## Gums & Stabilisers for the Food Industry June 3<sup>rd</sup> – 6<sup>th</sup> 2025

# **Book of Abstracts**

## Presentations

## PL Plenary lectures

# PL 1. Why a food is more than the sum of its ingredients: the role of foods structure in starch digestion of pulse-based foods

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Starch, a key hydrocolloid in foods, has often been demonized in public discourse due to its effect on blood glucose levels. However, starch holds a key position to enable sustainable and affordable energy for the growing world population. Importantly, not all starch is the same; its impact on the human body is influenced by factors such as molecular and granular structure, food macrostructure, and interactions with other food or meal components. This presentation focuses on strategies for food scientists and technologists to tailor starch digestion properties through formulation and processing as part of food design. Pulses, particularly lentils, will serve as a case study of how targeted thermal processing can modify the microstructure of the food, and in turn, lead to steered in vitro starch digestion properties. These insights were applied to develop lentil-based ingredients and solid foods (i.e., pasta) with distinct starch digestion properties.<sup>1-4</sup>

Most current in vitro digestion studies, including the work presented above, utilize static models. These models provide a simplified approach enabling the comparison of the susceptibility of starch in (structured) food systems to enzymatic hydrolysis. However, static models fail to capture the transient complexity of human digestion processes. To address this, a custom-made multireactor digestion system (MuReDi)<sup>5</sup> was employed, introducing physiologically relevant semi-dynamic simulation parameters. These parameters included (i) salivary amylase activity in the oral phase; (ii) dynamic gastric pH profiles; (iii) gradual enzyme secretion; and (iv) stepwise gastric emptying. This work showed the impact of salivary amylase activity and gastric pH profile on starch digestion in population groups showing clear differences in gastrointestinal conditions and requiring appropriate nutrition. The example of digestion simulations relevant for older adults (>65 years old) will be given, highlighting the importance of food structure on the digestion of starch-rich foods for this population group as well.<sup>7</sup>

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# PL 2. Tailoring plant protein aggregation to deliver functionality in complex food systems

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Promoting the use of plant proteins in food formulations can efficiently contribute to reducing the carbon footprint of the global food system. Currently, most of plant protein sources are derived from crops, used to produce non-protein ingredients such as oils, fibres or starches, leading to protein fractions with a variety of aggregation states and functional properties. The development of gentle extraction methods enables preserving the functionality of plant proteins, but in addition, formulation and processing steps can be considered and adjusted.<sup>1</sup>

In this presentation, we will first describe the use of high-pressure homogenization for improving the solubility and foaming properties of commercial pea protein isolate.<sup>2</sup> The shear induced by the process enables to break interactions occurring in protein powder, leading to free and aggregated pea protein fractions with different surface activity. In addition, we will show that a controlled heat aggregation of pea proteins induced by calcium addition can even be beneficial to generate protein aggregates able to reduce drainage in aqueous foams. Further, the importance of characterizing the interactions with water in soy protein doughs will be discussed in relation to their processability in wet extrusion conditions for producing texturized proteins. Finally, we will discuss the use of plant protein/polysaccharide mixtures for developing binding systems in plant-based meat analogues or for controlling the texture of heat-set seafood analogues.

All these examples will demonstrate the ability of plant protein ingredients to be widely used in complex food products when their functionality is well controlled.<sup>3</sup>

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## PL 3. Valorisation strategies to obtain hydrocolloids of interest for food and food packaging

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The food value chain generates huge amounts of by-products, losses and waste streams, many of which contain valuable fractions for potential applications as food ingredients or food packaging materials. In view of this situation, biorefinery strategies aim to maximize valorisation of biomass through targeted fractionation towards different applications, while minimizing food waste. Additionally, minimal processing techniques for waste biomass are being explored to develop innovative food packaging materials.

In this lecture, several examples of research works carried out in the group will be shown to demonstrate the relevance of understanding structural features to assess the technofunctional properties of new food ingredients and materials obtained from residual valorisation. By adopting a circular economy approach, waste valorisation can transform the food industry, fostering the development of sustainable ingredients and environmentally friendly packaging materials. These practices align with global sustainability goals and the transition towards a more resource-efficient and resilient food system.

# PL 4. Novel microalgae proteins and their structural, functional, nutritional and sustainability aspects

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The integration of novel production technologies with comprehensive sustainability assessments, incorporating nutritional, environmental, economic, and social indicators, offers a robust foundation for the holistic development of more sustainable food systems. This systems-based approach focuses on driving innovation, particularly in the production of more sustainable, nutritious and affordable protein-rich foods, such as those derived from emerging microalgae.

Microalgae protein-rich fractions have been studied for their diverse functionalities and properties. Cultivating microalgae through photoautotrophic, mixotrophic, and heterotrophic growth modes allows for variations in production efficiency, the utilization of side streams, and differences in macro- and micro-molecule composition, pigments, and biomass coloration. The relationships among structure, process, and properties are directly linked to the techno- and biofunctionality. In addition to technofunctional attributes such as surface activity, texturization, emulsification potential, and color, significant emphasis is placed on nutritional qualities, including bioaccessibility and bioavailability. Innovations like pulsed electric field processing integration have shown marked improvements in nutrient bioaccessibility. Heterotrophic microalgae-based meat substitutes hold particular promise for final products. Integrating up to 50% bright yellow microalgae protein concentrate, which contains significantly higher levels of micronutrients such as minerals, vitamins E, B1, B2, B3, and B6 compared to soy, highlights the potential of this approach.

Ensuring the affordability of these products involves addressing scalability, achieving economies of scale, and reducing capital (CAPEX) and operational expenditures (OPEX). Within this framework, solutions leveraging polyextremophilic microalgae for nutritious food production have been developed. For instance, Galdieria sulphuraria, a nutrient-dense microalgae species, thrives under extreme conditions such as low pH (0–3) and high temperatures (up to 56°C) without the need for cooling. Its unique habitat prevents the growth of competing microbial species, significantly reducing decontamination costs, simplifying processing, enabling circular residue stream utilization, and supporting continuous cultivation. Furthermore, the use of nanosecond pulsed electric field-based single-cell stimulation has demonstrated up to a 20% increase in biomass production compared to controls. Collectively, these innovations substantially lower CAPEX and OPEX, making microalgae-based protein rich foods more cost-effective and affordable.

Collaborative efforts with industry partners and start-ups underscore the practical impact and relevance of these science-driven innovations for the food sector.

# **PL 5.** Applications of partial hydrolysis for the structural analysis of polysaccharides

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The technofunctional properties of polysaccharides are decisively influenced by their molecular structure which is highly complex in most cases. Polysaccharides can be composed of various monomeric units which are interconnected by many different glycosidic linkages. Furthermore, a polysaccharide backbone may be substituted with side chains of varying composition and length. This structural diversity is a significant challenge for the analysis of polysaccharides. Methods such as monosaccharide analysis, linkage analysis, or NMR spectroscopy often only provide an incomplete picture of the molecular structure, because they yield no or limited information on, for example, side chain lengths or the presence of copolymers. In addition, their applicability in complex matrices is limited.

These limitations can be overcome by analyzing the oligo- and polymeric products which are obtained from partial hydrolysis of the polysaccharides. Although there are many possibilities for partially hydrolyzing polysaccharides, the use of highly specific enzymes is particularly promising. In most cases, the enzymes are used for the hydrolysis of defined glycosidic linkages within the backbone of homopolysaccharides. For example, the hydrolysis of dextrans with endo-dextranase yields isomaltose and glucose from the linear, a-1,6-linked areas, but also branched oligosaccharides. After the characterization of the branched oligosaccharides, an HPAEC analysis can rapidly provide detailed information on the structure of different dextrans. The fingerprints obtained from this approach can also be used to differentiate dextrans that can hardly be discriminated by using other methods. Furthermore, a combination of partial enzymatic hydrolysis with HPSEC and HPAEC can help to identify larger sections with other glycosidic linkages in the a-glucan backbone. Partial enzymatic hydrolysis in combination with different chromatographic techniques can also be applied for the analysis of bacterial fructans which also exhibit remarkable structural complexity. Furthermore, it is possible to analyze complex heteropolysaccharides after partial enzymatic hydrolysis, although their hydrolysis usually requires the application of multiple enzymes. Because the necessary enzymes are often not commercially available and because side activities must be avoided, it is in many cases necessary to produce them in recombinant form. The obtained enzymes can then be applied to gain detailed information on the structural architecture of, for example, hybrid carrageenans. Furthermore, specific hydrolytic enzymes can be applied to deepen the understanding of the structure of gum arabic. Although many polysaccharide-degrading enzymes have been described, it is sometimes not possible to apply this approach. This is especially true for heteroexopolysaccharides which show enormous structural diversity. However, partial chemical hydrolysis can be applied in such cases. Due to the high structural variability, it is typically possible to detect diagnostic fragments and to utilize them for structural elucidation. Hyphenated techniques such as HPAEC-PAD/MS are especially useful for this approach. Partial chemical or enzymatic hydrolysis can also be used to analyze the occurrence of different polysaccharides in complex food matrices such as yoghurt, beverages, or sourdough. Due to the high specificity of the hydrolytic enzymes, the sample workup can often be simplified. However, the application of suitable analytical techniques can also allow for the detection of individual polysaccharides in complex mixtures after less specific chemical hydrolysis. Altogether, partial hydrolysis offers a range of possibilities for the characterization and analysis of polysaccharides. The main challenge for the future will be to translate this potential into quantitative approaches for polysaccharide analysis.

## PL 6. A hydrocolloid approach to food and health

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To study the influence of hydrocolloids on human health, a multidisciplinary and comprehensive approach is necessary. In this talk, some examples are discussed.

1. A fundamental understanding is required for the treatment of cystic fibrosis using alginate containing a mixture of long and short chains, and to the problem of osteoarthritis with respect to interaction between short chain and long chain hyaluronan molecules in synovial fluids.

2. Although it is known that thickened fluids reduce the prevalence of aspiration, excessive thickening reduces the palatability leading to dehydration and malnutrition, and the residue at the oropharynx causes subsequent aspiration. It is therefore necessary to find the optimal condition for thickening and cohesiveness. The granular matter physics approach can be effective to understand the bolus rheology.

3. The application of capillary forces for reduced fat foods is expected to develop foods with reduced calories which can contribute to challenge the problem of obesity.

4. Understanding flavour release is the basis for salt reduction and studies should be carried out to find a better way to develop palatable and healthy foods.

Food hydrocolloids, therefore, make the ideal platform for enhancing collaboration among different fields and expertise.

## KL Keynote lectures

## KL 1. Dietary Fibre and Starch Structures affects Gut Microbiota and Metabolites

#### **Sushil Dhital**

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Dietary fibre, including the Resistant starch, is not digested and absorbed in the small intestine of the human body and thus excursed to the large intestine. The carbon polymers are fermented by microbes to produce beneficial metabolites such as short-chain fatty acids (SCFAs) and organic acids. These metabolites are known to have proven health benefits in diversifying the gut microbiota, leading to immunomodulation and reduction of infections and metabolic diseases. The presentation focuses on how the form (soluble vs insoluble fibres), types (resistant starches, intact plant cells) and size of fibre are related to the diversity of microbiota and metabolites, including the SCFA and other organic acids based on the in-vitro and in-vivo fermentation studies. Understanding the rate and extent of microbial fermentation can help to design functional food with tailored gut functionality.

## **KL 2.** Crafting sustainable polysaccharide ingredients for precision nutrition

## Mario M. Martinez

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Polysaccharides play a pivotal role in human nutrition and food structure design. Although they represent a large share of the commodity and ingredient market, they are vastly underutilised within the food industry. The complexity of polysaccharide structures and the intricacy of their interactions make the task of rational material design difficult, and sheds uncertainty to the practical question of what carbohydrates to eat to stay healthy. In this presentation, I will present featured research outputs on the design of polysaccharide assemblies for optimum functionality and metabolic response. Firstly, I will cover the design of enzyme-resistant starch within the food matrix (in situ), linking starch sequencing through enzymatic fingerprinting with in planta starch synthesis to moderate food texture and starch digestion rates in high-moisture starchy foods. Sometimes however, amyloplasts fail to synthesize our desired starch molecules, necessitating precision modification and transformation processes based on a deep understanding of starch structure. We addressed this limitation by using shear-induced fragmentation (via twin-screw extrusion) or our developed green esterification reaction (using specific foodcompatible deep eutectic solvents as chaotropic agents and reaction promoters). Secondly, and moving towards non-starch polysaccharides, I will go through some of the key aspects governing the successful integration of dietary fiber into food matrices. Last, I will cover our latest findings revealing how polysaccharides can interact with endogenous polyphenols (PPs) and the potential of these interactions to protect PPs from thermal degradation and cause sustained changes in gut microbiota ecology - aiming to design polysaccharide-PP assemblies for colonic health. These findings support creating nextgeneration, less-processed precision nutrition ingredients from upcycled fruit and vegetable pomaces rich in polyphenol-infused plant cell walls. This talk is intended to guide the development of targeted polysaccharide assemblies with programmable interactions, physiological responses, and techno-functional properties.

## KL 3. Mouthfeel challenges of plant proteins: Towards colloidal solutions informing future sustainable food development

## Anwesha Sarkar

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To ensure a continued supply of safe, pleasurable and healthy food to feed the growing population within the planet's environmental constraints, the transition from animal to plant-based foods is imperative. However, the major obstacle to consumer acceptance of plant proteins is their unpleasant mouthfeel, particularly with respect to textural perception i.e. astringency1, often linked to high oral friction. We have recently demonstrated that plant proteins offer different tribological properties2,3 dependent upon how they adsorb to oral-mimicking surfaces. This talk will cover our mechanistic understanding of astringency in plant proteins. We coupled well-established Rate All That Apply (RATA) sensory trial (n=100) with brain imaging using functional near infrared spectroscopy (fNIRS) and cellular response of plant proteins using mucin-producing cell lines. Results indicate that astringency mechanism of plant proteins resembles that found in polyphenols resulting from binding to salivary mucins. In addition, the jamming of tribological contact due to aggregation of plant proteins owing to surface hydrophobicity cannot be ignored. Finally, the talk will cover innovative colloidal strategies such as microgelation4,5 that may mitigate such astringency issues, informing the design of the next generation of pleasurable plant-based foods.

