong influence on how they interact with human fluids and cells. We aimed to assess the influence of the heat treatment in the digestibility of egg white proteins and the subsequent intestinal response generated by the digests.
Method. The digestion of ovalbumin (OVA), ovomucoid (OM), and lysozyme (LZ), untreated or previously heated, was performed *in vitro*. Digestibility of proteins was assessed by RP-HPLC and the response of Caco-2 cells exposed to peptides (< 10 kDa) generated during digestion by RT-qPCR.
Results. A remarkable amount of intact OVA and LZ remained after digestion of native proteins, while OM was completely degraded. Heat treatment at 65 °C for 30 min reduced digestibility of OVA and OM, while at 90 °C for 30 min resistant to degradation of OVA was increased. Digestibility of LZ was not affected by heat treatment. Peptides generated during digestion of the three untreated proteins induced IL-6 expression by Caco-2 cells while the ones obtained from treated OVA and OM reduced cytokine secretion compare to untreated proteins.
Conclusions. Heat treatment could favor the released of pro-inflammatory peptides during digestion of egg white proteins.

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Assessment of the effect of simulated gastrointestinal digestion on *in vitro* bioactivity of blue whiting (*Micromesistius poutassou*) protein hydrolysates.

Pádraigín A Harnedy-Rothwell[1, 2], Shaun Sharkey[3], Ryan A Lafferty[3], Finbarr PM O'Harte[3], Snehal Gite[4], Jason Whooley[4], Richard J FitzGerald[1, 2]

[1]University Of Limerick, Limerick, Ireland, [2]Health Research Institute, University of Limerick, , Ireland, [3]School of Biomedical Sciences, Ulster University, Coleraine,, Northern Ireland, [4]Bio-Marine Ingredients Ireland Ltd., Lough Egish Food Park, Castleblaney, Ireland

Protein hydrolysates from low-value underutilised fish species are potential sources of high-quality dietary protein and health-enhancing peptides. Six blue whiting soluble protein hydrolysates (BW-SPH-A_F), generated under different enzymatic hydrolysis conditions were shown to mediate anti-diabetic and antioxidant activity *in vitro*. The study aim was to assess the effect of simulated gastrointestinal digestion (SGID) on the stability of the BW-SPHs and their bioactivity. Degree of hydrolysis, molecular mass distribution, hydrophobicity and free amino acid analyses indicate that all BW-SPHs were hydrolysed during SGID. The *in vitro* antidiabetic (dipeptidyl peptidase–IV (DPP-IV) and insulin secretion from cultured pancreatic BRIN-BD11 cells) and antioxidant (oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP)) activity was affected to different extents. While a significant ($P<0.05$) reduction in DPP-IV inhibitory and insulin secretory activity was observed with BW-SPH-B, -D, -E and -F and BW-SPH-D and -F, respectively, following SGID their *in vitro* activity remained high. A significant increase ($P<0.05$) in ORAC activity was observed with BW-SPH-A, -B, -D, -F following SGID while the FRAP activity of all BW-SPHs decreased significantly during SGID ($P<0.05$). These results show that while the *in vitro* activity is modified following SGID, significant activity is predicted to be retained following oral ingestion.

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Pea seeds lacking anti-nutritional proteins have improved digestibility

Raquel Olias[1], Tracey Rayner[2], Clarie Domoney[2], Alfonso Clemente[1]
[1]CSIC, Granada, Spain, [2]John Innes Centre, Norwich, UK

The increasing demand on food production to meet the needs of a growing population, coupled to the need to protect the environment, makes it necessary to change the food production system. Legume crops are a great choice as food crops to fulfil many needs, including nurturing the human population and reducing the impact of agriculture on the planet. Peas are an excellent source of proteins, carbohydrates and micronutrients, but the presence of so-called anti-nutritional factors can reduce the bioavailability of nutrients. Among the anti-nutritional proteins are lectin, pea albumin 2 (PA2) and trypsin inhibitors (TI). We have investigated the impact of these proteins on protein digestibility and amino acid availability, using pea seeds lacking these proteins. Three genetic variants were used: i) wild type (WT); ii) seeds lacking trypsin/chymotrypsin inhibitors (TI mutant); and iii) seeds lacking TI, lectin and PA2 (Triple null mutant). *In vitro* digestions following the INFOGEST protocol were carried out. Significant differences in the degree of hydrolysis, protein profile and amino acid availability were found among pea variants. Proteins resistant to digestion were identified by LC-MS/MS. Our results indicate that pea seeds lacking certain proteins can be used in the development of novel foods with improved digestibility.

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Nutritional properties of a Colada drink supplemented with whey

Victoria García-Casas[2], Isabel Seiquer[1], **Raquel Olias[1]**
[1]CSIC, Granada, Spain, [2]University of Guayaquil, Guayaquil, Ecuador

Whey is an important industrial by-product of the dairy industry, resulting from cheese making, with a high nutritional value that has changed its consideration as an environmental problem to a source of bioactive compounds. In developing countries, like Ecuador, whey is discard without any treatment because of the high cost of processing it, creating an important problem for the environment and wasting a valuable product for a population with a high malnutrition issue. It is of great interest to find a direct use of whey in cheese factories as a food ingredient. Using whey as a substitute of water in a popular drink in Ecuador named Colada, made from fruit juice and cereals, will increase the nutritional value of the beverage and would require little processing for the industry. Using a Colada made with whey (70%), Maracuya juice (30%), barley (10%), sucrose (1%) and aromatic spices (1%) we have carried out *in vitro* digestions following the INFOGEST protocol. Analysis of the degree of hydrolysis, protein, fatty acid and amino acid profile after digestion revealed that this beverage is full of nutritional components that could have many health promoting properties.

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Impact of human saliva on proteolysis during *in vitro* gastric digestion of dairy products

Martine Morzel[1], Sylvaine Ramsamy[1], Gwénaële Henry[1], Steven Le Feunteun[1]
[1]INRAE - STLO, Rennes, France

Background: Saliva may play a role in digestion through its enzymatic content or physico-chemical impact on the food bolus or chyme. Salivary proteins also interact with tannins, which could counteract their effect on digestive enzymes. We here evaluated the impact of saliva on gastric proteolysis of solid or liquid dairy products, including one containing tannins.
Method: Swiss-type cheese (C), milk in water (M) or milk in black tea (T), all in presence of human saliva vs water, were digested using semi-dynamic (C) and static conditions (M,T), respectively. Proteolysis was monitored throughout gastric digestion by OPA assay in all samples. The degree of hydrolysis was also calculated for cheese using acid titration data.
Results: For cheese, the presence of saliva delayed slightly the initiation of proteolysis. OPA measurements suggested a small proteolytic inhibition by saliva. For milk, the presence of tannins increased gastric proteolysis (possibly by denaturing the pepsin substrates) but saliva had no substantial impact on OPA measurements, whether the matrix contained tannins or not.
Conclusion: In the conditions used, saliva had a moderate, if any, impact on gastric digestion of dairy products. Due to the documented effect of saliva on emulsions stability, the study could be extended to lipolysis.

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Improved estimation of *in vitro* protein digestibility of different foods using size exclusion chromatography

Anne Rieder[1], Nils Kristian Afseth[1], Ulrike Böcker[1], Svein Halvor Knutsen[1], Bente Kirkhus[1], Hanne Mæhre[1], Simon Ballance[1], Sileshi Wubshet[1]
[1]Nofima AS, Ås, Norge

Background: While the harmonized static INFOGEST model provides a physiologically relevant platform for simulated digestion, it needs to be combined with adequate analytical methods to enable quantification and comparison of protein digestibility in different foods.
Method: Digests of casein, chicken mince, beef entrecote, bread, pea protein concentrate and heated pea protein concentrate were analyzed by size exclusion chromatography (SEC) to estimate the proportion of small peptides potentially available for uptake. SEC was compared to other methods for protein digestibility determination including TCA or methanol soluble nitrogen and free NH2-groups.
Results: SEC could be combined with determination of total dissolved protein to calculate the % of short peptides per total protein as a physiologically relevant estimate of protein digestibility (DSEC). Absolute values and ranking of protein digestibility of samples differed between all methods, with DSEC values ranging from 87.6% (casein) to 57.8% (pea protein), which was between values calculated from TCA soluble nitrogen and free NH2-groups.
Conclusion: In contrast to other methods (TCA or methanol soluble protein, free NH2-groups), the proposed SEC based method gives separate insight into the two fundamental processes during protein digestion (solubilization and break-down), while maintaining the ability to rank digestibility of very different food proteins.

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Milk and egg white protein digests: *in vitro* DPP-IV-inhibitory activity and in situ insulinotropic activity of the absorbable fraction

Marta Santos-Hernández[1, 2], Maria Cermeño[1], Isidra Recio[2], Richard J. FitzGerald[1]
[1]Department of Biological Sciences, University of Limerick, Castletroy, Ireland, [2]Instituto de Investigación en Ciencias de la Alimentación, CIAL (CSIC-UAM), Nicolás Cabrera, 9, 28049, Madrid, Spain

Dietary proteins participate in the regulation of glucose homeostasis by different mechanisms, among others, by inhibiting dipeptidyl peptidase-IV (DPP-IV), enhancing the release of incretin hormones in enteroendocrine cells and/or inducing insulin secretion in pancreatic β-cells. Therefore, the *in vitro* and the in situ DPP-IV inhibitory activity, and the in situ insulinotropic activity of simulated gastrointestinal digests of milk and egg white proteins was evaluated. A two-tiered transport model containing a monolayer of Caco-2 cells, or a co-culture of Caco-2 and STC-1 cells, along with BRIN-BD11 cells on the basolateral side, was configured to develop a more physiologically relevant in situ assessment system. Comparable trends in the DPP-IV inhibitory profiles were obtained *in vitro* and in situ. The absorbed fraction of the intestinal digests from both egg white and whey proteins induced insulin secretion in BRIN-BD11 cells in the two-tiered cellular model containing Caco-2 and BRIN-BD11. However, gastric digests of the same substrates only triggered insulin secretion when the monolayer was composed of STC-1 and Caco-2 cells, potentially due to the insulinotropic effect of STC-1-origin released incretins. The results show the physiological relevance of these models and the importance of the cell composition when simulating the gastrointestinal epithelium at cell level.

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Milk protein digestion impact on intestinal TLR3 gene expression

Sarah Gilsenan[1,2,3], Lorraine Brennan[2,3,6], Paul D. Cotter[1,2,4,5], Kanishka N. Nilaweera[1,2,5]
[1]Food Biosciences Department, Teagasc Food Research Centre, Moorepark, Fermoy,, Ireland, [2]VistaMilk Research Centre, Teagasc, Moorepark, Fermoy, Ireland, [3]UCD School of Agriculture and Food Science, Belfield, UCD,, Ireland, [4]Department of Anatomy and Neuroscience, University College Cork, , Ireland , [5]APC Microbiome Ireland, University College Cork, Ireland, [6]UCD Institute of Food and Health, Belfield, UCD, Ireland

Background: Milk associated whey compared to casein protein alters the gut microbiota, and improves metabolic health in mice fed high fat diets. Given that Toll-like receptor 3 (TLR3) mediates the microbial effects on pro-inflammatory cytokine production, we investigated if increased whey consumption can influence its gene expression, along the GI tract.
Method: Gene expression was measured in the colon and duodenum across two studies. Differing in whey and casein protein, in study 1 mice consumed for 12 weeks, 55% fat and 30% protein, 55% fat and 10% protein, or 20% fat and 30% protein. In study 2 mice consumed, 45% fat and 20% protein for 10 weeks and then co-treated with antibiotics for 5 weeks.
Results: Independent of fat and protein intake, whey compared to casein protein, decreases colon TLR3 expression ($p<0.05$*), but not in the duodenum. Depletion of the gut microbiota by antibiotic treatment further reduced TLR3 expression in both casein ($p = 0.013$*) and whey protein fed mice ($p = 0.003$**).
Conclusions: These results suggest that the milk protein whey downregulates TLR3 gene expression in the colon. Moreover, the microbiota present following whey intake reduces this effect possibly by further breaking down the associated peptides or amino acids.

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Nutritional evaluation of meal-scale supplementation for the elderly

Angeline Duval[1], Thierry Sayd[1], Sylvie Bourillon[1], Laurent Aubry[1], Vinciane Mosconi[1], Vincenza Ferraro[1], **Veronique Sante-Lhoutellier[1]**
[1]Inrae QuaPA, Saint Genes Champanelle, France

The risk of deficiency increase with ageing. A diet adapted to specific nutritional needs is essential. This study aims to supplement each dish of a meal with a protein mix and evaluate micro and macronutrient availability.
Two meals (fortified with a protein mix or not) were studied. Each meal included 5 dishes as proposed in nursing home. Each dish was characterized at the nutritional level. Each dish of a same meal was sequentially introduced into the DIDGI® according to the elderly digestive conditions. Then nutrients, protein digestibility and amino acids were quantified on the digestates.
The protein level is significantly higher in the supplemented meals (+43%). More peptides and free amino acids are released at the end of digestion in the enriched meals. In addition, higher protein digestibility was found in the enriched meals and Cysteine, Leucine and Phenylalanine levels were higher at the end of digestion.
Supplemented meals provide more protein with higher digestibility, and a large increase in leucine released. This point is crucial for elderly to stimulate anabolic synthesis. This work demonstrated the how meal formulations can meet nutritional needs of elderly and the complexity of the meal and the interactions at stake in the digestive tract.