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# **KL 4. Legume cells: Exploiting their digestive breakdown for healthier food solutions**



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Dietary consumption of legumes such as beans, chickpeas, lentils and peas has long been associated with beneficial effects on human cardiometabolic health. However, legumes have traditionally mainly been consumed as whole seeds, which have limited appeal to modern consumers. How can we ensure that the nutritional quality and health benefits of legumes are preserved as these crops are transformed into processed convenience foods? Understanding the mechanisms underpinning the health benefits of whole legume consumption is important to ensure that their nutritional potential is preserved into endproducts.

Whole cooked legumes are comprised of intact cotyledon cells, in which the plant cell wall -a complex network of polysaccharides, encapsulates cytoplasmic nutrients such as starch and protein. Plant cell walls are resistant to mammalian enzymes of the human uppergastrointestinal tract and can thereby regulate the digestion and absorption of intracellular nutrients from legumes[1]. As legumes are increasingly consumed as processed food products, there is a need to understand the effects of processing on cell structure and its consequences for nutrient bioaccessibility.

We have recently investigated the digestive breakdown of chickpea meals with different degrees of plant cellular intactness through the human gastrointestinal tract. Differences in meal structure were consistently found to alter nutrient bioaccessibility, impacting on the human intestinal contents with direct effects on post-prandial glycaemia and gut hormone responses[2].

Recent findings highlight the value of preserving natural legume cotyledon cell structures into processed food products to support consumer health. PulseON (PulseOn Food Ingredients Ltd) is an example of a whole cell legume flour that has been developed for such applications, and has been studied in terms of ingredient properties [3], end-product compatibility[4], through to clinical trials[5].

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## **D** Oral presentations

# P1. Towards clean-label solutions for plant-based foods: combining commercial plant protein isolates and using enzymes to modify gel properties

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Commercial plant protein isolates are used in various plant-based food products. However, it is a general fact that these isolates have rather limited functionality due to their harsh production process. As a result, they underperform compared to dairy proteins.

In this project, we blended commercial legume plant protein isolate (fava bean, pea) to see if there are conditions (protein concentration, ratio, pH, homogenization treatment) at which we can have a synergistic effect and thus enhanced gel properties. However, we mainly found linear or anti-synergy behaviour upon mixing, indicating that the studied proteins had only limited interactions although no phase separation at the micron scale was observed. The results and understanding of protein mixing behaviour are relevant, as one can select conditions to either promote or prevent the gelling ability of multi-protein systems.

In contrast, we found that using a crosslinking enzyme was effective in improving the gel properties (fracture stress and strain) for fava bean and pea protein gels. The impact of crosslinking was closely related to the dispersibility of the protein isolates, and therefore homogenization greatly enhanced the impact of the enzyme treatment. We also evaluated a deamidating enzyme, which increased the dispersibility of the studied protein isolates by unfolding proteins and increasing charge. Deamidation mainly improved the gelation properties of our least dispersible protein isolate (mung bean). We hypothesize that the increase in dispersibility promotes gelation. But modification of already dispersible proteins can have an adverse effect on gelation properties due to increased charge that can limit hydrophobic interactions. Research is ongoing to determine the relations between enzyme activity, protein source, dispersibility and the gelation mechanism.

# P2. In vitro digestion and release of bioactive peptides from chitosan-alginate polyelectrolyte complexes

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In previous work we have characterized the physicochemical characteristics of polyelectrolyte complexes (PECs) formed from mixtures of chitosan (CS) and alginate (ALG), plus the effects of incorporating a mixture of low molecular weight bioactive peptides (BAPs) into these complexes.<sup>1</sup> In this work we investigate the effects of *in vitro* gastric and intestinal digestion conditions on the physical properties of the PECs and the concomitant release of the peptides from them. The molecular weights of the chitosan and alginate were 111 and 21 kDa, respectively. The *in vitro* gastric and intestinal digestion fluids were prepared according to the International Network on Food Digestion (INFOGEST) protocol with key enzymes pepsin, trypsin, and chymotrypsin. Dynamic light scattering (DLS) and mixed-mode phase analysis light scattering (M3-PALS) were used to assess the changes in the PEC size and structure. Ultra-high-performance liquid chromatography (UHPLC) was used to identify and quantify the peptides released under the digestion conditions and the bioactivity of the digestates was analyzed with respect to angiotensin-converting enzyme (ACE).

There are differences in the initial sizes of the PECs depending on whether they are formed in the presence of excess CS (molar charge ratio of ALG : CS = 0.5) or excess ALG (molar charge ratio ALG: CS = 10), as well as their initial zeta potentials but, in general, under gastric conditions (pH 3 + pepsin) the changes in the sizes of the PECs are minimal over 2 h digestion time. At the same time, there is a steady release of peptides over 2 h of up to  $60 \pm 5$  %. Under intestinal conditions (pH 7 + trypsin and chymotrypsin) the PECs formed with excess ALG are more stable in terms of size change than those formed with excess CS, but both PECs show almost complete (100%) release of the peptides after 2 h, though the release is slightly delayed with the latter. Thus, the PECs show reasonably good resistance to gastric digestion, but less stability to intestinal conditions. It should be noted that when digestive conditions are applied but in the absence of the enzymes, the complexes are more stable and peptide release tends to be reduce. Since the free nonencapsulated peptide itself shows susceptibility to intestinal enzyme breakdown (but of course not the CS or ALG) the overall release kinetics are the result of a complex interplay between the enzymes, the encapsulated peptide and the pH and salt conditions moderating the electrostatic interaction between the biopolymers and the peptides. Overall, these types of PECs show good promise for significant protection of the ACE bioactivity of the peptide on consumption.

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Keywords: alginate, bioactive peptides, chitosan, encapsulation, digestion.

# **P3.** Non-destructive Techniques for Hydrocolloid Characterization: from particle sizing to micro-rheology.

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The sensory experience of food is inherently multisensory, encompassing its visual appearance, aroma, texture, and taste. Hydrocolloids are integral to this experience, as they enable precise control over the texture, viscosity, stability, and appearance of food products. To meet the growing demands of consumers, significant research efforts are focused on developing hydrocolloids from novel natural ingredients.

The formulation and optimization of these innovative materials require accurate characterization techniques that span both microscopic and macroscopic properties. Optical methods provide a robust, non-destructive approach to analyze complex samples in native conditions, enabling quantitative assessment of colloid size, polydispersity, shape, and key rheological properties such as viscosity and elastic moduli.

This work introduces advanced techniques based on 3D static and dynamic light scattering which overcome traditional sample dilution requirements for the precise determination of size and shape in colloidal suspensions. Utilizing a custom machine learning algorithm, we can reliably distinguish mixed particle populations, detect aggregation, and identify the onset of instabilities in complex formulations. Additionally, Diffusing Wave Spectroscopy is presented as a powerful tool for determining critical rheological parameters, such as elastic moduli and gel points, in viscoelastic food systems. Practical examples include the characterization of gelatin across the fluid-solid transition, emulsions and dairy products.

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## P4. Impact of individual wet and dry fractionation processing steps on pea protein composition and in vitro digestibility

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Pea protein has gained attention in response to the growing global demand for foods due to its balanced amino acid profile, low cost, and hypoallergenic properties. Conventional wet fractionation methods of pea protein consume large amounts of water and energy, raising concerns about environmental sustainability. To address this issue, mild processing technologies, such as dry fractionation, have been developed to reduce the environmental footprint of fractionation processes. Dry fractionated proteins tend to show better technofunctional properties because the native protein state is preserved. In this study we sought out to study the effect of each step of the fractionation process (dehulling, extraction, drying, post-processing) of pea protein on nutritional composition, anti-nutritional factors, and *in vitro* protein digestibility. Dry fractionation resulted in protein content ranging from 87 to 91%. The INFOGEST 2.0 protocol with minor modifications was used to determine *in vitro* protein digestion. Preliminary analysis indicated that fractionation methods significantly influenced pea protein digestion. Data collection is still ongoing and results will be presented at the conference.

# P5. High temperature thermo-electric treatments as an innovative strategy to improve the techno-functional potential of vegetable proteins.

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Alternative proteins, particularly those derived from legume such as peas, chickpeas, and fava beans, have gathered significant interest from the food industry and scientific community as substitutes for more established plant proteins like soy or wheat. However, commercial proteins from these sources often exhibit poor solubility and functionality, which limits their broader use in food applications. To address this challenge, various modification techniques aimed at enhancing protein functionality have been explored. Physical modification methods such as electric and ultra-high thermal (UHT) processing methods have shown promise in modifying protein structure and improving functionality. This study investigated the potential use of an emerging thermo-electrical processing technology involving ohmic heating (OH), in UHT conditions. Commercial protein isolate samples - pea, fava and chickpea - were subjected to OH treatment at temperatures ranging from 100 °C to 150 °C, and the effects in structural and functional were assessed. Characterization of the treated proteins revealed that the thermo-electric treatments resulted in shifts in tertiary and secondary structures, consistent with structural rearrangements rather than unfolding. The treatments effectively dissociated insoluble aggregates in the commercial protein samples, significantly reducing particle size and enhancing solubility by 5- to 7-fold. Additionally, the antioxidant capacity of the proteins was markedly improved. Other functional properties, including emulsifying and foaming capacities, remained largely unaffected. The observed changes in protein composition were directly linked to the treatment temperatures, with chickpea protein exhibiting the highest thermal resistance, followed by fava bean and pea proteins. These findings highlight the potential of OH processing under UHT conditions as an innovative and effective approach for enhancing the solubility and functionality of alternative proteins. By improving their properties, thermo-electric processing could facilitate the implementation and application of these proteins in the food industry, driving broader adoption and fostering innovation.

# **P6.** Rheological characterization of in vitro digested tube feed to minimize aspiration risk

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**Background and Motivation**: Dysphagia is a possible indication for tube-feeding<sup>1</sup>. Vomiting and aspiration are complications known from tube-feeding individuals<sup>2</sup>. Vomiting food may cause aspiration by being transferred into the respiratory system following the path *stomach*  $\rightarrow$  *larynx*  $\rightarrow$  *respiratory system* or via *stomach*  $\rightarrow$  *oral cavity*  $\rightarrow$  *larynx*  $\rightarrow$  *respiratory system*. The motivation of this study is to approach the following vomiting-related questions with the help of rheology:

- 1. How to optimize the structure of tube feeding formulations, e.g. by a defined protein, fibre and carbohydrate composition to minimize the risk of aspiration?
- 2. How to identify time periods after feeding with increased risk of aspiration?

**Materials and Methods**: The break-down of food in the stomach involves peristalsis as well as enzymatic and acid hydrolysis<sup>3</sup>. Gastric motility and gastric secretions vary over time<sup>4</sup> and structural changes at the food-gastric-juice-interface are time dependent<sup>5</sup>. We apply bulk rheological characterization to commercial tube feed including milk, milk and soy proteins and banana juice that were digested using the following three approaches:

- 1. Ex-situ digestion using a rheometer cup equipped with a multi-blade stirrer
- 2. Ex-situ digestion using digestive solutions mimicking digestive fluids
- 3. In-situ digestion using a rheo-dialysis system consisting of a chamber through which fluid (e.g. water, acid) flows and which is closed at the top by a paper filter and a perforated plate. The tube feed sample is placed on this plate and the fluid can diffuse through the filter into the sample while the fluid is pumped through the chamber at a constant flow rate.

**Results and discussion**: Results from the three approaches are shown in the form of amplitude and frequency sweeps as well as time sweeps during in-situ digestion. We discuss the findings from the respective approaches regarding changes in sample structure by adding acid and digestive solutions, observable effects of dilution and energy input due to shear. In addition, we discuss the relevance of the observed rheological changes of the materials regarding the possible assessment of aspiration risk.

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# P7. Production of low viscous pectin-rich carrot dietary fibre by enzymatic hydrolysis and high-pressure homogenisation

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Dietary fibre (DF) has a high nutritional value since it can prevent several cardiovascular diseases and diabetes type 2 when the daily intake exceeds 25 g. However, in most countries, dietary fibre consumption is much lower; consequently, in the last decades, the fortification of food matrixes with DF has gained interest. While DF-fortified solid matrixes (such as baked goods) are broadly accepted by consumers, incorporating DF into liquid matrixes (*i.e.* beverages or yoghurt) is still under development since the sensorial properties of liquid matrixes are highly sensitive. Hence, tailor-made dietary fibre has to be produced with adequate solubility, low viscosity, small particle size and physical stability, among other properties. In this context, a combination of enzymatic hydrolysis and high-pressure homogenisation (microfluidization) has been proposed as a powerful tool to tune the functional properties of high cellulosic dietary fibre. Nevertheless, this processing approach has not yet been applied to pectin-rich dietary fibre sources (commonly produced in the fruit and vegetable derivates industry). Due to the complex chemical structure of pectin, selecting adequate enzymes is a critical step to be studied.

This work aimed to produce stable and low viscous pectin-rich dietary fibre-based suspensions. To that end, suspensions were prepared through enzymatic hydrolysis (30–240 min and 5% enzyme/substrate ratio) using a ternary mixture of enzymes (polygalacturonase, a 1:1 arabinanase-galactanase blend and cellulase; enzyme percentages within the mixture ranging from 5 to 90%) followed by high-pressure homogenisation (*i.e.* microfluidization). The hydrolysed and microfluidized suspensions were characterised in terms of stability against sedimentation, viscosity, particle size distribution and content of insoluble, alcohol-insoluble, and soluble fractions. Additionally, the mono-and disaccharide content of a selected subset of samples was determined.