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Multivariate correlation of IR fingerprint and molecular weight distribution with bioactivity of poultry by-product hydrolysates

Sileshi Wubshet[1], Anne Rieder[1], Nils Kristian Afseth[1], Shiori Koga[1], **Liudmila Sorokina[1,2]**
[1]Nofima, Ås, Norway, [2]University of Oslo, Oslo, Norway

Enzymatic protein hydrolysis (EPH) is a promising method of extraction of valuable proteins and peptides from food processing by-products. Adjustment of hydrolysis conditions allows to produce protein hydrolysates with different characteristics, including bioactive properties.
EPH of mechanically deboned chicken residue was performed using ten proteases and six periods. Chemical fingerprints of the hydrolysates were made using SEC and FTIR. Bioactive properties of the hydrolysates were evaluated using DPPH radical scavenging assay and ACE inhibitory assay. Principal component analysis and partial least squares regression (PLSR) were performed to establish relationship between processing conditions (enzyme, hydrolysis time), chemical fingerprints and evaluated bioactivities.
The hydrolysates demonstrated varied DPPH radical scavenging activity (0.08-2.8 µM in quercetin equivalents) and ACE inhibitory properties (35-74%) depending on enzyme and hydrolysis time. PLSR models were developed for the prediction of bioactivities based on FTIR fingerprints (r2=0.738, RMSECV=0.311 for radical scavenging; r2=0.908, RMSECV=2.754 for ACE inhibition) and molecular weight distributions (r2=0.749, RMSECV=0.304 for radical scavenging; r2=0.941, RMSECV=2.211 for ACE inhibition).
The hydrolysates possess DPPH radical scavenging and ACE inhibitory properties, which were strongly dependent on processing conditions. The developed PLSR models indicate that FTIR fingerprints and molecular weight distributions of the samples contain information that allows to predict bioactivity.

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Glycoxidation during thermal processing and *in vitro* gastrointestinal digestion of pork

Xiaona Tian[1], Els Vossen[1], Stefaan De Smet[1], Thomas Van Hecke[1]
[1]Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Sciences and Aquatic Ecology, Ghent University, Coupure Links 653, B-9000, Ghent,, Belgium

Oxidative reactions occur during heating and gastrointestinal digestion of meat. This study aimed to investigate the effect of glucose addition to meat on the formation of glycation and glycoxidation products during thermal processing and *in vitro* gastrointestinal digestion. Pork shoulder was minced with or without 5% glucose, after which meats were heated in an oven and subjected to an *in vitro* digestion model simulating the conditions in the human mouth, stomach, and duodenum. Glycation and glycoxidation in meat and digests were evaluated by the assessment of Maillard reaction products (MRPs, spectrophotometry), total fluorescent advanced glycation end products (AGEs, fluorescence spectroscopy), and pentosidine (HPLC-FLD). Protein oxidation was assessed by measuring protein carbonyl compounds (spectrophotometry). Depending on the heating conditions, glucose addition stimulated glycation and glycoxidation during heating and digestion of pork, as evidenced by the increased levels of MRPs, total fluorescent AGEs, and pentosidine. The addition of glucose also resulted in increased protein oxidation, demonstrated by higher protein carbonylation levels in pork and their digests.

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In vitro gastrointestinal digestion of meat and fish with alcoholic beverages: oxidation and fatty acid ethyl ester formation

Jasper Van Pee[1, 2], Els Vossen[1], Stefaan De Smet[1], Thomas Van Hecke[1]
[1]Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Sciences and Aquatic Ecology, Ghent University, Coupure Links 653, B-9000, Ghent, Belgium, [2]ILVO Flanders Research Institute for Agriculture, Fisheries and Food, Technology and Food Science Unit, Brusselsesteenweg 370, BE-9090, Melle, Belgium

Oxidative reactions during cooking and gastrointestinal digestion of meat and fish lead to the formation of various lipid-and protein oxidation products, some of which are toxic. The intake of alcoholic beverages with meat and fish, may have an antioxidant effect due to polyphenols present but, may also stimulate the formation of possibly toxic fatty acid ethyl esters (FAEE's). Therefore, the effect of different alcoholic beverages on the formation of oxidation products and FAEE's during *in vitro* digestion of meat/fish was investigated.
Cooked chicken, pork, beef or salmon was combined with ethanol, or alcoholic beverages (lager, dark beer, triple, white wine, red wine) in two volumes. These combinations were exposed to *in vitro* digestion, simulating conditions from mouth to small intestine. 4-hydroxy-2-hexenal, 4-hydroxy-2-nonenal, hexanal and propanal were determined via HPLC fluorescence; malondialdehyde, protein carbonyl components, polyphenol content and total antioxidant capacity (TAC) via spectrophotometer, and FAEE's via GC-MS.
Depending on their polyphenol content and TAC, most alcoholic beverages significantly reduced oxidation by approx. 25 to 90% during digestion of meat/fish, with red wine being the most antioxidative. FAEE's were formed during digestion of meat/fish with alcohol, corresponding to the fatty acid profile of the digested muscle.

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Milk extracellular vesicles; infant *in vitro* gastro-intestinal digestion and gut barrier response

Charlotte Oliver[1, 2], Anindya Mukhopadhya[2, 3], Lorraine O'Driscoll[2, 3], Andre Brodkorb[1], Linda Giblin[1]

[1]Teagasc Food Research Centre, Moorepark, Fermoy, Ireland, [2]School of Pharmacy and Pharmaceutical Sciences, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland, [3]Trinity St. James's Cancer Institute, Trinity College Dublin,, Dublin 2, Ireland

Background: The study aimed to track the fate of extracellular vesicles (EVs) in bovine milk during infant gastro-intestinal digestion and evaluate their effect on the intestinal barrier.
Method: Milk was digested as per INFOGEST protocol modified for the infant gut (Menard et al.2016) (no oral phase; gastric phase 1h, pH 5.3, pepsin 286U/mL; intestinal phase 1h, pH 6.6, trypsin 16U/mL, bile 3.1mmol/L). Sequential centrifugation was used to isolate EVs at various time points. Western blot and nanoparticle tracking analysis were employed for EV characterization. Caco-2 day 21 monolayers were treated with purified EVs ($1.07x10^{11} \pm 4.72x10^{10}$ particles/mL of milk) for 4h and the effect on tight junctions was monitored by transepithelial electrical resistance (TEER) and lucifer yellow permeability.
Results: Failure to detect the EV associated markers TSG101, XDH, CD9 indicated that EVs were disrupted in the intestinal phase, although they did survive the gastric phase. Yield of EVs significantly reduced post-digestion from $1.82x10^{10} \pm 1.34x10^{10}$ to $5.97x10^{8} \pm 6.25x10^{7}$particles/mL milk ($P<0.05$). Intact purified EVs did not disrupt Caco2 barrier integrity as determined by TEER and paracellular permeability data.
Conclusions: EVs yield and integrity is reduced by infant gastro-intestinal digestion. Any intact EVs that survive digestion are unlikely to negatively impact on gut barrier health.

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Monitoring the GABA content and viability of *Lacticaseibacillus paracasei* in GABA-enriched synbiotic yogurts over the gastrointestinal digestion

Farhad Garavand[1], David Daly[2], Laura Mascaraque[1]
[1]Teagasc Moorepark Food Research Centre, Fermoy, Ireland, [2]Abbott Nutrition, Cootehill, Cavan, Ireland

The current study was designed to produce gamma aminobutyric acid (GABA)-enriched synbiotic yogurts by incorporation of *Lactobacillus paracasei* and different prebiotics, i.e. galactofructose, inulin, soy protein isolate, or spirulina, compared with the probiotic and plain yogurt samples. The changes in GABA content and viability of probiotics were then monitored after the oral, gastric, and intestinal phases during the static gastrointestinal digestion. Spirulina and galactofructose-supplemented synbiotic yogurts showed the highest GABA production capacity (0.80 and 1.07 mM, respectively) compared to plain, and probiotic yogurts as well as other synbiotic yogurts supplemented with soy protein isolate and inulin. However the lowest viability was also observed in spirulina-supplemented synbiotic yogurt. Gastrointestinal digestion did not significantly change the GABA content in both gastric and intestinal phases, while the number of viable probiotics declined by about 5 to 6 log CFU/mL in the gastric and intestinal phases, respectively. These results provide valuable information regarding the stability of health-promoting fermentation metabolites and highlights a considerable loss of viability of beneficial bacteria when exposed to gastrointestinal conditions.

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Enzyme inhibitory and antioxidant peptides of MCC and SPC proteins released via INFOGEST method

Justyna Borawska-Dziadkiewicz[1], Anna Iwaniak[1], Piotr Minkiewicz[1], Damir Mogut[1], Maria Baranowska[2], Katarzyna Przybyłowicz[3], Bogusław Staniewski[2], Justyna Żulewska[2], Małgorzata Darewicz[1]

[1]Department of Food Biochemistry, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland, [2]Department of Dairy Science and Quality Management, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland, [3]Department of Human Nutrition, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Milk is a rich source of essential nutrients and also bioactive peptides. Biopeptides from milk proteins, can be generated by gastrointestinal digestion after consumption. Milk protein concentrates: MCC – casein and SPC – serum proteins concentrate, were *in vitro* digested and their bioactivity were analyzed.

The MCC and SPC were prepared by membrane filtration at the Department of Dairy Science and Quality Management, UWM. The *in vitro* digestion method according to INFOGEST consisted of the following steps: "oral", "stomach" – 1 hour, "duodenal "- 1 hour. Hydrolysates were analysed for their enzyme inhibitory (ACE and DPP-IV) and antioxidant activities. The hydrolysates were used in a screening for bioactive peptides by RP-HPLC-ESI-MS/MS method. Hydrolysates of milk protein concentrates showed ACE inhibitory, DPP-IV inhibitory and antioxidant activity. The difference between MCC and SPC samples were observed. The ACE inhibitory (eg. IPA, IR), DPP-IV inhibitory (eg. IPA, IR, PW) and antioxidant fragments (eg. IR, PW) were identified.

Milk protein concentrates are considered as an interesting source of peptides with biological activity, including ACE and DPP-IV inhibitors, as well as antioxidant peptides released after digestion.

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Wheat sensitivity and digestive discomfort: Identification of antinutrients in wheat.

Tora Asledottir[1], Tove G. Devold[1], **Gerd Vegarud[1]**, Anne Kjersti Uhlen[1]
[1]Norwegian University of Life Sciences, Aas, Norway

Incidence of wheat sensitivity is increasing worldwide, and the abdominal discomfort experienced after ingestion of gluten or wheat containing food, such as bloating, diarrhea, eczema and fatigue is often described as a non-celiac gluten sensitivity. However, the food components involved, and the mechanism behind the activity resulting in abdominal discomfort, is still unknown. Several components have been linked to wheat sensitivity and our aim was to identify wheat types low in components related to digestive discomfort through wheat sensitivity. Wheat samples were harvested from an experimental field at NMBU, Ås, Norway. The collected wheats represented both common wheat (*T. aestivum*) and ancestral wheat types (einkorn, emmer and spelt). Fructan content in the samples was measured and amylase-trypsin inhibitors were identified through extraction of low-molecular weight proteins followed by trypsination and purification prior to LC-MS/MS. Wheat samples were also cooked and digested *in vitro* with human gastrointestinal juices. The peptides released during digestion were identified and manually searched up against a wheat opioid peptide list. Although fructan content was mainly influenced by cultivation season and location, the ancestral wheat types released fewer sequences related to opioid activity after *in vitro* digestion. These results will be further assessed and presented.

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Development of *in vitro* model of GUT-lung axis to evaluate the immunomodulatory effect of probiotics on SARS-Cov2

Lidia Tomás-Cobos[1], Laura Soriano-Romaní[1], Elena Díez-Sánchez[1], Elisa Gallego[1], Blanca Viadel[1]
[1]Ainia, Paterna, Spain

The irruption of COVID-19 has been a great challenge for society. It is important to develop products to boost the immune system against these infections. There is growing evidence that the use of microbiota modulating compounds, such as probiotics or fructo-oligosaccharides, can prevent the development of dysbiosis, improving the susceptibility to infectious diseases.
The objective is the development of an integrated *in vitro* system based on a dynamic colonic fermentation digester and a cellular model of respiratory airways to assess the effect of a symbiotic on immune system against respiratory viruses.
Differentiated human pulmonary cell line A549 grown in co-culture with PMA-differentiated macrophage cell line THP1 were infected with human coronavirus 229E (ATCC® VR740™). The effect of a commercial symbiotic on the microbiota population and on the gene expression of cytokines was monitored.
The symbiotic led to a slight modulation of the beneficial gut microbiota. It was shown an anti-inflammatory effect on infected A549 by the reduction of the mRNA of IL-6, IL-8 and IL1-b, and an immunostimulant effect on THP1 by the increase of the mRNA of IL-6, IL-8, IL-1b and IL-10.
It was defined an experimental strategy to design oral products to prevent or mitigate respiratory virus infection.