Results showed that combining enzymatic and mechanical treatment reduces the particle size and insoluble mass, leading to stable low viscous suspensions (5 to 18 mPa·s, 1wt%). No stable suspensions were produced for a viscosity lower than 5 mPa·s. As a general trend, the longer the hydrolysis time, the lower the viscosity of the suspensions due to a decrease in the insoluble mass content and a decrease in the molecular weight of the soluble dietary fibre. Regarding the composition of the enzymatic mixture, a high percentage of cellulase and/or polygalacturonase reduced viscosity considerably. Presumably, the hydrolysis of the cellulosic network followed by microfluidization, apart from decreasing the insoluble mass, may result in a more open structure that facilitated the hydrolysis of pectin by decreasing the sterical hindrance. At a high content of polygalacturonase, the homogalacturonan regions of pectin were hydrolysed, potentially resulting in a severe reduction of the molecular weight. On the contrary, intense hydrolysis of the galactan- and arabinan- chains had a minor impact on the functional properties. In this case, the reduction of molecular weight may be too low.

The results of this work broaden the application of enzymatic hydrolysis and high-pressure homogenization to produce low viscous pectic-rich dietary fibres with the potential of being ingredients to fortify liquid food matrices that can be applied to up-grade fruit and vegetable industrial by-products.

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## **P8. Unveiling the effect of whey protein hydrolysates on gut microbiota**

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The gut microbiota is essential for human health, influencing metabolic functions, immune responses, and overall homeostasis. While dietary carbohydrates have been extensively studied for their impact on gut microbiota, the role of dietary proteins, particularly whey proteins, is less explored. Recent research indicates that bioactive peptides, generated during gastrointestinal digestion, can beneficially modulate the gut microbiota and promote the production of metabolites such as short-chain fatty acids (SCFAs)<sup>1</sup>. To harness these benefits more effectively, controlled enzymatic hydrolysis of whey proteins before gastrointestinal digestion has been proposed. This approach allows for the production of specific peptides with desired sequences and sizes, enhancing their bioactivity and possible gut microbiota modulation. At the same time, it improves protein digestibility and reduces allergenicity, making whey protein hydrolysates suitable for hypoallergenic diets<sup>2</sup>.

This study explored the impact of whey protein isolate (WPI) enzymatic hydrolysis on gut microbiota composition and metabolite production through an *in vitro* colonic fermentation model of whey proteins. Whey protein hydrolysates (WPHs) were produced using Alcalase and Protamex enzymes at varying hydrolysis times (10, 30, and 120 minutes). The samples were subjected to in vitro digestion using the standardised INFOGEST static methodology and then the undigested fractions of whey protein isolate (WPI, control) and WPHs were fermented. During digestion, hydrolysis enhanced the release of essential amino acids, including proline and asparagine, with distinct patterns depending on the enzyme employed, while preserving branched-chain amino acids crucial for muscle protein synthesis and energy metabolism. In the gut, WPHs promoted the growth of beneficial bacteria, such as Acidaminococcus intestini, while reducing pro-inflammatory species like Dorea longicatena, particularly in hydrolysates produced with Protamex. Fermentation of WPHs led to increased SCFAs, notably acetate and propionate, which are vital for gut and systemic health. Metagenomic analysis highlighted notable shifts in microbiota composition, with enriched populations of *Bifidobacterium* adolescentis and Bifidobacterium longum, two probiotics known for SCFA production, anti-inflammatory effects, and gut barrier enhancement. These changes were particularly evident in hydrolysates obtained after 10 and 30 minutes of hydrolysis with Alcalase.

These findings emphasised the potential of WPHs as bioactive substrates with applications in gut health. By tailoring enzymatic hydrolysis protocols, it is possible to enhance both digestibility and microbiota modulation, paving the way for the development of functional food products that support overall health and well-being.

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# **P9.** On the Possibility of Inter-Transglycosylation by the Branching Enzyme *Rhodothermus obamensis*

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Branching enzymes (GBE, EC 2.4.1.18) are vital catalysts in starch biosynthesis, driving the formation of a-1,6 glycosidic bonds. This process enhances the branching of starch polymers, significantly influencing their structural and functional properties. These enzymes operate via a transfer reaction that can occur in two distinct patterns: intra-transglycosylation, which involves the same starch polymer chain, and inter-transglycosylation, which involves interactions between different starch polymer chains.

A notable and rare subtype of intra-transglycosylation is the cyclisation reaction, documented exclusively in a limited number of thermostable branching enzymes. While the unique structural signatures of cyclisation can be readily identified, distinguishing between inter- and intra-transglycosylation remains a more elusive task. Consequently, this aspect has garnered limited research focus, despite its potential implications for the resulting polymer structures and their emergent properties.

In this presentation, we will investigate the potential for inter-transglycosylation in the branching enzyme Rhodothermus obamensis. By examining the structural differences associated with these reaction patterns, we aim to elucidate their respective contributions to polymer architecture. Additionally, we will highlight the opportunities these mechanisms present for designing polymers with tailored properties, paving the way for innovative applications in starch modification and beyond.

## P10. Boosting Starch-Phenolic Interactions to Modulate the Multi-Scale Structure of Starch and Reduce Digestibility

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Starch interacts with a range of ligands by forming inclusion (V-type) and non-inclusion complexes during processing operations that involve partial or complete loss of the native granular structure<sup>1</sup>. These interactions modulate the multi-scale structure of the starch system, leading to unique rheological, mechanical and microstructural characteristics and reduced digestibility<sup>2</sup>. This study aimed to investigate the extent and nature of rice starch complexation with phenolics (gallic acid and Myrciaria dubia polyphenols) after gelatinisation via hydrothermal (90°C, 20 min) and high hydrostatic pressure processing approaches (HHP; 500 MPa, 20°C, 20 min) and after storage (21 days, 4°C). The starchpolyphenol composites were characterised by rheological, XRD, FTIR, <sup>13</sup>C-NMR and SEM analysis. Digestibility (Englyst method) was also investigated. Complexation (5-13 mg phenolics/g starch) led to the formation of significantly softer starch composite gels (lower G' and G'' values), with reduced digestibility (p < 0.05) and a very distinct microstructure that was characterised by a remarkable reduction in pore size. All the starch ligands and gelatinisation approaches promoted the formation of V-type starch (diffractions peaks at  $2\theta = 13.0^{\circ}$  and  $19.9^{\circ}$ ). However, non-inclusion amylose-phenolics interactions were found to be predominant and, therefore, responsible for the multi-scale changes observed. HHP gelatinisation increased starch resistibility to digestion in the composites (26.58±1.71%) to a greater extent than the hydrothermal treatment  $(22.47 \pm 1.52\%)$  (p<0.05), which was attributed to enhanced non-inclusion interactions according to the FTIR and <sup>13</sup>C-NMR data. The microstructure and digestibility of the composites changed over time, indicating that starch-phenolic interactions did not remain stable during storage. This investigation provides valuable insights into the health profiles of starch-phenolic composites and highlights the potential of innovative processing technologies such as HHP to create novel starch systems with distinct microstructures and tailored digestion properties.

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## **P11.** Subcritical Water Treatment of Cereal Brans for fiber solubilization and enhanced functional properties

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Cereal brans comprise the outer layers of the kernels, being the main by-product of grain milling for white flour production. The bran fraction constitutes about 13-17% of the grain and it is an excellent source of dietary fiber (DF), minerals, vitamins, and bioactive compounds. However, the use of cereal brans is limited due to the presence of some antinutrients (like phytic acid) and insoluble and recalcitrant DF, which have adverse effects on the texture, hydration, and volume of wholegrain food products. In this sense, different treatments such as micronization and heat treatment significantly reduced phytic acid, while fermentation improved the antioxidant and flavor profile, but these treatments were insufficient to enhance fiber solubility. An alternative to these methods is subcritical water treatment due to its properties for biomass hydrolysis, which can be beneficial to increase the soluble DF and reduce anti-nutrients by adjusting the pressure and temperature of the water. This work aims to understand the effect of various treatments with subcritical water on the composition and functionality of bran fractions from rye, wheat, and spelt sources. The bran fractions were treated in a continuous hydrolysis plant with water at 170 bars for 4 s and three different subcritical water temperatures (200, 270, and 340 °C). Following these three treatments, the samples were filtered and fractionated into soluble and insoluble fractions. The samples were compositionally characterized, including fiber and mineral composition, phytic acid, and starch content as well as structurally analyzed using microscopic, chromatographic and FTIR tools. For functionality, foaming, water absorption, viscosity and solubility were evaluated. Increasing subcritical water temperature resulted in a higher ratio of soluble to insoluble fraction for the three cereal sources, with wheat bran showing the greatest solubility increase. Filtration after subcritical water treatment led to the removal of small-molecular weight compounds (i.e., sugars) as well as high starch depletion in the insoluble fraction, especially at 340 °C, compounds remaining in the soluble fraction. Insoluble fractions exhibited higher concentrations of fiber (~75%) and protein (9-12%) compared to the controls. While FTIR and chromatographic analyses also suggested that both proteins and polysaccharides (i.e., arabinoxylans) suffered from hydrolysis due to the treatment. Regarding microstructure, control brans had fibrillar and compact heterogeneous particles, while the insoluble fractions had less compact, honeycomb-like structures. The soluble fractions showed smaller and spherical particles with concavities, resulting from spray-drying after treatment. Regarding the mineral profile, the insoluble fractions exhibited higher iron concentrations, while the soluble fractions demonstrated higher potassium and phosphorus contents, the latter indicative of phytic acid solubilization. The concentration of phytic acid in the treated samples was lower than in the control samples, and increasing temperature resulted in a decrease in phytic acid content, especially abundant in spelt and wheat brans. Regarding functional properties, the insoluble fractions exhibited increased water absorption due to fiber solubilization, which was enhanced at higher temperatures, while viscosity development was reduced due to counteracting effect of starch removal. The foaming capacity of the soluble fractions was higher than the insoluble ones, especially at higher temperatures, but temperature had a negative effect on stability. Overall, treatment with subcritical water resulted in fiber solubilization and starch depletion, reducing antinutrients and resulting in a high fiber fraction that exhibited enhanced water absorption and reduced viscosity, which can be beneficial for various wholegrain bakery applications.

# **P12.** Modifying digestion in a dense wheat gluten network through the addition of cellular legume flour

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Pasta and noodles are staple foods and examples of food products that contain a dense gluten network, yet their nutritional protein quality may be limited by the amino acid profile of wheat. Specific demographic groups such as elderly require higher concentrations of complete proteins. The addition of complementary amino acids to pasta and noodles, from for instance legumes, could improve health outcomes. In addition, maintaining a low glycaemic response can offer benefits to cardiometabolic health and support the prevention and management of type 2 diabetes. A potential strategy to achieve higher protein quality, increased protein quantity and reduced starch digestion rates is to enrich wheat pasta with legume flours which have been processed to retain the cell wall integrity of the legumes during the grinding process.

Pastas containing cellular flours from different sources were produced, and the impact this has on metabolic responses was quantified. The cellular flours were obtained through hydrothermal treatment followed by grinding, freeze-drying and sieving. The cellular flour was compared to a 'broken flour', which was produced in the same way but included cryomilling and sieving steps. The legume pasta production, drying, and cooking times were optimized to enable comparison of pasta made with different legume flours to determine the influence of (1) cell wall integrity, (2) legume species, and (3) wheat-to-legume ratios on digestion. Starch and protein digestibility were assessed using the Englyst and the INFOGEST 2.0 protocols respectively. Pasta matrix properties such as degree of starch gelatinization and cell wall integrity were measured using differential scanning calorimetry and microscopy. Our findings provide insights on the metabolic impact of intact cellular flours in a real food system, and provide guidance on ways to tailor pasta formulations to optimize starch and protein digestion using legume flour.

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# P13. Hydrocolloid-Based Fat Analogues: Advancing the Texture and Taste of Plant-Based Meat Products

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The growing interest in sustainable and healthier meat substitutes has driven the need for innovative strategies to replicate the functionality and sensory characteristics of animal fat. This study investigates the use of hydrocolloids, including kappa carrageenan, gellan gum, methyl cellulose, and konjac gum, to develop structured fat systems specifically designed for meat analogue formulations. Thermo-irreversible gels with varying textural properties can be prepared by adjusting the mass ratio of different hydrocolloids, which enables precise control over gel strength and functionality<sup>1,2</sup>. By leveraging the gelation, emulsifying, and thermal stability properties of these hydrocolloids, oleogels were created with texture, fat distribution, and mechanical properties resembling those of animal fat. These structured fat systems effectively integrated with plant protein matrices, enhancing their thermal and mechanical behaviour as well as their cooking performance. This research highlights the potential of hydrocolloid-based solutions as sustainable and high-performance alternatives to animal fat in plant-based meat analogues, improving their appeal and acceptance among consumers.

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# P14. Sulfonated Cottonseed Hydrolysates with Adjustable Amphiphilicity as Environmental -Stress Stable Emulsifiers

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Cottonseed protein isolate (CPI) is a valuable agro-industrial waste with potential biotechnological applications. However, inadequate stability in water due to its characteristic hinders its widespread use. Therefore, a new sulfonation modification approach was developed to improve the amphiphilicity and structural flexibility of CPI. Structural characterizations confirmed the successful incorporation of sulfonate groups with structural and conformational changes. This significantly unfolded molecular-chain, and improved amphiphilicity, flexibility, and surface-hydrophobicity while reducing pI (5.1 – 1.7), and molecular weight (5745 – 2089 g/mol). The modified samples exhibited improved emulsification with higher amounts of absorbed proteins on the droplet interface, smaller droplet size, and a higher zeta potential. Additionally, they possessed good emulsification ability under acidic conditions. The nano-emulsions exhibited long-term stability ( $\geq$ 70 days) under different environmental conditions, with excellent fluidity. This study contributes to understanding sulfonation as a viable approach for improving protein properties, thus, opening up new possibilities for their application and maximizing their economic benefits.

# P15. Linking physicochemical properties to sensory perception of plant-based meat analogues patties: Retained water and released serum enhance juiciness and boost flavour intensity

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Textured vegetable proteins (TVPs) are key ingredients of plant-based meat analogues (PBMAs), as their ability to hold and release water during preparation and consumption, contributing to meat-like textures. This study aimed to investigate juiciness perception by exploring the relationships between physicochemical and sensory properties of PBMA patties. The patties were designed to vary in water holding and release properties by controlling TVP hydration level and particle size. Increasing TVP hydration increased water cooking loss, while fat cooking loss remained unchanged. Despite increased water cooking loss, higher initial water content still led to a 30% increase in patty water content after cooking. This elevated water content significantly enhanced serum release under compression, strongly influencing juiciness and fattiness perception. Increasing TVP hydration increased juiciness (+204%) and fattiness (+71%), decreased hardness (-53%) and crumbliness (-41%) intensity. TVP particle size effected texture perception, with larger TVP particles yielding harder (+62%) and chewier (+119%) patties. Network analysis showed that increased juiciness enhanced fattiness, savoury and garlic flavour and decreased hardness intensity. These sensory changes influenced liking, which was positively related to juiciness, chewiness and savoury flavour, negatively to beany flavour.

We conclude that adjusting TVP hydration level effectively alters patty composition and texture and enhances juiciness of PBMA patties, while varying TVP particle size primarily impacts patty texture without affecting composition. Juiciness in PBMA patties is driven by higher retained water after cooking, increased serum release under compression and a less stiff texture. Enhanced juiciness not only boosts flavour perception but also drives consumer liking.

# P16. Multiphase systems – new challenges for the stability and stabilizers evaluation

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Most food products can be considered as a complex colloidal system. For the final consumer, properties such as visual aspects, shape, texture, smell as well as the most important factor which is taste are the most important. However, in point of manufacturing, transportation, and long-term stability play a crucial role. The same aspects are important in the case of cosmetics, and biomedical applications (i.e. wound healing systems). One of the most frequently investigated systems is based on emulsion systems. Due to the low costs of oil in water systems are preferable. In our previous work, we reported that some of the artificial surfactants can be successfully substituted with natural ones [1]. Here, stabilizers based on saponins showed desirable properties. Soap nuts or Aesculus hippocastanum L. extracts [2], [3] showed a high stability effect in water in oil emulsion systems. The reduction of droplet size using a two-step homogenization process decreases the particle size into nanorange, which increases the system stability. Typical stability tests various scattering techniques, and apply dynamic light microscopic, and spectrophotometric analytical tests, which are supported by rheology, and storage condition evaluations.



Fig. 1 Idea of the multiphase system

Here, we present the concept of a multiphase stable system. This solution might be interesting in case of preventing the degradation of bioactive compounds such as vitamins and their active release in selected conditions. In the proposed multiphase system the O/W emulsion systems might be beneficial, due to final composition stability. As a final matrix of the emulsions with vitamines hydrogels will be applied. Our goal in this presentation is to open a discussion for:

- which factors/interactions should be considered in stability evaluation,

- what kind of stability tests/desirable properties tests should be applied for multiphase colloidal systems evaluations,

- how the natural stabilizers affect each phase in the complex/multiphase system.

During the presentation, some examples of testing techniques, such as microscopy (with digital microscopy support), DLS, and rheology will be discussed.

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## **P17.** Decoding meat analogues: insights into ingredient structure-function relationships

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Meat analogues play a pivotal role in the transition to more sustainable protein sources. Most meat analogues are primarily composed of textured vegetable protein (TVP), oil and binding agents. Each of these ingredients play a critical role in defining the functional properties of the meat analogue, including texture and serum release. Juiciness and flavour perception are particularly significant for consumer acceptance and largely depend on water and oil release upon consumption. Understanding the structure-function relationships of the ingredients is essential for optimising meat analogues.

This presentation delves into the intricate interplay between ingredient structure and functionality in meat analogues. For various TVPs, we quantified the structural characteristics, such as porosity, pore size and wall thickness using X-ray tomography. Our findings reveal how these characteristics influence water release, mechanical properties and protein digestibility of meat analogues. Oil release is less dependent on TVP structure, but is governed by oil droplet characteristics. We will demonstrate how modifying the interfacial characteristics and interactions between oil droplets and the meat analogue matrix can help to tune oil release dynamics.

Besides TVP and oil, also binding agents are important as they provide structural cohesion and influence texture and serum release. The most commonly used binding agent in meat analogues is methylcellulose (MC). Despite its widespread use, the gelation behaviour of MC in protein-rich systems such as meat analogues remains poorly understood. We will present how the gelation behaviour of MC is influenced by proteins, and how this is dependent on MC molecular weight and protein characteristics. Additionally, we will discuss the tribological properties of meat analogues and the serum released, offering insights into how different ingredients contribute to the lubrication of meat analogue surfaces.

Overall, this presentation provides a comprehensive overview of the complex relationships between structure and functionality, offering insights to advance the design and quality of meat analogues.

# P18. Use of cellulose microfibrils and potato protein to form double network gels

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The formation of double network hydrogel systems is investigated using cellulose microfibrils from citrus fibre and a thermally gelling potato protein. We study how the system transitions from a single, to a double network gel, as the potato protein is thermally denatured, and it forms a second network entangled within the network of cellulose microfibrils. The system is studied via oscillatory rheology, namely temperature and amplitude sweeps. We find that the contribution of the native potato protein on the single network cellulose microfibril gel is minimal. However, when the protein is thermally denatured, the cellulose microfibrils and gelled protein act synergistically to contribute to the storage modulus of the double network gel. At low protein concentrations, the addition of the cellulose microfibril network reduces the minimum protein concentration for gel formation. At low to moderate protein concentrations, the cellulose network interpenetrates the protein network, significantly increasing the elastic modulus. At high concentrations of protein, the protein gel network entirely dominates the rheological response. This is observed up to a certain ratio of protein to fibre. We link the observed rheological properties to the microstructure via confocal laser scanning microscopy. Flocs of the cellulose microfibrils are observed with the secondary protein network entangled throughout. These dense flocs are likely to be the key contributor to the increased mechanical properties of the double network system.

## P19. Improvement of Amphipathic Properties with Molecular Structure Unfolding and Activation of Cottonseed Protein as Ultra Stable and Safe Emulsifier

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The protein content of cottonseed meal, a by-product of cotton processing – cottonseed after shelling and oil extraction, is as high as 40 -50%. It is a plant protein resource with high nutritional value and good quality. They are excellent options for numerous applications due to their superior properties and lower cost. However, its complex folded structure and large molecular weight lead to lower reactivity and insufficient amphiphilicity. Cottonseed protein isolate (CPI) is less soluble in water. To address these challenges, we enhanced the amphiphilicity of CPI with associated hydrolysis, molecular structure unfolding, and activation by alkaline-induced deamidation (at 24, 36, and 72 h) and produced three cottonseed protein hydrolysates CPH 24, 36, and 72. Structural analysis via FTIR and circular dichroism measurements confirmed the conformational changes, including protein unfolding and shifts in the secondary structure composition. The deamidation process significantly reduced the particle size from 2503.4 nm to 771.8 nm and increased the surface hydrophobicity from 133.5 to 326.7, carboxyl content from  $1.13 \times 10^{-3}$  to  $2.09 \times 10^{-3}$ , and flexibility from 0.106 to 0.162. This indicated the hydrolysis, unfolding, and amphiphilicity improvement of CPI. These modifications led to improved emulsifying properties, with CPH72 exhibiting the most promising results. CPH 72 stabilized emulsions were long-term stable under various environmental stresses, including temperature, pH, and ionic concentration without visible phase separation after at least 40 days of storage except at pH 4. Compared to CPI, it had smaller emulsion droplets (939.3 to 264.9 nm) and larger absolute ζ-potential (-26.5 to -58.0 mV), indicative of enhanced emulsion stability. In addition, in-vitro cytotoxicity assay revealed that deamidated CPI is significantly safer than commonly used synthetic surfactants. This research introduces a sustainable approach for producing multifunctional emulsifiers from renewable resources, offering promising applications in the development of functional foods and non-foods.

# P20 Tuning Cellulose Microfibrill Containing Plant-Protein Gels by Shear

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Cellulose microfibrils (CMFs), derived from plants waste material offer a unique fibrillar structure<sup>1</sup> and serve as sustainable, natural and functional ingredients for dietary-rich, clean-label food products contributing to the texture and stability of these products.<sup>2,3</sup> However, the dispersion of CMFs is challenging due to their tendency to aggregate via OH-driven hydrogen bonding and van der Waals interactions.<sup>4</sup> While previous studies demonstrate that high-energy treatments of CMF dispersions in the presence of biopolymers improves homogeneity and reduces aggregation, the role of processing conditions in controlling these interactions and the resulting microstructural changes remains underexplored.<sup>1,4</sup> Understanding the influence of shear induced microstructural changes in these composite systems is crucial to tailor the texture of plant-based food products.

This study investigates the impact of processing conditions on the rheological and structural properties of composite CMF plant-protein systems. Model systems were prepared by dispersing CMF in presence of plant-proteins, followed by controlled shear treatments using a Microfluidizer varying the applied energy density. The findings demonstrate that alternating the processing conditions significantly influence the structural and rheological properties of CMF – plant-protein systems. These results provide a foundation for tailoring the continuous phase in plant-based food systems, optimizing texture and mouthfeel.

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# P21 Solubility influences the colloidal stability of lentil protein emulsions

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This study investigates the enhancement of thermal and colloidal stability of lentil proteinstabilised emulsions through high-pressure homogenisation (HPH) pre-treatments. Lentil protein dispersions were homogenised at pressures ranging from 0 to 150 MPa and subsequently used to formulate emulsions at total solid concentrations of 29% w/w. The results showed that HPH significantly (p<0.05) improved protein solubility, with an increase from 55.7% at 0 MPa to 93.2% at 50 MPa. This is agreement with the literature on the effect of HPH on the plant proteins ingredients<sup>1,2</sup>. When the ingredients were used to prepare an emulsion, the particle size distribution analysis showed a reduction in oil globule size, with the volume-weighted mean diameters decreasing from 1.4  $\mu$ m (0 MPa) to 1.19 µm (150 MPa), indicating an improvement of the interfacial properties of the lentil protein isolate. Indeed, soluble proteins can effectively adsorb at the newly created oilwater interface during homogenisation, therefore reducing interfacial tension and forming a layer around oil droplets and allowing for better physical and heat stabilisation of emulsions<sup>3,4</sup>. Emulsions prepared from HPH pre-treated dispersions exhibited improved physical stability, with separation rates decreasing from 16.75%/h (at 0 MPa) to 2.05%/h (at 150 MPa). Rheological analysis showed that HPH pre-treatments led to initial apparent viscosities ranging between 28.30 and 22.56 mPa·s for the samples HPH pre-treated at 0 and 150 MPa, respectively. After a thermal treatment at 90°C for 2 min, the pre-treated emulsions had a final viscosity of 34.88 mPa $\cdot$ s, which was significantly (p<0.05) lower than the one measured in the untreated samples (60.52 mPa·s). Confocal laser scanning microscopy images showed a more homogeneous distribution of oil globules and reduced flocculation after the thermal treatment in emulsions prepared from HPH-treated dispersions. Overall, the results indicated an enhancement of the colloidal and thermal stability of the HPH pre-treated samples, and this has been linked with the improved solubility of lentil protein isolates upon the HPH treatment. These findings highlight the potential of HPH as an effective pre-treatment to enhance the techno-functional properties of lentil protein-stabilised emulsions, supporting the development of stable and sustainable plant-based food products.

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## **P22 Impact of Protein Denaturation and Solubility on Structuring and Gelation of Plant Proteins**

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The structuring and gelation behaviour of plant proteins in food applications is influenced by factors such as solubility and molecular interactions, which are affected by protein structure and its degree of denaturation. We aimed to evaluate the role of these factors on structure formation in thermomechanical processing, heat-induced and enzymatic gelation.

Soy protein flours, with varying solubility profiles due to their different pre-treatments (toasted or untoasted), were subjected to thermomechanical processing by dry extrusion. Structural changes were monitored by Fourier-transform infrared (FTIR) spectroscopy. Soluble pea protein fractions were isolated to assess the aggregation behaviour of low processed versus high processed (commercial) fractions during thermal treatment. To investigate the effect of solubility on enzymatic gelation, a commercial pea protein isolate was separated into soluble and insoluble fractions, dialyzed, freeze-dried, and subsequently subjected to gelation tests to evaluate crosslinking potential.

After thermomechanical processing of soy protein, FTIR analysis revealed the appearance of an intermolecular  $\beta$ -sheet band when initially absent, suggesting that the degree of protein denaturation in the secondary structure had minimal influence on the structure formation within the extrusion process, as all samples formed comparable textures. For heat-induced gelation, low processed pea protein fractions formed temperature-dependent disulphide-bonded aggregates, while commercial products with pre-formed aggregates exhibited limited potential for further aggregation. Enzymatic gelation experiments revealed that fractionation enhanced crosslinking potential. Improved hydration from ion exchange and reduced incompatibility between soluble and insoluble fractions were proposed as mechanisms for better gel formation.

While the degree of denaturation played a minor role in structure formation, phase behaviour and tailored sample preparation significantly influence the functionality of plant proteins. Future work should focus on optimizing processing conditions and fractionation techniques to enhance gelation properties and broaden the application range of plant proteins in alternative food systems.

## P23. Casein stabilized interfaces, the role of molecular structure

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The future of dairy may lie in the development of animal-free milk, providing a more sustainable alternative to traditional dairy. Central in this development is the synthesis of casein by microorganisms, a key protein for dairy functionality. To anticipate the development of microbial casein, we need to answer fundamental questions such as: Are casein micelles necessary? Is casein phosphorylation essential? Can fewer casein variants achieve comparable functionality?