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Increasing confidence in peptide identification and quantification during *in vitro* digestion

Gijs Vreeke[1], Peter A. Wierenga[1]
[1]*Wageningen University, Wageningen, The Netherlands*

Background: While first protein digestion was described by loss of intact proteins, now detailed information on formation and degradation of peptides is used. Typically approaches from proteomics are used, in which peptide annotations and confidence in these annotations are based on fragmentation spectra. 50% reproducibility in repeat analyses has been reported even for annotations with high confidence. When analysing protein digests it is important to determine criteria that yield highly reproducible annotations.
Method: A structured approach was developed to determine these criteria, using UPLC-PDA-MS for untargeted peptide identification and absolute label-free quantification.
Results: Processing filters were set to ensure reliable annotations based on MS/MS fragmentation, while maintaining maximum amount of information. Peptides in individual hydrolysates above the limit of annotation represented 99% of total MS intensity and were 100% consistently annotated between four replicates. The amino acid sequence coverages for individual protein hydrolysates were 99-100%. Mixing the hydrolysates resulted in a loss of 11% of the peptide annotations above the limit of annotation and a 3% lower reproducibility for the remaining annotations, as well as more co-eluting peptides.
Conclusion: The proposed approach allows complete description of peptide composition with highly repeatable annotations and quantification of peptides even in mixed hydrolysates.

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Gastric emptying and nutrient absorption of pea protein

Julia Roelofs[1], Elise van Eijnatten[1], Diederik Esser[1], Anja Janssen[1], Patteela Prathumars[1], Paul Smeets[1]
[1]Wageningen University & Research, Wageningen, Netherlands

Background: Digestibility of plant-based proteins is generally lower compared to animal-based proteins. Heat treatment and food structure may affect digestibility *in vivo*. MRI is a promising tool to assess such characteristics. The aim was to compare gastric emptying (GE) and amino acid (AA) absorption kinetics between pea-protein based liquid and solid foods with and without heat treatment.
Methods: Fourteen healthy males participated in a randomized crossover trial. Iso-caloric and iso-volumetric treatments were a 420mL heated drink, 420mL unheated drink and 105g heated semi-solid food (gel) consumed with 315mL water containing 20g pea protein (99kcal). GE was measured with MRI over a period of 90 minutes. Blood samples were collected for five hours to measure AA absorption kinetics.
Results: No differences in GE were observed between the heated and unheated drinks (mean GE half time (t50)=54.5 and 55.8 min). However, the gel treatment showed faster initial emptying, which was due to the emptying of the water (mean GE-t50=40.2 min). Blood analysis showed a ~20 minute delay in AA absorption for the gel treatment compared to both drinks.
Conclusion: Our results demonstrate that food structure affects GE and AA absorption, but that heat treatment does not.

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Monitoring fatty acid oxidation of beef and fish meals during gastric digestion using an *in vitro* semi-dynamic model

Isabel Ferreira[1], Iskandar Azmy Harahap[2, 3], M. Madalena Sobral[1], Susana Casal[1], Susana Pinho[1], Joanna Sulibursk[1], Miguel Faria[1]
[1]LAQV/REQUIMTE - University of Porto, Porto, Portugal, [2]Poznan University of Life Sciences, Poznan, Poland, [3]National Agency for Research and Innovation (BRIN) , Jakarta, Indonesia

Background: Beef and fish meals present different fatty acid (FA) composition. Fish meals contain higher levels of polyunsaturated fatty acids (PUFA), which despite their health benefits makes them more prone to suffer oxidation. This study followed lipid oxidation and FA release from stomach to duodenum using an *in vitro* model.
Methods: Meals composed of 25% beef (BM) or fish (FM), 25% fried potatoes, and 50% sugar soft drink were digested using the INFOGEST semi-dynamic model. In each gastric emptying (GE), FA and glycerides were assessed, as well as lipid oxidation products (LOPs) formation, namely, conjugated dienes/trienes (CD, CT) and TBARS.
Results: During gastric digestion, triacylglycerols decreased and free FA increased notably. >50% FA release occurred in GE1. Total amount of FA released was similar in both meals, but saturated fatty acids and PUFA content differed between meals ($p<0.01$ and $p<0.0001$). Increased CD, CT, and TBARS formation occurred in the later GEs in FM.
Conclusions: The whole meal approach used evidenced higher formation of LOPs during digestion of FM compared to BM. Neverthless, FM digests that reached duodenum, still present higher content of unoxidized PUFAs than BM and a desirable ω3/ω6 PUFAs ratio, which corroborates their anti-inflammatory effects based on DII®.

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Buckwheat post-gastrointestinal digestion stimulates glucagon-like peptide-1 secretion from the enteroendocrine cell line STC-1

Leo Nunn[1, 2], Michael Callanan[2], Aylin Sahin[3], Elke Arendt[3], Eimear Gallagher[4], Linda Giblin[1]
[1]Teagasc Food Research Centre, Moorepark, P61 C996, Fermoy, Ireland, [2]Department of Biological Sciences, Munster Technological University,T12 P928, Bishopstown, Ireland, [3]School of Food & Nutritional Sciences, University College Cork, T12 K8AF, , Ireland, [4]Teagasc Food Research Centre, D15 KN3K, Ashtown, Ireland

Background: The objective of this study was to screen a range of plant-based proteins for their potential to modulate satiety hormone production/release from enteroendocrine cells as these proteins transited the upper gut.
Method: Test samples (Buckwheat, Quinoa, Lentil, Oat, Strong Wheat Flour, Teff, Inulin) were subjected to a static adult simulated gastrointestinal digestion (SGID) using the INFOGEST method. Glucagon-like peptide-1 (GLP-1) was quantified from murine enteroendocrine cells, STC-1, treated for 4 hours, with test samples (5mg protein/mL) from 3 distinct time points (pre-gastric G0, post-gastric G120 and post-intestinal I120). In parallel, mRNA transcripts of the satiety hormones PYY and CCK were measured by Real-time PCR.
Results: Buckwheat (15820.38 ± 2854.95 pg/mL), Oat Fibre (9916.85 ± 944.91 pg/mL) and Lentil Protein Isolate (9752.16 ± 642.75 pg/mL) significantly increased active GLP-1 secretion from STC-1 compared to KREBS control ($p<0.05$). Buckwheat increased its GLP-1 bioactivity post-SGID (G0= 9631.48 ± 1026.66). In contrast, other samples such as Strong Wheat Flour, lost their satiating potential as they journeyed through the gut.
Conclusion: Consumption of Buckwheat may prolong a feeling of fullness. Future work will focus on the satiating potential of Buckwheat-based bakery products.

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Whole meals digests impact on inflammation markers expression in human macrophages *in vitro*

Miguel Faria[1], Mafalda Ribeiro[1], Marta Silva[1], Rebeca Cruz[1], Zita Martins[1], Madalena Sobral[1], Olga Viegas[1], Susana Casal[1], Isabel Ferreira[1]
[1]LAQV/REQUIMTE-University of Porto, Porto, Portugal

Background: Diet plays a central role in the regulation of chronic inflammation with implications in several diseases. Tools for its *in vitro* assessment are crucial to implement a greater number of experiments needed to study diets complexity. This work presents an approach to assess whole meals effects in inflammation *in vitro*.
Methods: Meals were prepared representing western/vegetarian/pescatarian patterns, analysed (proximate) and digested (INFOGEST). Bioaccessible fraction detoxification was done by centrifugation (12000 g, 30 min), filtration (0.22 µm), ultrafiltration (30 kDa) and dilution (3x). THP1 cells were exposed to treated digests with/without LPS stimulation (22 hrs). RNA levels for IL6, IL-1β, IL10, TNF-α and NF-kB were measured (qPCR).
Results: Treated digest had no cytotoxicity in macrophages (MTT, LDH). Exposition of digests without stimulation promoted the expression of the majority of cytokines. However, the simultaneous treatment with digests and LPS induced the strongest response in mRNA levels with 2 to 300-fold increase for IL6 and IL-1β. Overall, digests effects seem to overlap the differential ones of diets.
Conclusions: The meal digest detoxification seems to be adequate to obtain an effect in THP-1 macrophages inflammatory markers, without cytotoxicity. However, new strategies to highlight the differential effects of meals are being exploited.

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Effect of *Alternaria* toxins on the adhesion of lactobacilli to intestinal cells

Stepanka Kozakova[1], Barbora Lampová[1], Ivo Doskočil[1]
[1]Department Of Microbiology, Nutrition And Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences., Třebíz, Česko

Mycotoxins are the common food contaminants negatively affecting human health. Lactobacilli can play a role, especially regarding microbial decontamination. However, it's not known how genus *Alternaria* spp. can affect the ability of the probiotic´s to adhesion to intestinal epithelial cells.
Our work aimed to find whether *Alternaria* toxins (alternariol AOH and alternariol monomethyl ether AME in concentrations 5 and 10 μM) can affect *Lactobacillus gasseri* and *Lactobacillus plantarum* adhesion on cell lines Caco-2 and HT29. Two methods were used the traditional cultivation method (TCM) on Petri dishes and the method using Fluorescein isothiocyanate for marking lactobacilli.
Adhesion on the cell line Caco-2 increased significantly in *L. gasseri* combined with AME (5 μM). When using the FITC, the adhesion was more than 40% higher than the cell line HT29 and the TCM ($P<0.05$). But otherwise, the FITC resulted in a reduced adhesion (30%) compared to the TCM. At the same time, higher concentrations of AOH and AME showed lower adhesion. Furthermore, using the FITC led to a significant reduction of adhesion compared to the TCM. Based on the outcomes, the FITC seems to be a more precise and effective method to determine the influence of mycotoxins on lactobacilli on intestinal cells.

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Untargeted peptidomics approach of sheep and goat infant formulas submitted to *in vitro* digestions

Mattia Casula[1], Olivia Menard[2], Marie-Françoise Cochet[2], Dr Didier Dupont[2], Simone Serrao[1], Cristina Manis[1], Viviana Garau[1], Barbara Manconi[1], Pierluigi Caboni[1]
[1]University of Cagliari, Cagliari, Italy, [2]STLO, INRAE, Institut Agro, Rennes, France

Background: Untargeted peptidomics are well established holistic disciplines enabling researchers to characterize functional characteristics of foods at a molecular level. The aim of this work was to employ a comparative peptidomics approach to identify and measure relative quantities of sheep and goat formula milk peptides after *in vitro* gastrointestinal digestion.
Method: Goat and sheep infant formulas were submitted to dynamic *in vitro* gastro-intestinal digestions using the DIDGI® system adapted to the newborn digestive conditions. High resolution nanoLC mass spectrometry analysis allowed the comparison of the kinetic of peptides released during digestions.
Results and Conclusions: Peptides deriving from osteopontin-1, amylopid A and lactotransferrin, GlyCAM1 were characteristics of sheep and goat formula milk, respectively. Additionally, 280 and 330 peptides were already present in sheep and goat milk before ingestion, indicating that proteolysis events occurred in milk during formula transformation and storage processes. Furthermore, milk peptides were also checked for their unique functional properties, such as anti-hypertensive, immunomodulatory and prebiotic activities.
Comparison of the peptides released from two proteins of animal origin in the context of infant digestion could give more knowledge using alternative protein sources as cow milk protein remain the main source in commercial infant formulas.

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Dipeptides of food origin structure-activity relationship

Damir Mogut[1], Anna Iwaniak[1], Małgorzata Darewicz[1]
[1]*University of Warmia and Mazury in Olsztyn, Faculty of Food Science Chair of Food Biochemistry, Olsztyn, Poland*

Food proteins can be a source of peptides with a variety of bioactivities, including antioxidant effect. This study uses a statistical model based on principal component analysis (PCA) to determine the impact of the structure of dipeptides on their antioxidant activity. Dipeptide sequences were obtained after digestion/hydrolysis. The numerical variables (22 in total) were the descriptors of physicochemical properties of each dipeptide amino acid (obtained from the AAIndex database). 47 dipeptides with antioxidant activity were subjected to chemometric analysis. The obtained results indicated that the first 4 components explained 79.9% of the total variance. The first component described the N-terminal and the second the C-terminal properties: (molecular mass, number of carbon atoms, polarizability, size, percentage of buried residues and amino acid composition. The third component described the C-terminal and fourth N-terminal residues (polarity, bulkiness, and Kyte-Doolittle hydropathy index.
Based on the PCA results concerning the structure- activity relationships of analyzed peptides, it was found that the presence of N-terminal amino acids such as Trp, Phe, Leu, Ile, Ala and C-terminal Pro, Val, Leu and/or His decided about the antioxidant bioactivity of dipeptide.