Hence, this research used bovine casein as a benchmark to investigate the effect of casein chemistry on the stabilization of air- and oil-water interfaces. We show that casein micelles are beneficial for stiffer viscoelastic interface at polar oils but individual casein fractions might be a good alternative. The phosphorylation is essential for creating interfacial networks by forming a space-spanning network with calcium. The location of phosphorylation will also affect the interfacial configuration which has a big effect on the sensitivity to calcium. In  $\alpha_{s^2-}$ , and  $\beta$ -casein, the phosphorylation creates a tail-train configuration which makes them highly calcium sensitive.

With this work we show that casein chemistry critically impacts interfacial viscoelasticity, enabling the tailoring of functional properties through selective use of casein fractions. Microbial casein should focus on phosphorylation to create interfacial networks, but using all four casein fractions in animal-free dairy might not be necessary.

# **P24.** Role of polysaccharides in ice cream texture and aroma release

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Ice cream's physical and sensory properties depend on its microstructure, which consists of the ice, air and fat phases. Understanding the interactions among these phases is essential for controlling the texture and flavour profile of ice cream. To enhance these attributes and maintain stability through shelf life, which may involve temperature fluctuations, stabilizers such as polysaccharides are used<sup>1</sup>. This study is based on a pilotscale system with a fixed 100% overrun and aims to examine how combinations of polysaccharides affect premix viscosity, and ice cream hardness, texture, melting behavior over a 12-month shelf life at various storage temperatures. The release of volatile organic compounds is also investigated.

Preliminary observations suggest that after seven days more viscous mixes produce softer ice cream, contrary to the usual assumption that higher viscosity leads to denser textures<sup>2</sup> For instance, samples with a high viscosity of 825 mPa·s prepared with alginate had a mean hardness of 334 ± 65g, while samples with the lowest viscosity of 218 mPa·s prepared with cassia-guar-carrageenan kappa had a hardness of 394 ± 120g. Rather than a simple "more viscous = firmer" relationship, these results point to how different polysaccharides, each with distinct molecular weights and hydration behaviors, can influence water immobilization and ice crystal formation in ways that do not solely depend on overall viscosity<sup>3</sup>. In addition, producing a softer, more deformable matrix could enhance volatile compound release and initial aroma intensity<sup>4</sup>. Planned PTR-MS and GC-MS analyses will clarify how formulation adjustments influence both immediate flavor release and long-term aroma stability.

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# P25. Linking adsorption dynamics and interfacial viscoelasticity to droplet formation using microfluidics: Insights from faba and whey protein mixtures

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The composition and viscoelastic properties of interfaces are key factors in imparting stability to emulsions. In mixed protein systems, the adsorption dynamics may affect the composition and interactions between proteins, with important consequences to the bulk properties. Microfluidics is proposed as a means for rapid screening of such interactions. In this work, we aimed to evaluate the viscoelastic properties of oil water interfaces obtained with a mixture of whey protein isolate (WPI) and faba protein isolate (FPI), measured via drop tensiometry, and relate such properties to the properties of emulsion droplets obtained using microfluidics.

Drop tensiometry revealed that all WPI/FPI mixtures effectively reduced the interfacial tension at the oil-water interface but displayed distinct interfacial adsorption dynamics: WPI-stabilized droplets exhibited slow adsorption and rearrangement rates, while FPI-stabilized droplets showed fast adsorption rates and rearrangement rates. Although both WPI and FPI formed viscoelastic interfaces alone, the interfacial viscoelasticity was significantly reduced when combinations of WPI and FPI were used, likely due to the difference in adsorption dynamics and, thereby, competitive displacement between the proteins for the interface.

The lack of interfacial stability in FPI/WPI mixtures was reflected in the behavior of the droplets formed with microfluidics. WPI (0.1 mg/mL) formed stable, elongated droplets, while FPI-stabilized droplets were prone to coalescence, forming large, non-spherical oil clusters, indicating that a higher concentration of FPI is required to obtain stable droplets. WPI/FPI mixtures showed increased instability compared to WPI alone. However, at low ratios of WPI (25/75 WPI/FPI) droplet stability was increased, indicating that small amounts of WPI may aid in droplet stability. Alternatively, small amounts of FPI caused significant instability reflected in the larger droplet sizes and more elongated droplet shape due to increased competition at the interface at low ratios FPI (75/25 WPI/FPI). The microfluidics results will be compared to bulk emulsion properties to demonstrate how microfluidic techniques can serve as a rapid screening tool for optimizing interfacial interactions and enhancing product formulation.

Overall, the findings highlight the critical interplay between protein-protein interactions, interfacial viscoelasticity, and droplet stability, providing the first evidence linking drop tensiometry with coalescence and shape eccentricity during microfluidic droplet formation.
### P26. The development of agar fluid gels for fat reduction in highsugar bakery fillings.

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Fats are important dietary components providing 25–30% of our daily energy intake. However, fats can also be controversial, with excessive consumption of trans- and saturated fats (in particular) being linked to conditions/pathologies that are detrimental to human health. Saturated fats are largely responsible for the mouthfeel and textural performance of many formulated food products, and so reduction in food products poses a serious technical challenge.

This work focusses on the development of fluid gels for the reduction of saturated fats in high-sugar bakery fillings such as custard, chocolate filling and whipped cream. Fluid gels are concentrated suspensions of gelled particles, formed when gelation of a biopolymer occurs under shear. Their combination of lubricating ability, paste-like rheology and deformable micron-scale microstructures has led to the suggestion that fluid gels may be suitable for fat replacement in food products. However, the presence of sugar in bakery fillings poses a problem, since the material properties of hydrocolloid gels are sensitive to such co-solutes<sup>1,2</sup>. While research exists in the context of quiescent gels, there is limited literature exploring the effect of sugar on fluid gel formulations. Thus, development of a fluid-gel based bakery filling requires exploration of the effect of sugar on the material properties of fluid gels, and translation into the practical implications this would have for mimicking fatty mouthfeel.

A combination of texture analysis, rheology and phase contrast microscopy have been used to explore the effect of sucrose (up to 60 wt%) on agar fluid gels produced in a cup and vane rheometer geometry, as well as a pin stirrer. Sucrose is found to increase the physical strength and stiffness of the gel network up to a critical concentration, possibly through enhanced stabilisation due to more pronounced hydrogen bonding between water, the used hydrocolloid and equatorial OH-groups present on the sugar. The mean fluid gel particle size is shown to decrease with sucrose addition, likely due to a combination of effects arising from an increased viscosity during gelation. An increase in viscosity and yield stress but reduction in elastic modulus is observed, which can be related to changes in the particle textural properties and microstructure.

Application of current models for predicting mouthfeel from rheological behaviour suggests that the presence of sucrose in these formulations may be beneficial for enhancing creaminess perception. This work confirms that the established view that the properties of fluid gels can be manipulated through variations to the processing conditions used for their manufacture, still persists in the presence of sugar, and demonstrates there is also the possibility to further tune fluid gel properties by changing the order of sucrose addition; i.e. addition prior or post fluid gel formation. Overall, this research presents both a fundamental and practical approach for developing fat-reduced formulations for high-sugar products using fluid gels.

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# P27. Common bean proteins: similar interfacial rheology, distinct interfacial structures and functionalities

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In the current protein transition, plant proteins have been extensively studied; however, their compositions are typically complex, and the direct relationships between composition, structure, and functional properties remain unclear. To investigate the impact of compositional and structural differences on plant protein properties, we focused on common bean (Phaseolus vulgaris L.), the most widely consumed legume worldwide. Proteins were extracted from three commercially available common bean varieties-red kidney bean, black turtle bean, and pinto bean. Proteomics analysis revealed that the primary component in all three varieties was a 7S globulin, phaseolin, and extracts had nearly identical protein compositions. Interfacial rheology at the air-water interface demonstrated no significant differences in adsorption kinetics or dilatational moduli among the three proteins, indicating the formation of solid-like interfaces with similar stiffness. However, Langmuir-Blodgett deposition combined with AFM revealed that all three proteins formed a unique strand-like structure at the interface, though different surface pressures or aging times were required to achieve these structures. More surprisingly, foam stability also showed significant variation, with the foam half-life time of black turtle bean globulin reaching 25.3 hours, over 3.7 times that of the other two proteins. These findings suggest potential structural differences among the protein extracts despite their nearly identical composition, which are insufficient to cause differences in interfacial rheology but can influence the formation of interfacial structures and foamability. This study provides new insights into the link between protein structure and interfacial properties, contributing to the understanding of plant protein functionality.

#### P28. Utilizing capillary forces to structure protein oleogels

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The ongoing protein transition puts high pressure on the food industry to reformulate their food products into plant-based analogues. Next to plant proteins, also the fat source needs to be plant-based. In many of these products, exotic fats, such as palm oil and shea butter are used, due to their solid character. However, there are several concerns related to deforestation and other sustainability aspects connected to the use of such fats. Alternatives for solid fats are thus desired. A novel category that would increase ingredient flexibility are protein oleogels, in which protein particles are used to provide a solid structure to liquid plant oils, such as sunflower, rape seed or olive oil. In such approach, different types of proteins, including whey, soy, pea or potato, are introduced into oil via an intermediate solvent approach, after which a small amount of water is added to create a protein network. The water forms small water bridges between protein particles, also known as capillary bridges. Capillary bridge formation is a powerful approach to modify the rheological properties of such oleogels. The small amount of water provides an attractive capillary force to induce protein particle network formation, which increases the gel strength by orders of magnitudes. The network, and the corresponding rheological characteristics, such as gel strength, critical strain and recoverability, largely depend on the amount of added water and the properties of the particles. Protein particles vary significantly in their properties, such as their ability to absorb water and swell, their roughness or particle size. Little is known about how variations in particle properties affect particle interactions and capillary bridge formation. In this research, we systematically varied particle properties to gain more understanding on the particle network formation. By imaging of spherical protein particles of relatively large size (up to 20 µm), we show that even though particles are able to absorb water, enough water was available to form bridges and to subsequently increase the network strength. In this presentation, we further discuss the specific effects of particle properties such as size, water absorption capacity and surface roughness on capillary suspension rheology. We demonstrate how modification of protein particle properties and variations in the amount of added water provide ample opportunities to control the rheological properties of the protein oleogels. In addition, we also discuss how a heat treatment facilitates further network rearrangements and therefore leads to additional alteration of rheological properties.

# P29. Unrefined pistachio shell powder as multifunctional stabilizing agent for emulsions and foams

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Nuts are currently investigated for their nutritional (fatty acids, polysaccharides, sugars, minerals) and bioactive (such as polyphenols) components. Used as a whole or transformed in flours, they are widely consumed due to their specific aroma and health benefits. Among the nuts, almonds, walnuts, cashews, pistachios and pine nuts are especially popular. Since only a part of the nuts is edible, shells (and hulls) are set aside. Those by-products are rising interest in literature for the formulation of biofuels, phytochemicals, carbon traps or biobased materials.

With a world production of 1.1 million tons in 20201, pistachios represent a big biomass deposit. According to literature, pistachio wastes are rich in antioxidants and some extracts were already associated to health benefits such as anti-inflammatory<sup>2,3,4</sup> and antibacterial<sup>4</sup>. However, the valorisation of pistachio shells is not explored for human nutrition applications. The present study aimed for the investigation of the potential of pistachio shells as a clean-label food ingredient. Pistachio shells were ball-milled without any extraction, fractionation or chemical modification. The powder displayed 44.3% of carbon, 0.17% of nitrogen and 0.95% of ashes. Thanks to the ball-milling process, the sizes of the dry and hydrated powder reached 9.3 ± 0.0 µm and 13.8 ± 1.4 µm respectively, with a soluble content of 12.5 ± 0.7% in water.

Pistachio shell powder (PSP) was used as sole stabilizer in emulsions (50% water + 50% oil) and foams. Despite a creaming index of 12% (aqueous phase layering below the emulsion phase after 24h), PSP was a successful emulsifying agent, as it created very thin droplets, cream-colored emulsions with no specific smell. Their average diameter (15.6  $\pm$  0.7 µm) was comparable to the one of the PSP particles in suspension, which is not possible for particle-stabilized emulsions, usually exhibiting a 10-fold-difference between droplets and particles. Even if some particles were cleared adsorbed around the oil droplets, Pickering mechanism could not be the only one at work. This was evidenced when studying the PSP soluble phase, which was able to stabilize emulsions, even though less efficiently than the whole powder. Finally, the PSP was tested as sole stabilizer of aqueous foams. With an overrun was comprised between 350 and 1300%, increasing with a decreasing PSP amount (from 13.7 to 2.3%), the PSP also displayed a high potential as foaming agent.

As a conclusion, pistachio powder can be valorised as an efficient stabilizing agent for the formulation of emulsions and foams. The next step of the study is to model the PSP potential as stabilizing agent with a predictive statistical tool. We will also tackle the increased complexity of formulation in order to get towards prototypes for food and cosmetic applications. We would like to present these results in an oral presentation.

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<sup>2</sup> Dreher, 2012. DOI: 10.1111/j.1753-4887.2011.00467.x

<sup>4</sup> Arjeh et al., 2020. DOI: 10.1016/j.tifs.2019.12.031

#### P30. Clean label oleogels from legumes as fat source in puff pastry

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The excess of saturated fatty acids in the diet is related to health problems, such as the increase of cardiovascular diseases, type II diabetes, metabolic syndrome and obesity. However, saturated fats are responsible for many high-quality attributes in the food we consume. This positive and fundamental contribution of saturated fatty acids to sensory properties and to the technological manufacture of food is the main reason why, the reduction of saturated fatty acids is so complex.

Oleogels are gaining everyday more important in the topic of saturated fatty acids reduction. Oleogelation confers structure to a liquid oil and provides texturing, oil binding, rheological organoleptic and stabilising properties like those provided by saturated fats. For the formation of an oleogel, an oil gelling or oil structuring agent, forming a three-dimensional network capable of conferring structure to the liquid oil is required.

Substances mostly used as gelling agents in the production of oleogels are mainly hydrophobic in nature, like fatty acids, fatty alcohols, mono-, di-, tri-acyl glycerides, ceramides, waxes, phytosterols, alpha-oryzanol, sorbitan derivatives, and polymeric gelators, like ethylcellulose and chitin. Hydrophilic oleogelators, like hydrocolloids have also been used to obtain oleogels by indirect approaches (Wang, Q et al.). Soy protein isolate oleogels were used by the emulsion template approach (2), although proteins have been usually combined with polysacharides to improve oleogel stability.

One limitation of the oleogelators employed so far is the fact that they are additives, which are largely criticized by consumers who want to consume clean label foods free of synthetic ingredients. This demand has motivated our research on new oil-structuring systems, which might fulfill these expectations.

The aim of the present work was to investigate the feasibility of aquafaba from chick pea and faba bean as natural, clean label oleogelators. Rheological, microstructure and oil retention properties of the legume oleogels were investigated. In addition, the legume oleogels were employed to replace 100% shortening in the preparation of croissant, as model of puff pastry food and the croissant quality properties evaluated.

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2 Tavernier, I., Patel, A.R., Van der Meeren, P., Dewettinck, K. (2017) Emulsion-templated liquid oil structuring with soy protein and soy protein: κ-carrageenan complexes. *Food Hydrocolloids*, 65, 107–120.

# P31. Techno-functionality of pigeon pea proteins and their interfacial properties at the air-water interface

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Pigeon pea (*Cajanus cajan*), an underutilised pulse native to tropical and subtropical regions, holds significant promise as alternative protein source. Despite this potential, its techno-functional properties remain largely unexplored. Therefore, This study investigates the extraction and characterisation of pigeon pea proteins using conventional alkaline processing followed by isoelectric point precipitation. Two protein fractions were obtained—globulin-rich and albumin-rich—and their physicochemical, structural, and techno-functional properties were analysed.

The globulin-rich fraction exhibited a higher protein purity (above 80%) compared to the albumin-rich fraction (20%). SDS-PAGE analysis revealed prominent 7S-vicilin subunit bands at 47 and 64 kDa in the globulin-rich fraction, with smaller 11S legumin subunit bands at 20, 25, and 35 kDa. The albumin-rich fraction displayed more low molecular weight bands (<20 kDa). Both fractions featured a complete amino acid profile, though the amount of methionine and tryptophan was limiting. Secondary structure analysis showed a dominance of  $\beta$ -sheets over a-helices, unlike typical animal-based proteins.

Surface hydrophobicity was at least three times higher in the globulin-rich fraction, indicating greater hydrophobic residue exposure. Solubility testing revealed that the globulin-rich fraction was highly soluble (>80%) at neutral and alkaline pH but showed minimal solubility near its isoelectric point (pH 4). In contrast, the albumin-rich fraction demonstrated excellent solubility across a wide pH range. Gelation experiments identified the least gelation concentration of the globulin-rich fraction at approximately 6% w/w, with yield stress and storage modulus values of  $3.66 \pm 0.52$  Pa and  $162.02 \pm 2.69$  Pa, respectively. Dilatational rheology assessed interfacial properties at the air-water interface. The albumin-rich fraction increased surface pressure more rapidly due to its smaller molecular size, lower surface charge, and reduced energy barrier for adsorption. It also exhibited lower dilatational moduli but a greater linear deformation range, indicating a more stretchable but less stiff interfacial film compared to the globulin-rich fraction. Frequency sweep results suggested that protein elasticity at the interface depended on inplane interactions. Notably, the albumin-rich fraction demonstrated superior foaming capacity and stability relative to the globulin-rich fraction.

This study provides valuable insights into the interfacial and techno-functional properties of pigeon pea proteins, highlighting their potential applications in the food industry.

# **P32.** Mimicking the melting profile of adipose tissue through a controlled coalescence in dense emulsions

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The adipose tissue is a densely packed system of fat cells around and in between muscle tissue. In meat products this adipose tissue is responsible for the flavour and the perceived juiciness of meat. Mimicking these properties is essential to create meat analogues with good sensory quality. We could mimic the structure and mechanical properties of adipose tissue at elevated temperatures by binding oil droplets with pea proteins. However, the challenge is to mimic the system hardness at ambient temperatures and melting behaviour during heating. When liquid oil was used in the oil droplets, no melting profile was observed, while when crystalline fat was used, we could represent the animals fat melting profile, as measured with rheology. Using laser diffraction and confocal microscopy, it was found that after a heating-cooling-heating circle, fat crystals protrude from the oil droplet interface, bridging neighbouring droplets and leading to droplet coalescence, which is responsible for the decrease of viscoelasticity (melting). By changing the crystalline fat content we could influence the extent of coalescence either by reducing the crystal protrusion into the continuous phase or by modulating the cluster size of aggregated crystal droplets. Controlling this coalescence rate of the oil droplets in dense emulsions yields melting profiles similar to those of animal fat, which can be the key to creating accurate adipose tissue mimics.

#### **P33. Microbubble powders from freeze-dried Pickering emulsions**

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Microbubbles are a widely used micromaterial in food formulations, as well as various biomedical and pharmaceutical applications. Typically, microbubbles that are stabilized by surfactants tend to coalesce, break, and coarsen quickly <sup>1</sup>. In contrast, microbubbles stabilized by solid nanoparticles, so-called Pickering stabilized microbubbles, can have much better stability than typical ones. Conventional Pickering-stabilized microbubbles are produced by directly dispersing gas into the water phase and allowing the dispersed nanoparticles to adsorb at the air-liquid interface. However, this direct process results in a significant loss of gas, and the resulting bubbles often have large sizes and a wide size distribution, which contributes to Ostwald ripening<sup>2</sup>. To improve this, we draw on the wellstudied case of Pickering-stabilized oil droplets, which lead to oil-in-water (O/W) emulsions that are known for their high stability and uniform size distribution <sup>3</sup>. We hypothesize that since the production of O/W Pickering emulsions is a reliable and controlled process, it may be advantageous to generate stable microbubbles starting from these emulsions rather than relying on the more challenging process of directly stabilizing air-water interfaces with particles. This approach requires the use of a volatile oil, with the water phase evaporating along with the volatile oil phase, resulting in microbubble powders instead of microbubble dispersions. This is followed by resuspension to obtain standard microbubble dispersions.

With this in mind, our current work focuses on producing microbubble powders using Pickering emulsion templates. Both hydrophobic nanoparticles and volatile oils are essential materials for achieving stable and sustainable microbubbles. In our initial experiments, we used modified hydrophobic silica nanoparticles and cyclooctane oil. As anticipated, we produced microbubbles that remained stable for up to a week in both water and plant protein solutions. To explore a more sustainable option, we then experimented with modified hydrophobic calcium carbonate (CaCO<sub>3</sub>) nanoparticles and cineole oil as our primary materials. Surprisingly, we achieved a series of even more stable microbubbles that remained intact for over a month. In summary, the Pickering emulsion template method proves to be an excellent approach for preparing completely sustainable and food-grade microbubbles.

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# P34. Stabilization of gluten-free starch-based model systems via arabinoxylan-protein networks

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The increasing prevalence of gluten-related disorders and the rising demand for glutenfree (GF) products requires innovative strategies to enhance their structural and functional properties. One promising approach involves the network-formation of cereal arabinoxylans (AX) and proteins, facilitated by interactions between their key functional groups (polyphenolics, tyrosine, cysteine). However, the extent of these cross-linking reactions and their influence on the textural properties of GF systems remain insufficiently understood.

In this study, commercially available (c. AX) and self-extracted (s. AX) arabinoxylan extracts (2,6 % based on starch content), along with corn gluten meal (CGM) (3 % based on starch content), were incorporated into GF starch-based systems (pH 5.5, 1:1 ratio of maize starch and buffer, 25 °C) and subjected to enzymatic treatment with laccase (0.5 U/mg substrate). Rheological evaluations, including amplitude ( $\gamma$ =0.01-100 %, 1 Hz) and time sweeps ( $\gamma$ =0.02 %, 1 Hz, 30 min), demonstrated that s. AX exhibited significantly higher storage modulus (G') values compared to c. AX. This indicated greater shear stability and continuous cross-linking over time. The superior performance of s. AX could be attributed to its higher phenolic content, which additionally enhanced the enzymatic cross-linking response, resulting in a more robust and stable network. Additionally, s. AX displayed more effective interactions with CGM than c. AX, further contributing to network formation.

Future research will focus on integrating these AX-protein networks into complex GF dough formulations to evaluate their impact on gas retention, baking stability, and the textural properties of model GF breads.

# **P35.** Predicting emulsion viscosity by encoding neural networks with physics; slowly removing the A from AI

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#### <u>Aim:</u>

The main aim is to better predict food properties on the basis of their ingredient properties by constructing models that make use of artificial intelligence (AI), while minimizing the number of data required and maximizing causal underpinning.

A major problem in using AI for predicting food properties is the scarcity of large amounts of data and, consequently, still the huge laborious experimental efforts to collect them. Another problem is the statistical character of AI, which neglects causality. We report on a novel route that addresses these two problems at the same time, which is a potential and novel tool to predict food functionality.

#### Method:

We have addressed a specific problem of predicting the viscosity of dairy- and plant-protein stabilised emulsion systems. The goal is to predict emulsion viscosity based on oil volume fraction and the type of protein used. We encoded a neural network with physics-based information before its statistical optimization. This physics information resembles the existing physical hierarchical structure within the system. After this encoding with physics information, we optimised this so-called Physics encoded Neural Network (PeNN).

#### **Results:**

We show that the mean squared error (MSE) of the PeNN is always smaller than that of the 'normal' NNs, in the order of a factor of thousand. Furthermore, the PeNNs capture extrapolation and interpolation very well, contrary to the 'normal' NNs, thus showing the improved accuracy and efficiency of our newly developed PeNN. This is most probably due to the inherent higher causality in the network by means of encoding it with (causal) physics information.

#### **Conclusion:**

By encoding physics information into a neural network, we can obtain a predictive model that is substantially more accurate than 'normal/traditional' neural networks (without the physics information). Furthermore, PeNNs need a much smaller dataset size for training than the 'normal' NNs to achieve similar accuracy. This extraordinary finding shows the potential to use PeNNs when data is scarce or laborious to collect. The newly presented PeNNs are the next generation of predictive AI models for food systems. The methodology is scalable and in principle also should better allow to connect different domains quantitatively.

### P36. Quantification of Anisotropic Microstructures in Gluten Network Formed by Addition of CMC

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Many food hydrocolloids are commonly used to enhanced textural properties in food products, they do so by interacting with other ingredients. These enhanced textural attributes are the result of hierarchical structures formed at multiple length scales by the hydrocolloids and their interactions with other ingredients. Currently, there is a gap in the understanding of how hydrocolloids form specific structures at the microscopic scales, this is in part due to the lack of detailed studies at that scale and the limited incorporation of image analysis tools to obtain quantitative data from microscopic images. In this presentation, it will be shown how using quantitative analysis of microscopic images helps gives new insights into the rheological changes seen in a well-known food material. Gluten and Carboxy Methyl Cellulose (CMC) are two commonly used ingredients for texture enhancement specially in plant-based meat analogues. In meat analogues, both gluten and CMC interact with many other ingredients, however their interaction and texture enhancing mechanism are largely unknown. While gluten is known for forming a 3D viscoelastic microscopic network, CMC has been reported to modify that the gluten network at the microscopic scale (Correa et al., 2014). However, this has not been quantified or measured before. In this work, we have delved into the mechanism of gluten/CMC interaction at the microscopic level. To this end, we have prepared wheat dough with increasing levels of CMC and quantify their microstructural changes. From the micrographs, it can be seen that CMC appears to disrupt the gluten network into a fibrillar microstructure, with aligned strands. Effectively going from an 'isotropic' network to 'anisotropic' fibrils. The degree of isotropy/anisotropy of the microstructures was quantified using a nematic order parameter (NOP). NOP is a value used in the field of liquid crystal physics, where the quantifying the alignment of microstructure is crucial for determine crystalline structures (Serra et al., 2011). Here we found that the NOP increases as the level of CMC increases until a certain level (5% CMC), after which CMC seems to form its own phase and does not modify the gluten phase anymore. These values of nematic order parameter correlate with the rheological changes seem in the dough. Overall, the quantification of these isotropic/anisotropic microstructures formed by addition of different hydrocolloids will help us unveil the mechanisms through which they modify texture in novel foods.



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#### P37. Tribology and rheology of model water-in-water emulsions

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Water-in-water (W/W) emulsions, derived from phase-separating mixtures of hydrophilic macromolecules, have attracted a great deal of attention for encapsulation, drug delivery, biotechnical separation and the development of stable 'oil-free' emulsions. Although their formation and stabilisation mechanisms have been studied in depth, evaluation of their unique rheological and tribological performance remains in its infancy. This study aimed to investigate the microstructural, rheological and tribological properties of model W/W emulsions composed of dextran (D) and poly(ethylene oxide) (P) at a fundamental level. Rheological analysis revealed that pure D exhibited pronounced shear-thinning behaviour compared to pure P. For D-P W/W emulsions, increasing the P concentration [P] resulted in increased viscosity ( $\eta$ ), whilst increasing the D concentration [D] intensified shearthinning behaviour, likely due to changes in the quantity and size of D-based droplets. Confocal laser scanning microscopy (CLSM) demonstrated a significant increase in the average droplet size with higher [D] or [P]. A striking tribological result was that the W/W emulsions demonstrated an unusually flat mixed lubrication regime, with friction coefficients ( $\Box$ ) < 0.01 over a considerable range of sliding contact speed (~10 to 100 mm s<sup>-1</sup>, of physiological relevance) before the onset of the elastohydrodynamic lubrication (EHL) regime. This was guite unlike the behaviour demonstrated by solutions of the individual polymers on their own. Such concentration-dependent behaviour was attributed to W/W emulsion droplets entering the tribological gap, flattening and reducing the viscosity of the entrained lubricants, thus delaying the formation of a fluid film. Overall, this detailed study shows how fabrication of W/W emulsions via phase-separating polymers can offer unique lubrication characteristics that could provide advantageous aqueous lubricants for biomedical applications.