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Trout protein digests - antioxidant and angiotensin I-converting enzyme inhibitory activity

Małgorzata Darewicz[1], Justyna Borawska-Dziadkiewicz[1]
[1]*University of Warmia and Mazury in Olsztyn, Faculty of Food Science, Department of Food Biochemistry, Olsztyn, Poland*

Trout may be the source of bioactive peptides in the human diet. Row and high temperature treated trout myofibrillar and sarcoplasmic proteins were examined as a bioactive peptides precursors after digestion. The computer tools available at UniProt database and BIOPEP-UWM database were use in silico part of the study. Then the digestion was carried out in three steps [2]: (1) "chewing" 3 min; (2) "stomach" with a gradual lowering of pH i.e. 7–5–2.5/2 h; (3) "duodenal" - pH adjusted to 7.0/1 h. Human gastric juice and duodenal juice were used for *ex vivo* digestion, and pepsin and Corolase PP were used for *in vitro* digestion. Digests were analysed for their ACE inhibitory and antioxidant activities. Digests of trout myofibrillar and sarcoplasmic proteins obtained with different methods showed ACE inhibitory and antioxidant activities. The difference between samples were observed. The temperature treatment had a beneficial effect on the samples bioactivity. The ACE inhibitory and antioxidant fragments selected based on the results of in silico studies were identified in the digests via RP-HPLC-ESI-MS/MS method. It was concluded that trout proteins can be the source of ACE inhibitory and antioxidant peptides after thermal treatment.

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Meal pH and glycemic responses: studying the digestion of starch-rich meals *in vitro* to better understand the determinants glycaemic responses *in vivo*

Daniela Freitas[1], Laura Gómez Gómez-Mascaraque[1], Steven Le Feunteun[2], Andre Brodkorb[1]
[1]Teagasc, Fermoy, Ireland, [2]INRAE, Rennes, France

Numerous studies have reported a 20-50% reduction of the glycaemic response to starch-rich meals by low-pH drinks or foods. Although different possible explanations have been put forward, our work using bread and lemon juice (pH≈2.3) as a case study has pointed to the acid-induced inhibition of salivary α-amylase as the most likely underlying mechanism. Therefore, our aim was to study the digestion of a different subset of such meals to further investigate this hypothesis.
Four meals have been replicated in the lab both with and without their pH-lowering ingredient. Their initial pHs were measured and they were then digested *in vitro* using the semi-dynamic INFOGEST protocol. Each digestion included oral, gastric and intestinal phases and experiments were conducted in triplicate. Samples were collected at different time-points to monitor the extent of starch hydrolysis and the MW weight distributions of the polysaccharide hydrolysates throughout the experiments.
Low pH meals exhibited a marked reduction of the proportion of starch digested compared to their neutral-pH counterparts. The agreement between these results with those of previous human studies confirms our earlier observations and offers new perspectives for the development of strategies to improve the glycaemic responses to starch-rich foods.

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Structural and functional relationships of plant allergenic proteins during gastrointestinal metabolism

Julia Bräcker[1], Jens Brockmeyer[1]
[1]*University of Stuttgart, Stuttgart, Germany*

Food proteins frequently result in severe allergic reactions after gastrointestinal digestion. Little is known about characteristics of plant-based food allergens and their resistance against digestion on a molecular level so far.
Human gastrointestinal digestion was simulated with a standardized in-vitro digestion model (COST Infogest) for four matrices. The resulting highly complex sets of degradation products to different time points of digestion were analyzed by LC-HRMS/MS on a Q-TOF instrument in a software assisted proteomics approach.
Dealing with huge data sets (480 samples) and several thousand identified peptides we developed a multistage post-processing approach using Python, taking into account further input data such as protein assignment, secondary structure features and known epitopes of the given allergens. This approach allows detailed visualization of protein degradation and digestion product formation in the course of gastrointestinal digestion on a global scale. For the first time we were able to draw structure-function relationships between structural characteristics of food allergens, their stability to the gastrointestinal environment and immunological properties.
The development of a multistage analysis and data processing approach using Python allows to characterize plant-based food allergens during gastrointestinal digestion, providing insight into highly stable regions in context with secondary structure features and immunological functions.

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In vitro glucose polymers degradation study: microbial preference for osidic linkages and molecular weights

Adrien Désidério[1], Charlotte Verschueren-Midy[1], **Caroline Perreau[1]**, Laetitia Guérin-Deremaux[1], Clémentine Thabuis[1]
[1]Roquette, LESTREM, France

Following daily fiber recommendation is positively correlated to the decrease in chronic diseases prevalence. The role of fibers is crucial for digestive health, for some of them through their interaction with gut microbiota. In the present study, we aimed to mechanistically investigate this interaction with the purpose of new prebiotics conception.
A colonic fermentation model was set up order to investigate the degradation process of several glucose polymers: the resistant dextrin NUTRIOSE® (linkages 1-4 + 1-6) and 3 substrates with atypical linkages: Dextran (α1-6), Pullulan (α1-4 + α1-6) and Curdlan (β1-3) were compared. A rat microbiota was challenged during 72h to use those substrates as only carbon source. Microbiota evolution was monitored using qPCR and DGGE on one hand. The structural degradation of the substrates was monitored using molecular weight measurement, and osidic linkage consumption by the bacteria along the fermentation process.
Our study showed that bacteria used at first the small molecular weights. By challenging bacteria with 72-h fermentations, we were able to observe specific linkage preference depending on the substrate and linked to α- and β- glucosidase activity mainly. These bacterial "tastes" could allow us to design new prebiotics with a strong specificity and targeted health benefits.

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Insights into gut microbiota metabolism of dietary lipids: the case of linoleic acid

Zongyao Huyan[1], Nicoletta Pellegrini[1, 2], Wilma Steegenga[1], Edoardo Capuano[1]
[1]Wageningen University and Research, Wageningen, the Netherlands, [2]University of Udine, Udine, Italy

Background: Compared to dietary fibre and proteins, microbial metabolism of dietary lipids by human gut microbiota is poorly explored. Here we investigated the differences in microbial metabolism of lipids, particularly of linoleic acid (LA), induced by the chemical forms of the lipid and the presence of the plant matrix.
Method: Free LA, glyceryl-trilinoleate, soybean oil, intact and damaged predigested soybean cells containing the same amount of LA were fermented *in vitro* using human faecal inoculums. Two conjugated-LA (9z11e-CLA and 9e11e-CLA) and 12hydroxy,9z-C18:1, were identified and monitored for 48h. Short-chain fatty acids (SCFAs) were evaluated to get insight into microbial activity.
Results: Free LA addition produced the highest amount of LA metabolites but reduced SCFA concentrations compared to trilinoleate and oil. Cellular integrity loss impacted CLA, hydroxy-C18:1 and SCFA production and free-fatty-acid release only in the first 24 hours. Compared to intact or damaged cells, free oil addition produced the highest amount of hydroxy-C18:1. The content of several fatty acids decreased during fermentation suggesting a substantial conversion in microbial metabolites. Besides, LA metabolites were identified in fermentation pellets suggesting incorporation in bacterial cells.
Conclusions: This study expands our understanding of microbial metabolism of dietary lipid with special emphasis on food-related factors.

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The formation of sulfur metabolites during *in vitro* gastrointestinal digestion of fish, white and red meat is affected by fructo-oligosaccharides

Nuria Elias Masiques[1], Els Vossen[1], Stefaan De Smet[1], Thomas Van Hecke[1]
[1]*Ghent University, Ghent, Belgium*

Rodent feeding studies indicate that consuming heme-Fe or red meat may induce a colonic microbial shift, accompanied by the formation of sulfur-metabolites, which may be modulated by the addition of fructo-oligosaccharides (FOS). High levels of H2S are hypothesized to disrupt the intestinal mucus barrier promoting inflammation. This study assessed the formation of sulfur-metabolites during *in vitro* enzymatic gastrointestinal digestion and fermentation of different muscle protein sources (beef, pork, chicken and salmon), with or without 20% FOS. The model simulated conditions of the human mouth, stomach, small- and large intestines. Following incubations, ammonia, indole, phenol, H2S, methanethiol and dimethyl x-sulfides were determined by chromatographic techniques. Levels of H2S and dimethyl x-sulfides were not significantly different amongst muscle foods, whereas salmon digests displayed a 4 and 3-fold higher level of methanethiol compared to chicken and beef, respectively. Protein fermentation was significantly reduced by the addition of FOS, as demonstrated by significantly lower levels of ammonia, indole, and phenol. In addition, the presence of FOS almost completely prevented the formation of various dimethyl x-sulfides, whereas methanethiol was not significantly affected. Surprisingly, H2S levels increased significantly with FOS addition, which could be related to the lower pH of the digests including FOS.

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Use of an *in vitro* gastrointestinal model to evaluate the potential impact of a vegetal extract on human intestinal health

Elizabeth Goya-Jorge[1], Pauline Bondue[1], Irma Gonza[1], Caroline Douny[1], Marie-Louise Scippo[1], Jeoffrey Christyn de Ribaucourt[2], Fabienne Crahay[2], Ambre Riba[2], Véronique Delcenserie[1]
[1]University of Liège, Liège, Belgium, [2]ORTIS S.A., Elsenborn, Belgium

There are numerous medicinal plants and fruits traditionally used to treat gastrointestinal disorders. However, the effects caused by these vegetal products on the intestinal microbial populations are poorly understood.
METHODS: An *in vitro* simulator of human digestion (SHIME®) was used to analyze the intestinal effects of two weeks of treatment with three increasing doses of a vegetal extract. Gut microbiota community and metabolites were studied on ascending (AC), transverse (TC), and descending (DC) colons using qPCR and SPME-GC/MS methods, respectively.
RESULTS: A significant increase of acetic acid in TC, and of butyrate in all colons were observed by the end of treatment, while propionate levels remained unchanged. On 11 targeted microbial populations, most decreased in DC during the treatment, and Bacteroidetes decreased in all colon compartments, while Firmicutes increased. Bifidobacterium increased in AC even two weeks after completing the treatment. *Akkermansia muciniphila* increased in TC and DC following treatment with the higher doses. During the two weeks after completing the treatment, Bacteroides-Prevotella populations significantly increased in TC and DC regions, probably as a residual effect induced by the vegetal extract. Overall, the studied vegetal extract increased health-promoting bacteria which could have a beneficial impact on gastrointestinal health and gut barrier.

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Impact of food additives on human gut microbiota and intestinal inflammation

Irma Gonza Quito[1], Elizabeth Goya-Jorge[1], Caroline Douny[2], Victoria Mohring[3], Marie-Louise Scippo[2], Edouard Louis[4], Véronique Delcenserie[1]
[1]Food Quality Management, Food Science Department, FARAH. University of Liège, Liège, Belgium, [2]Food Analysis, Food Science Department, FARAH, University of Liège, Liège, Belgium, [3]Master in Biomedical Sciences, Université Catholique de Louvain, , Belgium, [4]Department of Gastroenterology, University and CHU of Liège , Liège, Belgium

Some food additives have been related with adverse effects on health by the onset of microbial dysbiosis in animal models. However the effects of food additives on human intestinal microbiota composition and function are less known.
Six common food additives (polysorbate 80, maltodextrin, titanium dioxide, sodium nitrite, sucralose and carrageenan) were tested *in vitro* in batch culture models of intestinal microbiota for 72 hours. Changes in microbiota and short-chain fatty acid (SCFA) production were assessed using qPCR and SPME- GC/MS methods.
After 72 hours, the most relevant changes were for polysorbate 80, who dramatically decreased butyrate and propionate production. This was consistent with qPCR results, where significant decreases of *Clostridium* cluster XIVa (butyrate producing bacteria) and *Bacteroides*|*Prevotella* spp. (propionate producing bacteria) were observed. Maltodextrin increased acetic acid, propionate, butyrate and total SCFA production, which could be explained by the increase of *Bifidobacterium* and the preservation of *C. leptum* species. Both compounds promoted the growth of *Ruminococcus*, *Enterococcus*, *Lactobacillus* and *Streptococcus* genus, that include several species associated with intestinal inflammation or fibrosis. The use of some food additives could enhance the growth of bacterial groups considered deleterious for human health and potentially increase the risk of inflammatory bowel diseases or fibrosis.