#### P38. Revisiting Pectin in Food Applications

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Pectin is a naturally occurring polysaccharide present in most plants. Pectins are mostly extracted from citrus or apple, and are widely used in food products where they perform as thickeners, stabilizers, or gelling agents. Unlike common food hydrocolloids, pectins are well-perceived by consumers, and are increasingly found in fruit products, confectionary or acidified dairy products, but also in pharmaceutical applications or in cosmetics<sup>1-4</sup>. The structure of pectins is very complex and is closely related to its properties. For example, the degree of methoxylation (DM) impacts the gelling mechanism of pectin and the final gel macroscopic properties.<sup>5, 6</sup> DM also affects the interactions between pectin and proteins and, ultimately, the stabilization of protein aggregates under acidic conditions.<sup>7, 8</sup> We found that rationale selection of pectin polysaccharides based on their structure enabled the design of novel systems, outside of the classical functionality of pectins in food products. The relationship between the fine structure of pectin and the physico-chemical properties of the final formulations was systematically studied, and was correlated to sensory attributes. This work demonstrates that careful consideration of pectin structure enables novel formulations using this well-known hydrocolloid.

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# P39. pH-induced conformational changes of lupin protein-pectin mixtures and their effect on air-water interfacial properties and foaming functionality

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Lupin protein isolate (LPI) has high nutritional value and good foaming properties around neutral pH; however, its functionality becomes poor at acidic pH, due to reduced protein solubility. The addition of pectin to LPI can increase its solubility at acidic pH and hence improve protein functionality. Here, we investigated the air-water interfacial and foaming properties of LPI-pectin (1:1) mixtures at pH 3.5-7.0. We used interfacial shear and dilatational rheology, characterized the air-water interfacial microstructure with AFM of Langmuir-Blodgett films, and linked the results to the foaming properties of the LPI-pectin mixtures. Based on the phase diagram, LPI and pectin formed co-soluble mixtures at pH 6.0 and 7.0, while LPI-pectin electrostatic complexes were formed at pH 3.5 and 4.0. In the co-soluble mixtures, proteins diffused faster towards the air-water interface than the electrostatic complexes, due to smaller particle sizes of the proteins. Their air-water interfaces showed distinct differences with respect to microstructure and mechanical properties. The interfaces stabilized by co-soluble mixtures were dominated by protein aggregates, leading to weaker interfaces in response to shear and dilatational deformation, while the complexes formed thicker and denser polymeric air-water interfaces that were stiffer and more solid-like. As a result, the complex-stabilized foams were more stable than those stabilized with co-soluble mixtures. Findings from this study indicate that soluble LPI-pectin complexes formed at pH 3.5 and 4.0 were more efficient in improving interfacial and foaming properties of LPI than the co-soluble mixtures at pH 6.0 and 7.0, which can be used to tailor the properties of acid aerated products stabilized by LPI.

# P40. Low methoxyl pectin-based milk gels: Understanding structuring mechanisms in neutral dairy environments

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Low methoxyl (LM) pectin is widely utilized in the food industry for its ability to form gels in the presence of divalent cations, such as calcium [1-3]. While LM pectin has been extensively studied and valorized in acidic and sugar-rich matrices, its behavior in neutral dairy environments presents unique challenges and opportunities, particularly in the development of functional and texturally appealing dairy products. Indeed, alongside some traditionally used food hydrocolloids in neutral dairy environments, LM pectin which is highly appreciated by customers and consumers [3], has emerged as a promising hydrocolloid candidate for such food applications. This is justified by the fact that LM pectin can undergo gelation over a wide pH range (2.6 - 7) [2], and that dairy environments provide the necessary conditions (neutral pH, high calcium content) for LM pectin gelation [1-3]. Additionally, the presence of proteins (such as casein micelles and whey proteins) and other milk components (such as fats) constitutes significant factors that can influence and modulate the gelling properties of LM pectins in neutral dairy environments. However, although LM pectins can be employed in neutral dairy environments similarly to some traditionally used hydrocolloids, such as carrageenan, little work has been conducted to understand and control the "structure-function" relationships of LM pectins in neutral dairy systems. Thus, the objective of this study is to better understand the structuring mechanisms (gelation, interactions, etc.), both under static conditions and under shear, of milk gels formed using LM pectins with varying chemical and macromolecular characteristics.

Using an original approach that combines rheological and chemical analyses, this research highlights the critical role of interactions between LM pectin and milk proteins in the formation of neutral milk gel networks. These interactions are further modulated by the presence of fat and calcium bridges, which play a non-negligible role. The study shows that these factors strongly influence the microstructure and mechanical properties of the resulting milk gels, which are also highly impacted by the chemical and macromolecular characteristics of the pectin. By bridging the knowledge gap in this area, this work provides an in-depth understanding of the mechanisms underlying LM pectin gelation in neutral dairy environments, offering strategies to design innovative tailormade neutral dairy desserts with desirable properties.

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# P41. Decoding Gum Arabic: Unraveling the Connection Between Structure and Functionality.

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Gum arabic (GA), the exudate from Acacia senegal, is commonly used to stabilize aroma oil and beverage emulsions due to its amphiphilic character, high emulsifying capacity, and very low viscosity in aqueous solution. GA emulsions are able to prevent coalescence over extended storage periods. Despite its wide use and overall excellent technofunctional properties, the molecular reasons for the functional differences among various GA batches remain relatively unclear. Long-term emulsion stability, which is crucial for the quality of a GA batch, is mostly determined through an acceleration test, in which the change in droplet size distribution of a GA emulsion, after heating the emulsion over the course of a week, is observed and evaluated.<sup>1</sup> Previous studies on individual GA samples have postulated that certain GA glycoprotein fractions, especially those characterized by a high protein content and significant spatial expansion, are crucial for initial emulsion stability.<sup>2,3</sup> However, quality markers for unfractionated GA and correlations with structural parameters have not been established. Therefore, our aim was to investigate the structural properties and techno-functional characteristics of 20 GA samples of varying quality in detail to clarify the interrelation. To obtain valid results, analytical methods such as the protocol for monosaccharide analysis were refined. Furthermore, new approaches were developed which provide rapid and detailed insights into the molecular properties of GA.

The gum samples were first analyzed for their monosaccharide composition, protein content, molecular weight, and molecular weight distribution. O/W emulsions with weighted orange oil were prepared from all samples, and their initial droplet size distribution and viscosity were determined. By using Pearson correlation tests, we demonstrated that rhamnose content and the rhamnose/galactose ratio positively correlate with molecular mass. The molecular mass itself correlated with the viscosity of the emulsion, while the protein content negatively correlated with the droplet size distribution. Especially the molecular mass/viscosity ratio appeared to have a subtle impact on batch quality. To gain a deeper understanding of the relation between quality (i.e. long-term emulsion stability) and molecular structure, selected GA samples were fractionated by using hydrophobic interaction chromatography according to their protein content in different glycoprotein fractions, known as arabinogalactan (AG), arabinogalactan-protein (AGP) and glycoprotein (GP). The fractions were analyzed similarly to the native samples and also further characterized after partial enzymatic and chemical hydrolysis. This enabled detailed insights into the structural differences and similarities between the samples to be obtained, as well as the identification of the decisive factors for batch quality.

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# P42. Phase separation in aqueous mixtures with multiple components.

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Phase separation plays an important role in food and polymer technology, but also in many other fields like hematology, wastewater treatment, archaeology and forensics. Predicting phase behavior numerically is difficult, especially when many (macro-molecular) components are involved. Being able to calculate phase behavior in mixtures of many components is relevant for predicting effects of polydispersity in foods. One of the approaches to describe phase separation is in terms of a (Edmond-Ogston like) model based on a virial expansion up to second order of the concentrations of the components. Here recent and sometimes surprising results for two and three biopolymers are discussed.

For mixtures of two or more components [1,2,3], we found for example, that it is impossible to extract the second virial coefficients directly from an experimentally determined binary phase diagram, something that is due to a degeneracy in the second virial coefficients. However, it turns out that this becomes feasible by simultaneously analyzing multiple phase diagrams with shared components. In this way, we were able to generate a database of second virial coefficients without doing any experiments, but by relying on phase diagrams obtained from literature. Currently, alternative approaches are being studied for situations in which even less *a priori* information is available.

For mixtures of three components [4], we found that associative phase separation (where one of the phases is enriched in two of the components) does not require an attractive interaction (where the second virial coefficient is negative) but can also occur for repulsive interactions (where the second virial coefficient is positive). This contrasts with what is commonly reported in literature.

The approaches used for two and three component mixtures can be surprisingly extended to mixtures with a very large number of components N >> 1. It is possible to obtain explicit expressions for the spinodal and critical manifolds for N components, that can be evaluated through a relatively simple algorithm [5]. The calculation of the composition of the coexisting phases is still a challenge.

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# P43. Improving the stability of pea protein emulsions by phospholipids: a structure-dependent approach.

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Although plant proteins usually have good emulsifying properties, they migrate slowly to the interface and do not form a viscoelastic interfacial film, which facilitates the coalescence of oil droplets and lowers product stability. Phospholipids can be added for faster interfacial stabilisation, assuming they do not hinder the protein from adsorbing and interacting at the interface, as this would not only reduce the interfacial viscoelasticity but increase the emulsion's propensity to coalescence. The fatty acyl chain of the phospholipid seems to determine whether the phospholipid and protein can co-exist at the interface or if the PL inhibits protein adsorption and protein-protein interactions. This correlation is not well understood for plant protein and phospholipid mixtures.

Therefore, this study aimed to increase the stability of pea protein emulsions by a structure-dependent phospholipid selection. Phospholipids with different degrees of saturation have been used (saturated vs. unsaturated). The adsorption of protein and phospholipids was analysed by tracking the interfacial tension via drop tensiometry. In addition, the viscoelasticity of the protein-phospholipid interfaces was studied using dilatational and interfacial shear rheology. Then, the stability against coalescence was analysed using microfluidic experiments. Finally, the storage stability of the pea protein emulsions was studied via emulsification experiments, where emulsions were produced and stored for seven days.

At short time scales (microfluidic study) relevant to the emulsification step, the coalescence stability of protein-stabilised emulsions could be increased due to phospholipid addition. Here, the type of phospholipid did not seem to impact the coalescence stability of the emulsion much. On longer time scales (dilatational and shear rheology), the stability of the pea protein interface could only be increased in the case of saturated phospholipids due to the formation of a viscoelastic protein-phospholipid interfacial film. In contrast, unsaturated phospholipids reduced the viscoelasticity due to competitive protein displacement and or by interfering with interfacial protein-protein interactions. In the same way, in the emulsion system (emulsification experiments), reduced storage stability was observed in the case of the unsaturated PL, while saturated PL seemed to increase the storage stability.

In short, the emulsion stability of pea protein emulsions was improved due to the addition of saturated PL, while unsaturated PL lowered product stability. The PL must, therefore, be carefully chosen, depending on the product requirements.

### P44. Protein isolate from cryo-milled quinoa seeds: a strategy for the design of plant-based soft materials

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Quinoa protein isolate (QPI) is a promising food ingredient due to its complete essential amino acid composition and the ability to control its solubility and gel strength. Protein denaturation and loss of functionality during milling of quinoa seeds to produce flour and subsequent processes for protein extraction pose challenges in the utilisation of this ingredient in food formulations. Here, quinoa seeds were cryo-milled aiming to protect the protein fraction against heat damage during seed grinding. Then, QPI was extracted by wet fractionation using acetic acid or citric acid as the precipitation acids, which are more kosmotropic and better protect the native protein structure than the commonly used HCl. The protein content and solubility of the isolates were measured prior to heat-induced gelation, which was assessed by small deformation measurements. QPI extracted with the same acids from commercial quinoa flour, which was produced by flaking and milling of the guinoa seeds, were used as reference. Although the protein content of all three QPI extracted from cryo-milled seeds was higher (p < 0.05), the solubility of the isolates at pH 7 was reduced when compared to that of QPI extracted from commercial flour. When assessing the gelation properties, QPI precipitated with acetic acid and citric acid from cryo-milled seeds produced gels 4- and 7-fold stronger (higher final elastic moduli), respectively, than their counterparts prepared from commercial flour. The gel strength of QPI precipitated with HCl remained statistically similar (p > 0.05) regardless of the type of flour and was lower than the achieved with the other two acids. While there where only minor differences in the gel microstructures of the three QPI prepared from commercial flour, confocal laser scanning microscopy of QPI extracted from cryo-milled seeds revealed that the citric acid-precipitated QPI resulted in a homogeneous protein network, while the QPI precipitated with HCl formed a heterogenous and weak structure. The acetic acidprecipitated QPI showed a mixed gel structure with many aggregates included in the protein network. This research demonstrates that cryo-milling of guinoa seeds coupled with protein precipitation using citric acid can be used to recover a highly functional ingredient for the formulation of plant-based foods, an approach that likely has broader applicability to other plant sources.