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Food from agroindustrial waste: Impact of sugarcane xylooligosaccharides on protein fermentation and microbial functionality

Wilian Marcondes[1], Samira Prado[2], Pauline Seeburger[4], Victor Castro Alves[3], Valdeir Arantes[1], Robert Brummer[2], Tatiana Marques[2]

[1]University of Sao Paulo, Sao Paulo, Brazil, [2]Örebro University, School of Medical Sciences, Örebro , Sweden, [3]Örebro University, School of Science and Technology, Örebro, Sweden, [4]Food and Health Research Center - Orebro University, Orebro, Sweden

Xylooligosaccharides (XOS) are considered emerging prebiotics, although already commercialized in some countries. They have been gaining more attention due to their characteristics when compared to other prebiotics, such as the need of smaller daily doses for a beneficial health effect, and higher specificity to certain beneficial bacterial groups. XOS extracted from sugarcane bagasse (XOS-SCB) in a more sustainable way without using enzymes can present some structural differences when compared to commercial XOS, such as side acetyl groups. These side groups result in branched XOS with specific characteristics such as slower rate of fermentation, lower gas production and a potential to suppress protein fermentation, making this XOS-SCB a suitable candidate for patients with irritable bowel syndrome (IBS). The aim of this study was to evaluate and compare interactions between XOS supplements with different proteins, determining their effects on microbial functionality. Therefore, high concentration of proteins, from animal and plant sources, were pre-digested following the INFOGEST protocol and batch fecal fermentations were performed with different XOS supplements, both from commercial sources and XOS-SCB, in a Gas Endeavour system. Microbial functionality was determined by measuring fermentation characteristics such as production of gases, ammonia and short and branched chain fatty acids.

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Polysaccharides and polyphenols derived from Australian seaweeds enhance microbial abundance and short chain fatty acid production in a simulated gut-model

Emer Shannon[1, 2], Michael Conlon[2], Sarah Hotchkiss[3], Maria Hayes[1]
[1]Teagasc Food Biosciences, Ashtown Food Research Centre, Ireland, [2]The Commonwealth Scientific and Industrial Research Organisation, Health and Biosecurity, Kintore Avenue, Adelaide, Australia, [3]CyberColloids Ltd, Strandhaven, Curraghbinny, Ireland

Background: Diet is known to affect the composition of the human gut microbiota and bacterial production of short chain fatty acids (SCFA), which impacts the overall health of the host. Whole seaweeds (WH) contain prebiotic components such as polysaccharides (PS) and polyphenols (PP) that can be digested by gut bacteria.
Method: WH, PS and PP extracts from *Phyllospora comosa*, *Ecklonia radiata* and *Ulva ohnoi*, harvested in Australia, were assessed for their potential prebiotic activities using an *in vitro* model with human faecal inoculum. The relative abundance of bacteria was determined by 16S rRNA sequencing. SCFA were quantified by gas chromatography.
Results: After 24 hr, compared to the inulin (INU) and epigallocatechingallate (EGCG) controls, WH, PS and PP extracts significantly enhanced the abundance of bacterial taxa positively associated with SCFA production, microbiota homeostasis, gut mucosal barrier regulation, immunity and anti-inflammatory effects. These included Lactobacillales, Bifidobacteriaceae, Eggerthellaceae, *Faecalibacteria*, *Blautia* and *Barnesiella*. The Firmicutes/Bacteroidetes ratio, as well as species diversity and richness were increased by all seaweed extracts. Seaweeds increased total and individual SCFA production up to three-fold, particularly butyrate, acetate and propionate.
Conclusions: Seaweeds and their extracts may have potential as prebiotic functional foods to maintain normal gut function and alleviate dysbiosis.

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The interplay between dietary protein and fibre in colonic *in vitro* fermentation

Samira Prado[1], Victor Castro-Alves[1], Rebecca Wall[1], Robert Brummer[1], Tatiana Marques[1], Tuulia Hyötyläinen[1], Victor Castro-Alves[1]
[1]Örebro University, Örebro, Sweden

After consumption of a meal part of dietary proteins (DP) and dietary fibres (DF) may reach the colon and be fermented by the human gut microbiota. DP fermentation leads to the production of branched-chain fatty acids (BCFA), phenols, ammonia and indoles, whereas DF fermentation to short-chain fatty acids (SCFA). DP fermentation metabolites are usually related to deleterious effects on health, in contrast to DF metabolites. However, the interplay between DP and DF fermentation is poorly defined as well as the role of animal- and plant-based proteins. We aim to determine by *in vitro* batch colonic fermentation how DP affects microbiota and microbial-produced metabolites using plant- and animal-based proteins combined with wheat bran DF. Pre-digested wheat bran (WB), and isolated beef (BP) and pea protein (PP) were used as substrates for the *in vitro* fermentation with samples collected at 0, 4, 8 and 24h. UHPLC/Q-TOF-MS was used to determine polar and non-polar metabolites and 16s rDNA to determine microbiota composition. SCFA with statistical significance were butyrate for WB and WBPP 8h and total BCFA for PP and PPWB 8h. Although the subject's characteristics strongly determined microbiota composition, metabolites changes during the fermentation were dependent on the treatment and the fermentation time.

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In vitro digestion of plant protein extrudates: dry matter content drives degree of digestibility and amino acid release.

Shanna Bastiaan-Net[1], Anouk Boudewijn[1], Dianne BPM van den Berg-Somhorst[1], Walter H Heijnis[1], Ron TM van den Dool[1], Harry J Wichers[1]
[1]*Wageningen Food & Biobased Research; Wageningen University & Research, Wageningen, The Netherlands*

Background: Plant protein extrudates are gaining popularity as meat analogues particularly for sustainability reasons. Texturization by high moisture extrusion (HME) is applied to transform proteins into meat-like fibrous texture. The aim of our study was to investigate the digestibility of extruded soy protein concentrates (SPC) and investigate effect of structural properties.
Method: SPC-based extrudates were either milled or exposed to simulated mastication, and hydrolyzed using an adapted Infogest *in vitro* static digestion protocol. Uptake of digestive fluids (swelling behaviour), degree of free amino groups (NH2) and free amino acids (AA) were determined and correlated to textural properties and dry matter (DM) content.
Results: 'Masticated' extrudates with high structural texturization showed the highest swelling behaviour with over 50% weight gain. In addition, high-texturized extrudates displayed a higher degree of free NH2 and AA at the end of intestinal phase. Comparing milled versus 'masticated' samples, a smaller particle size increased digestibility slightly for two out of three extrudates.
Conclusions: The digestibility of SPC (HME) extrudates was up to 55%. The degree of digestibility at the end of intestinal phase was determined predominately by the DM content, while the effect of particle size (milled versus 'masticated') had minimal effect on digestibility.

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In vitro bioaccessibility of oil-soluble vitamins (A, D, E) in plant-based emulsions: influence of gurum seed oil droplet size

Emad Mohamed Ali Karrar[1]

[1]National Engineering Research Center for Functional Food, Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, School of Food Science and Technology, Jiangnan University, Wuxi 214122, China

This study systematically examined the effect of the droplet diameter of gurum seed oil (≈0.17, 1.8, and 13 m) on the bioaccessibility of three oil-soluble vitamins (vitamin A, vitamin D, and vitamin E) encapsulated within gurum seed oil-in-water emulsions. Due to a reduction in oil-water interface area, lipid digestion kinetics decreased with increased droplet size. Droplet size decreased the bioaccessibility of vitamins from 0.17 to 13 m: from 89 to 40% for vitamin A; from 77 to 46% for vitamin D; from 79 to 20% for vitamin E. Vitamin bioaccessibility also decreased as their hydrophobicity and molecular weight increased, which is probably because they tend to remain inside oil droplets and/or be poorly dissolved by mixed micelles. As well as vitamin A (~92%) and vitamin E (~4%), esterified vitamins are hydrolyzed under gastrointestinal conditions. Vitamin-rich functional food products should therefore be created using emulsion-based delivery systems carefully designed for their composition and structure.

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Improving the *in vivo* bioavailability of provitamin A carotenoids from *Dunaliella salina* using nanoemulsions formulated with different emulsifiers

Júlia Teixé-Roig[1], Gemma Oms-Oliu[1], Isabel Odriozola-Serrano[1], Olga Martín-Belloso[1]
[1]*University of Lleida, Lleida, Spain*

Dunaliella salina algae is a natural source of provitamin A carotenoids that can be converted to retinol in the intestinal mucosa. However, carotenoids are easily degraded and show low bioavailability. Nanoemulsions can protect and increase their bioavailability but the efficiency depends on their interfacial composition. Hence, this work aimed to encapsulate a carotenoid-rich extract from *Dunaliella salina* in nanoemulsions containing 20% corn oil and 8% emulsifier (lecithin, whey-protein or sodium-caseinate) to evaluate their effect on the *in vivo* bioavailability of provitamin A carotenoids. Nanoemulsions were orally administered to rats, which were sacrificed to obtain digesta from the gastrointestinal tract and blood. All nanoemulsions increased retinol levels in plasma compared to the suspension (carotenoids in water). The maximum retinol concentration was observed using whey-protein (473.18 ± 23.01 µg/mL) as emulsifier, probably due to the dispersed droplets present in the duodenum that could be rapidly digested and incorporated into the mixed micelles. The presence of coalesced and strongly aggregated droplets in the duodenum, observed through microscopy when using lecithin and sodium-caseinate, seems to reduce β-carotene absorption and its conversion to retinol. Thus, the election of emulsifiers in nanoemulsion formulation has a strong impact on the *in vivo* bioavailability of provitamin A carotenoids.

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Bioaccesibility of acrylamide in cereal and potato-based products. Study on isolated foods and combined meals

Lucía González-Mulero[1], Marta Mesías[1], Francisco J. Morales[1], **Cristina Delgado-Andrade[1,2]**

[1]Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), Madrid, Spain, [2]Estación Experimental del Zaidín (EEZ-CSIC), Granada, Spain

Background: Acrylamide is a carcinogenic processing contaminant formed in foods under high temperatures and low moisture conditions by the asparagine-reducing sugars reaction. Little information concerning its bioaccesibility and possible interaction with food components is available. Our purpose was analysing acrylamide bioaccesibility in different food sources, including isolated foods and combined meals.
Method: Food systems (biscuits, biscuits + milk, breakfast cereals, breakfast cereals + yogurt, French fries, French fries + roasted beef steak, "patatas a lo pobre" (potatoes cooked in oil), scrambled eggs-"patatas a lo pobre") were *in vitro* digested (INFOGEST protocol). Acrylamide was determined by LC-ESI-MS/MS in foods and in the bioaccesible and non-bioaccesible fractions obtained after different stages of the digestion.
Results: Acrylamide bioaccesibilities ranged between 90-105%, except in breakfast cereals, with values of 75% and lower recoveries. Acrylamide was not detected after digestion of meals including patatas a lo pobre, probably due to its low initial amount.
Conclusions: Accessible acrylamide generally increased after oral and gastric stages in isolated foods, possibly because the breakdown of structures trapping the contaminant during these phases. Released acrylamide was probably blocked up in subsequent interactions, which could explain the decreased accessibility levels after the intestinal phase in these systems.

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Influence of carrier oil nature on lipid digestibility and β-carotene bioaccessibility in lecithin-stabilised nanoemulsions

Isabel Odriozola-Serrano[1], Ariadna Gasa-Falcón[1], Gemma Oms-Oliu[1], Olga Martín-Belloso[1]
[1]*University of Lleida, Lleida, Spain*

Using lipophilic antioxidants as functional ingredients is still limited due to its low solubility in aqueous-based matrices, poor stability and bioavailability. To overcome these issues, nanoemulsions can be used as delivery systems to solubilise and protect antioxidant compounds. However, oil properties might strongly influence on delivery of encapsulated lipophilic antioxidant compounds. Hence, the objective of this work was to prepare β-carotene-enriched nanoemulsions with different carrier oils (OO: olive, CO: corn, and WO: walnut oil) and determine lipid digestibility (%FFA) and bioaccessibility of β-carotene. After *in vitro* digestion, nanoemulsions presented different lipid digestibility results, decreasing in the following order: CO≈WO > OO. Nanoemulsions with lower particle size (CO and WO) were digested more rapidly and presented the highest lipid digestibility (≈100% FFA) compared to those nanoemulsions formulated with OO (≈94% FFA). Bioaccessibility of β-carotene was similar for CO and OO nanoemulsions (≈13%), while for WO nanoemulsions was significantly lower ≈9%. High oleic acid content from olive oil might have enhanced β-carotene transfer to mixed micelles. Furthermore, highly unsaturated fatty acid content in WO nanoemulsions, would have been unstable during *in vitro* gastrointestinal tract passage, leading to the formation of several oxidation products which might have ultimately affected to β-carotene stability.

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β-carotene bioequivalence in theoretical healthy young females quantified by model-based compartmental analysis.

Tsitsi Chimhashu[1], Micheal H Green[2], Marieke Verbakel[1], Joanne Green[2], Alida Melse-Boonstra[1]

[1]Wageningen University and Research, Wageningen, Netherlands, [2]Department of Nutritional Sciences, College of Health and Human Development, The Pennsylvania State University, Pennsylvania , USA

Introduction: When used in combination with stable isotope dilution techniques, model-based compartmental analysis can quantify bioequivalence of β-carotene (provitamin A) to retinol from a mixed diet.
Objective: To develop a method to determine the bioequivalence of dietary provitamin A in theoretical subjects using compartmental modelling that can later be used in free-living subjects fed mixed diets.
Methods: We used a previously-developed compartmental model and simulated group retinol kinetic parameters and plasma retinol using the Simulation, Analysis and Modelling (WinSAAM) software in theoretical women who ingested a single dose of [13C10]retinyl acetate and then sequential daily doses of VA supplements (0, 400, 800, or 1600 µg retinol) starting on d 14. Thereafter, we calculated each supplementary group's retinol specific activity (SAp) and predicted bioequivalence.
Results: The rate of retinol transfer from hepatic stellate cells was controlled to homeostatically control plasma retinol concentration. Initiation of VA supplements on day 14 resulted in differences in SAp between groups, allowing the calculation of bioequivalence.
Conclusion: We hypothesise that, given homeostatic control of plasma retinol and using sequential dosing with VA supplements studied here using WinSAAM should be translatable to predict the bioequivalence of β-carotene to retinol from a mixed diet in real subjects.

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Bioaccessibility of omega-3s and vitamin D in supplements, naturally rich and fortified foods using a static *in vitro* gastrointestinal model

Stylianos Floros[1], Evaggelia Pasidi[1], Patroklos Vareltzis[1]
[1]Aristotle University of Thessaloniki, Thesasloniki, Greece

Background: Modern dietary habits have created the need for the design and production of functional foods enriched in bioactive compounds for a healthy lifestyle. However, the fate of many of these bioactive compounds in the human gastrointestinal (GI) tract has not been thoroughly investigated.
Method: In this study, the bioaccessibility of omega-3 fatty acids and Vitamin D was examined. Eight different foods, naturally rich or fortified with the bioactive components, and seven supplements underwent simulated digestion following the INFOGEST protocol. Furthermore, the effect of the gastric pH on the bioaccessibility of Vitamin D was investigated. Oxidation of PUFAs was followed using peroxide value and TBARS and by quantifying individual omega-3 fatty acids using GC-FID. Vitamin D was quantified by HPLC and LCMS. The final bioaccessibility values of omega-3 fatty acids and Vitamin D were determined.
Results: Digestion led to profound oxidation of omega-3 fatty acids, giving rise to both primary and secondary oxidation products. Oxidation rate was strongy correlated to each fatty acid's initial concentration. Emulsified lipids were better protected than non-emulsified lipids.
Conclusions: Stomach conditions seemed to exert the most significant effect on the integrity of both bioactive substances, significantly decreasing their bioaccessibility.

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Effect on starch bioaccessibility and glycaemic response of incorporating chickpea cellular powder into white bread

Natalia Perez-Moral[1], Peter Ryden[1], Jennifer Ahn-Jarvis[1], Ana Pinto[2], Peter Ellis[2], Sarah Berry[2], Balazs Bajka[2], Cathrina Edwards[1]
[1]Quadram Institute Bioscience, Norwich, United Kingdom, [2]King's College London, London, United Kingdom

Background: High glycaemic food contributes to the increasing incidence of type 2 diabetes. Reducing the glycaemic potency of white wheat bread is challenging because bread-making conditions facilitate starch gelatinisation and its digestion. In chickpea cellular powder resistant starch is encapsulated by intact plant cell walls that act as a physical barrier to amylase and limit the gelatinisation, rate and extent of starch digestion.
Methods: We replaced wheat flour with chickpea cell powder in white bread and evaluated its resilience to baking conditions and the effects on starch digestibility, glycaemic responses and released gut hormones.
Results: The integrity of cell wall fibre in chickpea powder was preserved after baking. This caused a ~40% reduction in *in vivo* glycaemic responses after 120 min in white bread rolls when 30%-60% (w/w) of wheat flour was replaced with intact cell powder. Starch digestibility analysis and microscopy confirmed the importance of cell integrity in attenuating glycaemic responses.
Conclusions: Using a combination of *in vitro* and *in vivo* techniques we demonstrated that plant cell integrity is the critical factor limiting the bioaccessibility of starch from pre-processed powders and that using chickpea cell powder in a conventional white wheat bread recipe reduces its glycaemic response.

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Effects of mastication on anthocyanin bioaccessibility in fresh blueberries: application of harmonised INFOGEST protocol

Zicheng Zhang[1], Iván Escobar-Martínez[2], Zahra Malik[1], Balazs Bajka[1], Peter Ellis[1], Cristina Torres-Fuentes[2], Manuel Suárez[2], Ana Rodriguez-Mateos[1]
[1]Department of Nutritional Sciences, School of Life Courses Sciences, Faculty of Life Sciences & Medicine, King's College London, London, United Kingdom, [2]Nutrigenomics Research Group, Department of Biochemistry and Biotechnology, University of Rovira I Virgili, Tarragona, Spain

Background: Little evidence exists on the effects of mastication on anthocyanin bioaccessibility (release) during digestion, especially when consuming fresh blueberries.
Method: *In vivo* mastication of blueberries performed by 3 healthy participants was compared to the *in vitro* oral phase of the INFOGEST harmonised protocol. Human chewed (HC) and simulated chewed (SC) samples were further digested using the INFOGEST gastric and duodenal phases. Anthocyanin bioaccessibility was determined by HPLC-MS.
Results: Masticated boluses analysed by mechanical sieving demonstrated that particle sizes in HC tissues were larger than in SC samples. Most blueberry skin cells remained intact, whereas ruptured cells at the peripheral edges resulted in anthocyanin release. Oral bioaccessibility of anthocyanins from blueberries was higher in SC tissues (7.84%) compared to HC samples (2.54%), potentially due to increased oral processing time in SC or biotransformation by the salivary microbiome in HC samples. Following simulated gastric digestion, both HC and SC tissues contained stable and bioaccessible anthocyanins (29.44% and 43.15%, respectively). However, after duodenal digestion, recoveries of anthocyanins in HC and SC samples were markedly reduced to 7.29% and 9.31%, respectively, likely due to the high pH environment.
Conclusion: Different mastication patterns of fresh blueberries may affect anthocyanin bioaccessibility during further digestion processes.

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Chemical and functional assessment of residual gluten immunogenic peptides after gastrointestinal digestion of real wheat food matrices with glutenase-E40

Gianfranco Mamone[1], Maria Cristina Comelli[2], Katharina Kessler[2], Luigia Di Stasio[1], Serena Vitale[3], Ilaria Mottola[3], Linda Cavaletti[4], Carmen Gianfrani[3]
[1]Institute of Food Science - National Research Council of Italy, Avellino, Italy, [2]Nemysis Limited, Dublin, Ireland, [3]Institute of Biochemistry and Cell Biology - National Research Council of Italy, Naples, Italy, [4]Fondazione Istituto Insubrico Ricerca per la Vita, Gerenzano, Italy

Background: Glutenases highly efficient in degrading gluten proteins, are attractive candidates for the development of a pharmacological treatment of gluten intolerance.
Method: Validated proteomic LC-MS/MS, competitive/sandwich R5-ELISA, and IFN-γ production in intestinal T cells from Coeliac disease-(CeD) patients were used to identify gluten immunogenic peptides(GIPs) surviving into gastric (G) and gastrointestinal (GI) digesta of liquid and solid gluten-based real food, containing soft or durum wheat. The Infogest 2.0 protocol was applied to the *in vitro* multicompartmental model, including oral, gastric and duodenal phases. The residual GIPs and T-cell immunogenicity was assessed in G and GI digesta after the addition of E40 (Endoprotease-40, Nemysis Limited), at increasing enzyme:gluten ratio.
Results: Since the gastric phase, the addition of E40 demonstrated an extensive (95%) dose-dependent detoxification of whole gluten in real complex food matrices. Overall, the residual gluten content was found at or even below the 20 ppm gluten-free threshold for soft and durum. Differently from untreated-GI digesta, none of the known toxic α-gliadins derived GIPs survived in E40-treated digesta. Traces of ω- and γ-derived GIPs were still detected in E40-GI digesta, but unable to stimulate CeD-intestinal T cells.
Conclusions: E40 is a promising candidate for the oral enzymatic therapy of gluten intolerance.

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Glucosinolates contents and bioaccessibility in *Brassica rapa* vegetables obtained by organic and conventional cropping systems

Fernando Cámara-Martos[1], Sara Obregón-Cano[2], Antonio de Haro-Bailón[2]
[1]Departamento de Bromatología y Tecnología de los Alimentos, Universidad de Córdoba. Campus Universitario de Rabanales, Edificio C-1, Cordoba, Spain, [2]Departamento de Mejora Genética Vegetal. Instituto de Agricultura Sostenible (IAS – CSIC), Cordoba, Spain

Background: Glucosinolates are a group of phytochemicals unique to vegetables belonging to Brassicaceae family. They are some of the most important secondary metabolites in these vegetables. Glucosinolates are associated with beneficial health effects (e.g. inhibiting tumor formation).
Method: *Brassica rapa* L. subsp. *rapa* (turnip greens and turnip tops) were grown under conventional and organic conditions in two Farms in Southern Spain. Glucosinolates contents and bioaccessibility were studied
Results: The total glucosinolate content ranged between 1.28 – 13.23 μmol/g and 13.36–20.20 μmol/g for turnip greens and turnip tops respectively and it was significantly higher ($p < 0.05$) in vegetables grown in conventional conditions. Bioaccessibility of the total glucosinolates analysed was high, with mean values of around 73% and 66% for turnip greens and turnip tops. Bioaccessibilities of predominant glucosinolates (gluconapin, progoitrin and glucobrassicanapin) were also high, with medium values of 78, 72, and 67%, respectively, in turnip greens, and 67, 49 and 78%, respectively, in turnip tops. All these glucosinolates are categorized as being aliphatic ones.
Conclusions: The fact that *Brassica rapa* vegetables were grown under organic conditions does not guarantee a higher content in glucosinolates. On the contrary, these compounds tend to be more abundant in the conventional system.

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Microencapsulation of selenium by spray-drying as a tool to improve bioaccessibility in food matrix

Fernando Cámara-Martos[1], Filipa Guerreiro[2], Joao Pedro Almeida Lopes[3], Ana Grenha[2]
[1]Departamento de Bromatología y Tecnología de los Alimentos, Universidad de Córdoba. Campus Universitario de Rabanales, Cordoba, Spain, [2]Centro de Ciências do Mar, CCMAR. Faculdade de Ciências e Tecnologia. Universidade do Algarve. Campus de Gambelas, Faro, Portugal, [3]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, Lisboa, Portugal

Background: Microencapsulation is the process by which compounds of interest are surrounded by a coating, resulting in particles that may range from submicrons to several millimeters in size. The presence of the shell provides a physical barrier that permits the protection of the encapsulated compounds from external agents, while enabling controlled of the encapsulated compound release and preventing interactions with other food ingredients.
Method: Se (sodium selenite) was microencapsulated by spray – drying and added to a food matrix (yogurt) to study the potential improvement of its bioaccessibility. Yogurt samples were also supplemented with Se in free salt form.
Results: The supplementation of yogurt with Se in the form of free sodium selenite had a low effect on improving the bioaccessibility of this micronutrient (1%). In turn, Se microencapsulation with mannitol or mannitol/gastro-resistant polymer (Eudragit©) had a strong impact on bioaccessibility results. After the gastric phase, Se bioaccessibility reached values of 21 and 40% for the microencapsulated formulations, respectively. This percentage rose to 55% at the end of intestinal phase, showing no differences between both formulations.
Conclusions: Results show the relevance of microencapsulation as an effective tool to improve the bioaccessibility of micronutrients when they are used in food supplementation.

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Ca, Se contents and bioaccessibility in *Brassica rapa* vegetables obtained by organic and conventional cropping systems

Fernando Cámara-Martos[1], Sara Obregón-Cano[2], Javier Martínez-Castro[1], Antonio de Haro-Bailón[2]
[1]Departamento de Bromatología y Tecnología de los Alimentos, Universidad de Córdoba. Campus Universitario de Rabanales, Edificio C-1, Cordoba, Spain, [2]Departamento de Mejora Genética Vegetal. Instituto de Agricultura Sostenible (IAS – CSIC), Cordoba, Spain

Background: Plant species belonging to the Brassicaceae were one of the first plant groups cultivated and domesticated by humanity. It has been pointed out that Brassicas are a good dietary source of bioaccessible Ca due to a low content of some chelating agents such as oxalates. In additon, Brassicaceae species can accumulate high concentrations of Se with little or no ostensible impairment to the plant
Method: Vegetables were grown under conventional and organic conditions in two Farms in Southern Spain. Ca and Se contents and bioaccessibility were studied
Results: Average Ca total and bioaccessible contents ranged between 14.6 – 23.4mg/g; 8.9 – 12.0mg/g for turnip greens and 6.4 – 8.9mg/g; 4.3 – 4.8mg/g for turnip tops. According to these concentrations, an intake of 100 – 200g of the *Brassica rapa* studied fulfills Ca DRI considering data of total content and complies with 72 – 100% Ca DRI percentage considering bioaccessible data. Se concentrations ranged between 0.061 – 0.073 µg/g and 0.039 – 0.053 µg/g for turnip greens and turnip tops respectively. Se bioaccessibility values were high, with percentages of around 90%.
Conclusions: Vegetables studied have proven to be an excellent dietary source of Ca and Se with a high bioaccessibility.

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Digestibility of protein made from cricket flour

Barbora Lampová[1], Ivo Doskočil[1], Štěpánka Kozáková[1]
[1]*Czech University of Life Sciences Prague, Prague, Czech Republic*

The world population is currently rapidly growing. As the population grows, so does the demand for animal protein. Due to the fast life cycle and high reproduction rate, the use of insects as food seems to be the promising solution.
The aim of this work was to compare the digestibility of cricket protein (Cricket protein powder for cooking & baking) with whey protein. For this experiment, both tested proteins were first digested using the INFOGEST static *in vitro* simulation of gastrointestinal food digestion. The samples were taken for amino acid composition analysis at predetermined time intervals of the intestinal digestion phase. Digestibility was determined by comparing the amino acid composition of digested and undigested samples.
In this work, it was found that the digestibility of cricket protein increases during the intestinal phase. At the beginning of the intestinal phase it ranges from 40 to 50%, at the middle of the intestinal phase it ranges from 71% to 77% and at the end of the intestinal phase digestion ranges from 78 to 83%, and therefore this protein source can be considered as suitable. This is despite the fact that digestibility was lower than that of whey protein by about 15%.

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Collagen hydrolysate bioavailability: does the molecular weight affect the absorption? a pilot randomized clinical trial

Nicolina Virgilio[1], Yvonne Mödinger[2], Christiane Schön[2], Bastiaan van der Steen1, Catarina Ferreira da Silva[1], Janne Prawitt[1]
[1]Rousselot BV, Ghent, Belgium, [2]BioTeSys GmbH, Esslingen, Germany

Background: Collagen hydrolysate (CH) is widely used as food supplement to maintain an adequate nutrient intake and/or to support specific physiological functions. CH represents a valuable alternative to whole protein intake due to the enhanced digestibility and bioavailability.
This study aimed to evaluate the bioavailability of low vs. high molecular weight bovine-based CH in healthy human individuals.
Method: A randomized, double blind cross-over clinical study was carried out involving 6 healthy participants. Subjects took 10g of low molecular weight (LMW) (~ 2000 Da) or high molecular weight (HMW) (~ 5000 Da) CH in 200 mL of water, with a washout period in between. Pharmacokinetic parameters of specific CH markers were assessed in blood samples to evaluate the bioavailability of the different CHs.
Results: The analysis of Hyp, Gly, Pro, Hyp-Gly, Pro-Hyp showed no significant differences in pharmacokinetic endpoints between LMW and HMW ($p>0.05$) and that ~ 40% of bioavailable Hyp is peptide-bound.
Conclusion: Markers for CH intake showed a comparable bioavailability of LMW and HMW and that CH is absorbed as free amino acids (AA) and peptides. These evidences suggest that the range of MW analyzed is not limiting the absorption of collagen pivotal AA/peptides in healthy subjects.

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In vitro digestion for assessing the effects of processing on proteinaceous nutrients bio-accessibility

Chiara Nitride[1], Giovanni D'Auria[1], Gianfranco Mamone[2], Gianluca Picariello[2], **Pasquale Ferranti[1, 2]**

[1]University Of Naples Department of Agricultural Sciences, Napoli, Italy, [2]Institute of Food Science, CNR, Italy, Avellino, Italy

Background: The ongoing growth of world population has made the evaluation of alternative sources of high valuable proteins a priority. Novel sources of plant proteins are likely to be accepted by consumers, however, suffer from low availability and accessibility of essential amino acids because of the antinutrients.
Method: We studied the digestibility of isolated proteins, of processed and fermented model foods using the INFOGEST method implemented with the jejunal digestion phase carried out by the brush boarded membrane enzymes (BBM). The products of digestion (peptides and amino-acids) were monitored by advanced proteomic approaches to identify positive effects associated with the processing.
Results: Roasting improves digestibility of tree nuts, also reducing the number of immunogenic peptides detected after BBM step. Physical removal of cellulose and polyphenols improved the digestibility of proteins derived from plant sources as *Moringa* leaves and hemp seeds. Germination can increase the amino-acid bioavailability of flour chickpea. The digestion of kashk, a fermented dairy by-product, showed the generation of peptides with positive bioactive potential.
Conclusions: The proteomic characterisation of the digestome can guide the selection of processing approaches successful in improving the protein's bio-accessibility and availability.

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Encapsulation of DHA oil as Pickering emulsion improved DHA release during digestion and substantially promoted oxylipin profiles of rat tissues.

Jun Wang[1,2], Jordane Ossemond, Olivia Ménard[1,2], Gwenaële Henry[1,2], Yann Le Gouar[1,2], Françoise Boissel[2,1], **Didier Dupont[1,2]**, Frédérique Pédrono[2,1]
[1]INRAE 1253 STLO, Rennes, France, [2]Institut Agro Rennes-Angers, Rennes, France

Background: Docosahexaenoic acid (DHA) is an essential polyunsaturated fatty acid (FA) mainly involved in cognitive and cardiac functions. Nutritional intake is nevertheless globally insufficient in many countries. Food fortification alternatives should be developed to meet nutritional needs, while avoiding lipid oxidation.
Methods: Pickering emulsion was prepared with heat-denatured whey protein particles to encapsulate DHA oil composed of DHA-enriched triacylglycerols. Then omelets were cooked with either non-encapsulated or encapsulated DHA oil. Each food matrix was digested according to the INFOGEST *in vitro* static model and tested *in vivo* on a rat model as food supplementation. The effect of encapsulation was investigated on bioaccessibility, bioavailability, tissue accretion and metabolism of DHA.
Results: *In vitro*, DHA oil was not hydrolyzed during the gastric phase, on the contrary to the other triacylglycerols from eggs. In the intestinal phase, encapsulation of DHA oil promoted DHA release from triacylglycerols as free FA (52% vs 40% of total DHA). *In vivo*, encapsulation increased rat growth by enhancing food consumption, without globally modulating the tissue FA profile. In contrast, it drastically impacted profiles of oxidized derivatives of FA in plasma, heart and even brain.
Conclusion: The intake form of DHA oil greatly influenced DHA metabolism in tissues.

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In vitro digestion and storage stability of riboflavin-loaded WPI nanostructures towards foods fortification

Daniel Madalena[1], Raquel Gonçalves[1], João Fernandes Araújo[1], António Augusto Vicente[1], Ana Cristina Pinheiro[1]
[1]Centre of Biological Engineering, University Of Minho, Braga, Portugal

The consumption of fortified foods incorporating bioactive compounds as a way to promote a healthier lifestyle has gain particular interest in research community and food industry. However, due to their chemical instabilities, bioactive compounds' bioavailability can be compromised during post-processing, storage, and digestion. Their encapsulation/association in nanostructures offers a good strategy to enhance bioactive compounds' bioavailability.
Whey protein isolate (WPI) nanostructures were developed to associate riboflavin (Rb), aiming at its incorporation in foods, and their storage stability and digestion behavior were evaluated. Rb bioaccessibility was determined through spectrofluorimetry by quantifying Rb concentration in the soluble fraction after digestion, that was performed using INFOGEST static *in vitro* gastrointestinal model. Also, storage stability was evaluated by assessing nanostructures size and polydispersity (PdI) through dynamic light scattering, over 45 days at 4 °C and 25 °C.
Rb-loaded WPI nanostructures showed no statistically significant differences in terms of size (ca. 120 nm) and PdI (0.2) during storage period, at both temperatures tested. Rb showed a bioaccessibility of 56 % when associated in WPI nanostructures, enhancing Rb bioaccessibility. These results contribute to improve the knowledge on the use of WPI nanostructures as effective encapsulating systems to augment hydrophilic bioactive compounds' bioaccessibility, towards food fortification.

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Evaluation of *in vitro* bioaccessibility of Ni and Pb in enteral formulas: risk assessment

Javier Martínez-Castro[1], Alicia Moreno-Ortega[1], Rafael Moreno-Rojas[1], Fernando Cámara-Martos[1]
[1]*Departamento de Bromatología y Tecnología de los Alimentos, Universidad de Córdoba. Campus Universitario de Rabanales, Edificio C-1, Cordoba, Spain*

Background: Enteral nutrition is the name given to the system that delivers nutrients directly into the gastrointestinal tract. Food can become contaminated with toxic metals.
Methods: 8 enteral nutrition formulas were analyzed to determine Ni and Pb content and its bioaccessibility. A probabilistic model was developed to estimate the intake levels for Ni and Pb derived from consumption of 1200–1500mL
Results: Ni and Pb total contents ranged between 2.9 – 8.7µg/100mL and 3.7 – 18.0µg/100mL respectively. Results obtained from the simulation of the probabilistic model showed that Ni and Pb intakes for 50th percentile with these enteral formulas were 68.4 and 75.1µg/day respectively; and for 95th percentile (the most unfavorable situation) were 114 and 246µg/day. In this latter case, the Tolerable Intake (TI) percentage for Ni and Pb (cardiovascular effects) for a mean body weight of 70kg person were 58% and above 100%. This represents a toxicological risk. Regarding Ni and Pb bioaccesible data, we found values of 33.0 and 51.5µg/day which represent a TI percentage of 17% and 49% (95th percentile) for both heavy metals.
Conclusions: According to the gastro-intestinal model developed, patients fed with these nutritional formulas could be exposed to a toxicological risk for Pb.

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Cr content and bioaccessibility in enteral nutrition formulas: Influence of protein and fat content and contribution to Dietary Reference intake

Javier Martínez-Castro[1], Alicia Moreno-Ortega[1], Rafael Moreno-Rojas[1], Fernando Cámara-Martos[1]
[1]Departamento de Bromatología y Tecnología de los Alimentos, Universidad de Córdoba. Campus Universitario de Rabanales, Edificio C-1, Córdoba, España

Background: Enteral nutrition is the name given to the system that delivers nutrients directly into the gastrointestinal tract. Its purpose is to maintain a proper nutritional status in patients who are unable to meet their nutrient needs with a regular diet. Different studies have indicated Cr (III) as an essential element, necessary as a glucose tolerance factor for the efficacy of insulin.
Methods: 8 enteral nutrition formulas were analyzed to determine Cr content and bioaccessibility, following an in-vitro gastro-intestinal digestion model
Results: Cr total and bioaccessible contents ranged between 10.4-16.8 µg/ 100 mL; 1.4 – 2 µg/ 100 mL respectively. A daily intake of 1.5 L of enteral formulas fulfills Cr dietary reference intake (DRI). Cr bioaccessibility showed dialyzability percentages between 10 – 18 %. Protein and fat contents ranged between 3.7-8.0 and 1.3-11.9 g / 100 mL respectively. Protein and fat contents did not influence Cr bioaccessibility in studied enteral formulas.
Conclusions: According to the gastro-intestinal model developed, patients fed with this nutritional support get the DRI established for this trace element

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Effect of processing of infant milk formula on growth performance, protein digestibility and gut barrier physiology *in vivo*

Cathal Dold[1], Yihong Chen[1], Hazel B. Rooney[3], Nora O'Brien[2], Simona L. Bavaro[1], André Brodkorb[1], Peadar G. Lawlor[3], Linda Giblin[1]

[1]Teagasc Food Research Centre, Moorepark , Fermoy, Ireland, [2]School of Food and Nutritional Sciences, University College Cork, Cork, Ireland, [3]Teagasc Pig Development Department, Teagasc Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Ireland.

Background: Infant milk formula (IMF) was produced at pilot scale by cascade membrane filtration (IMF-CMF) as an alternative to high temperature (IMF-HT) processing.
Methods: At weaning, 20 piglets were randomly assigned to two treatment groups (1) IMF-HT OR (2) IMF-CMF. Piglets were fed twice daily and water was available ad libitum for 28 days. Piglets were slaughtered 3 hours after their final feeding.
Results: Piglets fed IMF-CMF had significantly higher average daily feed intake from day 0 – 7 in comparison with IMF-HT fed piglets (269.49 V's 200.87 ± 17.85 g/day; P <0.001). Piglets fed IMF-HT had significantly lower average daily gain in contrast with piglets fed IMF-CMF (140.20 V's 200.82 ± 22.63 g/day; P = 0.013). The degree of protein hydrolysis was significantly higher (P = 0.027) in the duodenum of piglets who received IMF-CMF (1530 ± 136 µmol of NH2/mg of protein) versus IMF-HT (1174 ± 124 µmol of NH2/mg of protein). The IMF-CMF fed piglets had a significantly higher number of goblet cells in the jejunum versus the IMF-HT fed piglets (17.4 V's 11.7 ± 1.9 goblet cells: P = 0.028).
Conclusions: IMF-CMF increases feed intake, milk protein hydrolysis during duodenal digestion, and goblet cells in jejunum.

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Iron, zinc and carotenoids bioaccessibility in biofortified and non-biofortified foods based on millet, cowpea and orange fleshed sweet potatoes

Mbeugué Thiam[1,2], Adama Diouf, Nelly Fioroni[2], Christèle Vernière[2], Sylvie Avallone[2], Olouwafemi Mistourath MAMA Ba[1], Nicole Idohou Dossou[1], Jean Michel Medoc[3], Claire Mouquet-Rivier[2], Christèle Humblot[2]

[1]Laboratoire de Recherche en Nutrition et Alimentation Humaine (LARNAH), Université Cheikh Anta Diop , Dakar, Senegal, [2]IRD, UMR Qualisud, Montpellier, France, [3]CIRAD, UPR Recyclage et Risque, 18524 , Dakar, Sénégal

Background: Despite micronutrient supplementation, food fortification and dietary diversity strategies carried out for decades, micronutrient deficiencies remain prevalent among women of reproductive age and children under 5 years old in rural area in Senegal. In this context, the OR4FOOD project was implemented as a preventive and long-term approach to reduce maternal and child malnutrition through biofortification. This study aimed to assess iron, zinc and carotenoids bioaccessibility in biofortified and non-biofortified foods, to better estimate the impact of their consumption on the micronutrient status of the target population.
Method: Static *in vitro* digestion was performed in triplicate according to the INFOGEST method. Food samples (porridges and mashed) were prepared from millet, cowpea and orange fleshed sweet potatoes (OFSP). Lemon juice and non-enriched vitamin A oil were added to optimize iron/zinc and carotenoids bioaccessibility, respectively. Dialyzable iron/zinc were measured using dialysis membrane containing a PIPES buffer. Minerals and carotenoids were extracted and measured (in progress) with an ICP-OES and HPLC.
Results: Dry matter content in OFSP was 37% versus 28% for the conventional variety.
Conclusions: Through this work, we expect a better comprehension of the bioaccessibility of micronutrients in biofortified foods to estimate their potential to fight micronutrient deficiencies in rural area.

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Effect of feeding and pulsed electric field on lipid and protein hydrolysis of seabass fillets subjected to *in vitro* digestion

Giorgia Antonelli[1], Gianfranco Picone[1], Francesca Lombardi[1], Francesca Danesi[1,2], Mattia Di Nunzio[3], Alessandra Bordoni[1,2], Francesco Capozzi[1,2]
[1]Department of Agricultural and Food Sciences (DISTAL), University of Bologna, Cesena, Italy, [2]Interdepartmental Centre for Agri-food Industrial Research (CIRI Agrifood), University of Bologna, Cesena, Italy, [3]Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Milan, Italy

Fish is the most important source of long-chain omega-3 fatty acids in human diet. Therefore, technologies are being developed to increase its quality and stability. This study aimed to evaluate the impact of an innovative organic diet and pulsed electric field (PEF) technology, an emerging food preservation method, on the digestibility of European sea bass fillets. Sea bass juveniles were fed ad libitum with the conventional or organic diet, and the fillets resulting from the corresponding adult fish were randomly divided into two groups, treated or not with PEF after brine salting. Samples underwent static *in vitro* digestion [1], and digested samples were analyzed by NMR spectroscopy and GC to evaluate their metabolome and fatty acid composition. No significant differences were found between the two feeding groups, indicating that the different diets did not differently affect the digestion of fillets. The application of PEF treatment did not negatively modify the fillets' quality, confirming its potential for preservation of fish and its possible exploitation by the fishing industry.

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Impact of *in vitro* digestion on the phytochemical profile of *Salicornia ramosissima* and *Sarcocornia fruticose*

Juliana Oliveira[1], João Sousa[1], M. Rosário Bronze[1, 2, 3], Ana Teresa Serra[1,2]
[1]iBET - Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901 Oeiras, Portugal, [2]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, , Portugal, [3]iMed ULisboa, Faculdade de Farmácia, Universidade de Lisboa, Av. Gama Pinto, 1649-003 Lisboa, , Portugal

Halophyte species are recognized as promising natural ingredients, having several culinary applications, as they provide an alternative to common salt. Halophytes are rich in phenolic compounds known for their health-promoting properties. However, there are few information regarding the fate of these compounds after gastrointestinal digestion.
This study aimed to investigate the impact of *in vitro* gastrointestinal digestion of phytochemical compounds present in *Salicornia ramosissima* (SR) and *Sarcocornia fruticose* (SF) by applying INFOGEST protocol.
Plants were lyophilized, extracted and characterized in terms of phenolic content/composition and antioxidant activity using several analytical methods (HPLC-UV-DAD/ED, Folin-Ciocalteu's method, ORAC and HOSC). *In vitro* bioaccessibility of phenolic compounds was followed under simulated gastrointestinal conditions (oral, gastric, and intestinal) of the dried plants.
Results showed modifications in the phenolic profile of both plants during *in vitro* gastrointestinal digestion, with a considerable decrease in the total and individual phenolic compounds. Phenolic compounds present in SF were more bioaccessible than those from SR and the highest total phenolic content and antioxidant activity were identified in the gastric phases (TPC: 2.93 mg GAE/g dw; ORAC: 116.78 µmol TEAC/g dw; HOSC: 118.07 µmol TEAC/g dw).

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The effect of popping, a sustainable form of processing, on antinutritional factors in pulses

Joseph Godrich[1], Joanne Gould[1]
[1]*University of Nottingham, Nottingham, United Kingdom*

Background: Pulses are gaining traction as a staple food due to their desirable nutritional profile, accessibility, and sustainability. However, the presence of antinutritional factors (ANFs) in pulses can negatively impact the bioavailability and utilisation of nutrients, with food processing a common strategy to reduce the ANF content. Current processing methods are seen as unsustainable due to high water and energy consumption. The aim of this study was to investigate the effect of popping, a high temperature short time (HTST) form of processing, on the ANF content of two widely consumed pulses.
Methods: Whole chickpea and red kidney beans were subjected to several processes: soaking, roasting, boiling and popping. Samples were then dried and ground to fit a 425 micron sieve before being analysed for ANFs. Megazyme K-PHYT assay and the vanillin-HCL method were used to quantify phytic acid and condensed tannins respectively.
Results: Popping caused a significant decrease in phytic acid and a significant increase in condensed tannins in chickpea and red kidney bean.
Conclusion: Popping should be considered for food processing to enhance the bioavailability and utilisation of nutrients in pulse ready-to-eat snacks.

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Quinoa peptides as DPP-IV inhibitors: an *in silico* approach

Maria Lilibeth Manzanilla Valdez[1], Alan Javier Hernandez Alvarez[1], Caroline Orfila[1], Christine Bosch[1], Maricela Sarita Montaño Valdez[2]
[1]University Of Leeds, Leeds, United Kingdom, [2]Universidad Autónoma de Sinaloa, Culiacan, Mexico

Diabetes mellitus type 2 (T2DM) is characterized by hyperglycemia and insulin resistance. DPP-IV inhibitors are a new drug family to treat T2DM, as any pharmacology treatment it has many side effects such as pancreatitis and joint pain. Functional ingredients like bioactive peptides are an alternative to treat T2DM. Although, peptides are effective they are expensive to produce, for that reason bioinformatic analysis is an alternative to reduce costs and time. Methods: NCBI data base was used to retrieve FASTA sequences of 11S seed storage globulin (> AAS67037.1) Chenopodium quinoa, and BIOPEP was used for simulated hydrolysis with papain (EC 3.4.22.2), three peptides were selected QESWR, DKDYPKR and HVIKPPSSR for molecular docking using PyMol software and HPEPDock server. Molecular target DPP-IV (PDB 2P8S) was retrieved of PDB data base. Results showed that QESWR presented the highest number of bonded sites Arg 125, Arg 125, Ser 209, Tyr 547, Pro 550, Gln 553, Tyr 585. While DKDYPYR presented one binding site Ser 209 and HVIKPPSSR none. These findings are similar to those reported by a potent DPP-IV inhibitor named Sitagliptin. Quinoa peptides could be an alternative to treat T2DM, more studies are needed to support this information.

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Iron, zinc and β-carotene bioaccessibility in biofortified and non-biofortified foods based on millet, cowpea and orange fleshed sweet potatoes

Mbeugué Thiam[1,2], Adama Diouf[1], Nelly Fioroni[2], Christèle Vernière[2], Sylvie Avallone[2], Olouwafemi Mistourath MAMA Ba[1], Nicole Idohou Dossou[1], Jean Michel Medoc[3], Mouquet-Rivier[2], Christèle Humblot[2]

[1]Laboratoire de Recherche en Nutrition et Alimentation Humaine (LARNAH), Université Cheikh Anta Diop , Dakar, Senegal, [2]IRD, UMR Qualisud, Montpellier, France, [3]CIRAD, UPR Recyclage et Risque, 18524 , Dakar, Senegal

Background: Despite micronutrient supplementation, food fortification and dietary diversity strategies performed for decades, micronutrient deficiencies remain prevalent among women of reproductive age (WRA) and children under 5 years old in rural area in Senegal. In this context, the OR4FOOD project was implemented as a preventive and long-term approach to reduce maternal and child malnutrition through biofortification. This study aimed to assess iron, zinc and β-carotene (BC) bioaccessibility in biofortified foods, to estimate the impact of their consumption on the micronutrient status of the target population. Method: Static *in vitro* digestion was performed in triplicate according to the INFOGEST method. Food samples (porridges and mashed) were prepared from millet, cowpea and orange fleshed sweet potatoes (OFSP). Dialyzable iron/zinc were measured using dialysis membrane containing a PIPES buffer. Minerals and BC were measured with an ICP-OES and HPLC. Results: Total bioaccessible BC, iron and zinc in porridge were more than 20%, 30% and 60%, respectively. An edible portion of 200 g of porridge containing OFSP provide 30% of the recommended dietary allowances of vitamin A both in children 6-23 months old and WRA. Conclusions: These results support the introduction/promotion of complementary biofortified food to tackle micronutrients deficiencies in rural area in Senegal.

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Effect of Zn and Se biofortification on macro and microelement *in vitro* bioaccessibility in fresh wheatgrass juice

Andrijana Rebekić[1], Sanja Grubišić[1], Marija Kristić[1], Miroslav Lisjak[1], Katarina Mišković[2]
[1]Faculty Of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, 31000, Osijek, Croatia, [2]Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Josipa Huttlera 4, 31000, Osijek, Croatia

Background: Wheatgrass represents young wheat plants, which are in the form of fresh wheatgrass juice or wheatgrass powder used as a dietary supplement. The aim of this research was to examine the effect of Zn and Se biofortification on the total and *in vitro* bioaccessible concentrations of Ca, K, Mg, Fe, Mn, Zn, and Se in fresh wheatgrass juice.
Methods: Conventional and biofortified (Zn and Se) wheat grains of two wheat cultivars were used for wheatgrass production. Plants were grown in controlled conditions for 6, 8, and 10 days. Simulation of *in vitro* digestion was carried out according to Minekus et al., 2014 (https://doi.org/10.1039/C3FO60702J).
Results: A K concentration increased by 13 %, Zn for 19 %, while Se concentration increased by 3.8 fold under the effect of biofortification in comparison to control.
Conclusion: This research indicates that besides biofortification, a cultivar, and plantlets age also have a significant effect on total and *in vitro* bioaccessible mineral concentrations in wheatgrass juice. Accordingly, cultivar selection and suitable agricultural practice can have a significant effect on total and *in vitro* bioaccessible mineral concentrations, and thus increase the nutritional value of fresh wheatgrass juice.

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Bioaccessibility and cellular uptake of free carotenoids and esters from orange peel: comparison between conventional and ionic liquid mediated extractions

Daniella Murador[1], Leonardo de Souza Mesquita[1], Bruna Neves[1], Paula Martins[1], Ana Braga[1], Leila Zepka[2], **Veridiana De Rosso[1]**

[1]Universidade Federal De São Paulo, Santos, Brazil, 2Universidade Federal de Santa Maria, Santa Maria, Brasil

Oranges are commonly regarded as the most complex natural source of free carotenoids and carotenoid esters. The aim of this work was to investigate the bioaccessibility and cellular uptake of free carotenoids and carotenoid esters from orange peel obtained by conventional acetone extraction and an alternative method using ionic liquid (1-n-butyl-3-methylimidazolium chloride ([C4mim]Cl)). The carotenoid extracts were emulsified and submitted to an *in vitro* simulated digestion model, according to the protocol adapted from INFOGEST, followed by the uptake by Caco-2 cells. After digestion, 22.9% of total carotenoids obtained by acetone and 24.2% from [C4mim]Cl were bioaccessible. Significant differences were not noted between the bioaccessibility of the acetone and [C4mim]Cl extracts within each class of carotenoids (xanthophylls, carotenes, monoesters and diesters). The uptake by Caco-2 showed that contents of xanthophylls were 15.78 and 15.50 ng carotenoids.mg-1 cellular protein, carotenes were 35.02 and 69.58 ng carotenoids.mg-1 of cellular protein, esters were 36.35 and 51.42 and ng carotenoids.mg-1 cellular protein, total carotenoids were 118.07 and 183.39 ng carotenoids.mg-1 cellular protein, for the acetone and [C4mim]Cl extracts, respectively. The results showed that free carotenoids presented better bioaccessibility and cellular uptake compared to the esters, regardless of the extract were well absorbed by Caco-2 cells.

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