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# P45. Antioxidant-loaded electrostatic hydrogels with *Hibiscus* Sabdariffa extracts

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Hydrogels are three-dimensional, cross-linked polymeric networks that can retain significant amounts of water, making them versatile materials for various pharmaceutical, biomedical, agricultural, and food applications. In this study, we developed antioxidant electrostatic hydrogels using halal-grade commercial bovine gelatin (CBG), gelatin extracted from Eland skin (EG), and carrageenan, employing a cold gelation method (in situ gelation using GDL acid). Hibiscus extract (Hibiscus sabdariffa), known for its high polyphenol content-primarily anthocyanins-and excellent antioxidant and coloring capacities, was incorporated into the hydrogel delivery systems through three sequential extractions (1:5 w/v ratio, 3 hours) by mixing dried Hibiscus calyces with distilled water. The resulting hydrogels were characterized based on several physicochemical properties, including color, strength, water-holding capacity, and swelling capacity. Additionally, we analyzed the total phenolic content and antioxidant capacity of the extracts from the three concentrated hydrogels. As expected, the total polyphenol content decreased from the first to the third extraction, with values ranging from 65.86 to  $79.11 \ \mu g$  GAE/mg sample. Consequently, the phenolic content (ranging from 14.36 to 22.01 µg GAE/mg sample) and antioxidant capacity (ranging from 5.60 to 8.49 µg AAE/mg sample) of the hydrogels prepared with both types of gelatins also diminished. The hydrogels' strength ranged between 0.54 N and 1.07 N, while their water-holding capacity (WHC) varied from 62.57% to 90.29%, and swelling capacity ranged from 11.04% to 20.56%. All these properties decreased as the extracts became more concentrated compared to the control hydrogel (without hibiscus extract). However, the loading efficiency of polyphenols and antioxidant capacity for the three systems exhibited only minor differences across concentrations, with approximate values of ~25% and ~16%, respectively. Our findings suggest that incorporating hibiscus extracts can enhance the properties of hydrogels, making them a promising system for improving antioxidant capabilities in various industrial sectors and for future research applications.

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# P46. Zooming in on structural properties of mealworm protein gels with and without added $CaCl_2$ – a study combining rheology and SAXS

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In 2022 frozen, dried and powder forms of yellow mealworm were officially added to the list of authorised novel foods in the European Union (Implementing Regulation (EU) 2017/2470). However, corresponding research on mealworm protein and its techno-functional properties is only just emerging and does not yet provide a sufficient understanding of the structural properties of these gels on all relevant length scales.

To this purpose, our study aimed to elucidate on changes to structural properties at molecular, floc and gel length scales by combining FT-IR-, fluorescence- and UV-Vis spectroscopy with SAXS and rheological measurements. In an additional set of samples, we investigated the impact of the addition of a divalent salt (CaCl<sub>2</sub>).

From SAXS we derived the existence of multiscale structures as indicated by a lack of plateau in the low Q region. On the molecular length scale, difference spectra from FT-IR spectroscopy indicated an increase in inter- and intramolecular  $\beta$ -sheet structures upon addition of CaCl<sub>2</sub>. The gelation process led to a decrease in amide I and II bands for both samples indicating an overall decrease in ordered structures as well as a slight loosening of  $\beta$ -sheets upon heating and cooling. This corresponded to a slight red shift in fluorescence spectroscopy of diluted solutions indicative of increased exposure of tryptophane residues and was accompanied by an increase in the UV-vis absorption at 290 nm which was related to aggregation, especially if CaCl<sub>2</sub> was added.

For the characterisation on the floc length scale, we derived fractal dimensions from the scattering exponents in the lower Q region. These indicated mass fractals in both unheated samples, with larger values implying denser flocs if CaCl<sub>2</sub> was added. After gelation, the structure at the floc length scale in samples without CaCl<sub>2</sub> could be described as a surface fractal with rough structure but could still be characterised as (even denser) mass fractals in the presence of CaCl<sub>2</sub>.

On the gel length scale scaling of rheological results according to Wu and Morbidelli showed contributions of both, inter- and intrafloc interactions with an increasing contribution of intrafloc interactions and higher storage modulus in samples with added CaCl<sub>2</sub>, again indicating an overall denser structure of these samples also on the gel length scale.

In summary, we were able to obtain a deeper understanding of the structural properties of heat induced mealworm protein gels on all relevant length scales and how they were affected by a change in environmental conditions. In future, this knowledge could be used towards customising gel properties for specific applications.

# P47. <u>Oral presentation abstract</u>: Sodium alginate – a promising material for the encapsulation of next-generation probiotics

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The next-generation probiotics (NGPs) have appeared in recent years with an abundance of significant therapeutic potential. However, some of these strains have been identified to be extremely oxygen-sensitive (EOS), which leads to various challenges for their production, processing, storage, and colonic delivery. Sodium alginate (SA) has appeared as a promising encapsulating material for EOS probiotics using ionotropic gelation with calcium ions. It confers excellent oxygen barrier properties as well as optimal release behavior in simulated gastrointestinal fluid (SGF). The present study comprehensively examines the effects of SA structural characteristics, molecular weight (MW), and Mannuronic/Guluronic (M/G) ratio on its functional properties as an encapsulating material for the protection and controlled release of 3 NGP strains including *Lactiplantibacillus plantarum*, *Bifidobacterium longum* subsp. *infantis* and *Faecalibacterium duncaniae* (EOS strain).

The results show that the rheological properties of SA are notably influenced by molecular weight but not by the M/G ratio. Autoclaving SA powder significantly reduces the viscosity of SA solution. However, the MW and M/G ratio do not affect the oxygen barrier properties of SA films. Although cross-linking with divalent cations slightly increases oxygen permeance, this still provides a high protection level for encapsulated microorganisms. High MW and low M/G ratio SA beads form stronger gels due to effective crosslinking of the G blocks with divalent cations. The M/G ratio affects SA bead swelling, beads with a high ratio of M blocks display higher swelling in SGF than those with a high ratio of G blocks. The MW determines solubility, beads with low MW dissolved in SGF after 4 hours. SA with low MW and high M/G ratio appears as optimal for swelling, solubility, and probiotic release. Although viability tests showed that pure SA does not protect enough probiotics in SGF, adding sodium carbonate and L-cysteine to the polymer matrix preserves *F. duncaniae* viability up to 6 logs CFU·mL<sup>-1</sup> after exposure to SGF.

**Keywords:** Sodium alginate, functional properties, probiotic encapsulation, next-generation probiotics, gastrointestinal tract, colonic delivery.

#### **Reference:**

Phùng, T.-T.-T., Đinh, H.-N., Ureña, M., Oliete, B., Denimal, E., Dupont, S., Beney, L., & Karbowiak, T. (2024). Sodium Alginate as a promising encapsulating material for extremely-oxygen sensitive probiotics. *Food Hydrocolloids*, 110857. https://doi.org/10.1016/J.FOODHYD.2024.110857

# P48.\_Protein-dense droplets to colloidal ingredients: Developing colloidal plant protein powders

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Pursuing sustainable and efficient plant-based protein ingredients has stimulated research into innovative processing methods, including Liquid/Liquid Phase Separation (LLPS). This study utilises LLPS, inherent to biopolymers, to develop high-quality plant protein ingredients from diverse legumes. We leveraged the phenomenon of coacervation by altering environmental conditions to induce phase transitions, thus forming spherical, protein-rich coacervates from minimally processed materials such as legume flours. Demonstrating that this process occurs consistently across various leguminous plant protein fractions underscores its viability for developing ingredients from diverse sources. Following coacervation, we developed colloids from these phase-separated protein fractions. The colloids underwent heat treatment for pasteurisation and stabilisation, followed by spray drying to mirror industrial processing conditions.

The colloidal concentrates derived from these protein-rich droplets exhibit a very high internal protein content of 35%w/w. Notably, CLSM and SEM imaging revealed significant morphological distinctions from lab-based commercial controls, highlighting the distinctive structural properties conferred by our processing technique. A critical discovery is the monomodal particle size distribution and the porous nature of the colloidal samples, suggesting a deviation from traditional vacuole formation during the spray drying processes. Instead, these samples display a porous structure throughout the colloidal particle. Furthermore, these colloidal concentrates demonstrate substantially increased density and nearly double the water-holding capacity compared to standard commercial preparations.

In assessing potential industrial applications, the dispersibility of the concentrates significantly exceeds that of control samples, achieving rates up to ten times faster. Additionally, these colloidal systems exhibit viscosities up to ten times lower than those of conventional samples, providing a substantial advantage in formulating high-protein dispersion for further processing or developing protein-rich, low-viscosity plant-based beverages. This characteristic permits higher concentrations of protein while maintaining optimal flowability.

This research not only underscores the viability of LLPS in creating superior plant protein concentrates but also paves the way for their practical application in a range of food products. Moreover, using industrial processing conditions in our methods facilitates straightforward scalability, ensuring these innovations seamlessly integrate into large-scale production environments.

### P49. Next-generation prebiotics: Oligosaccharides-protein Maillard-conjugates for selective targeting of proteins to probiotic bacteria in the colon

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Prebiotics consumption may promote gut-probiotics, and improve human-health. Current prebiotics are predominantly carbohydrates. However, great competition exists among gut-microbes for the scarce protein in the colon, as most consumed-protein is absorbed in the small-intestine. Still, no protein-containing-prebiotics are commercially-available. Here, we developed and evaluated *in-vivo* the next-generation of prebiotics: proteincontaining-prebiotics, for selectively-targeted delivery of protein to colonic-probiotics, to boost their growth. The system is based on micellar-particles, made of Maillardconjugates of 2'-Fucosyllactose (2'-FL) shell, engulfing protein (Lactoferrin or potato protein) peptic-then-tryptic hydrolysate (LFH or PPH, respectively). This core-shell structure lowers protein-core digestibility, while the prebiotic-glycans are hypothesized to selectively-target colonic-probiotics. In-vivo mice-study showed that the consumption of 2'-FL-LFH Maillard-conjugates significantly increased the colonic-concentration of shortchain-fatty-acids (SCFAs), compared to the unconjugated-components or to saline, by promoting SCFAs-producing bacterial-families and genera (Ruminococcaceae, Lachnospiraceae, Odoribacter and Prevotella). Plasma-levels of inflammatory lipopolysaccharides were significantly lower in the 2'-FL-LFH group compared to the unconjugated-components and the saline groups, indicating lower pathogen-induced gut wall permeability. We found that 2'-FL-LFH can serve as novel protein-containingprebiotics, beneficially-modulating the composition and metabolic-activity of gutmicrobes, thereby contributing to host-health more effectively than carbohydrate-only prebiotics. Varying the oligosaccharide or protein parts may allow targeting different probiotics and providing their essential amino-acids. These possibilities would enable tailoring the product for desired health-benefits or target consumer-populations.

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# P50. Nonlinear rheological behaviour of mixed plant-dairy matrices: the influence of protein solubility

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The rising global population has substantially increased the demand for protein sources, prompting the exploration of innovative approaches. One such strategy involves partially replacing animal proteins with plant proteins in mixed food systems<sup>1</sup>, including emulsion filled gels (EFGs). Limited information is available on how technological approaches designed to modify protein solubility influence the rheological behaviour of food systems<sup>2</sup>, despite the well-established impact of solubility on rheological properties<sup>3</sup>. Therefore, the aim of this study was to investigate the impact of protein solubility on the nonlinear rheological behaviour of pea, whey and 1:1 mixed pea:whey EFGs. The solubility of pea proteins was modified by pre-treating protein suspensions (6% total protein content) with high pressure homogenization (HPH; 6-125 MPa), with the pre-treated suspensions used to formulate EFGs. Solubility, particle size distribution and rheological behaviour in the linear (SAOS) and nonlinear viscoelastic regions (LAOS) were evaluated.

The results showed that HPH progressively increased the solubility of pea protein, from 22% in the untreated sample to 96% at 125 MPa. On the other hand, the solubility of the mixed pea:whey suspensions ranged from 53 to 58% across all the pressure range. Interestingly, the mixed pea:whey EFGs showed an increased storage modulus (G') in the SAOS region when pre-treated at high pressure, increasing from 5184 Pa in the untreated EFGs to 27203 Pa in those treated at 100 MPa. No significant (p < 0.05) changes in G' were observed in pea EFGs formulated with pea suspensions at different solubility, with G' ranging from 27203 to 29313 Pa in untreated and 100 MPa treated samples, respectively. In the Lissajous curves of LAOS analysis, all samples exhibited a similar mechanical transition from solid-like to liquid-like behavior as the applied strain amplitude increased. This transition was characterized by the transformation of tight ellipses into progressively open ellipses, eventually approaching a rectangular shape. To elucidate differences among samples, nonlinear elastic and viscous responses (as a function of strain amplitude and shear rate, respectively) were quantified using the Chebyshev decomposition method<sup>4</sup>. Both pea and mixed EFGs exhibited strain stiffening (S > 0) and shear thinning (T < 0) behaviour with increasing strain amplitude or shear rate. The results of this study highlighted a positive impact of HPH on the rheological behaviour of mixed EFGs, despite having minimal impact on protein solubility in the mixed suspensions. These findings contribute new fundamental understanding and advancement of developing mixed protein systems.

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#### P51. a-Lactalbumin Nanotube as a Novel Delivery System for Food Bioactive Compounds

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Food Sourced Protein nanotubes (PNTs) as biocompatible nanocarriers are promising to deliver functional ingredients in nutritional foods. a-Lactalbumin accounts for 20% of whey protein and has anti-inflammatory, anticancer, and immune-enhancing effects. In our Lab, the well-defined nanotubes were obtained by self-assembly of partially hydrolysed alactalbumin peptides by Bacillus licheniformis protease (BLP) specially at Glu and Asp sites, the amphiphilic peptides can form nanotubes through Ca<sup>2+</sup> ions bridging coordination<sup>1</sup>. In this presentation, the molecular self-assembly mechanism and structural evolution of alactalbumin nanotubes will be discussed, laying a theoretical foundation for the application of a-lactalbumin nanotubes in nutritional delivery systems<sup>2</sup>. At the same time, it was also found that a-lactalbumin nanotubes can self-assemble to form nanospheres after removing  $Ca^{2+}$  or lowering pH. Nanospheres can also be transited into nanotubes under certain conditions, thereby realizing the reversible transformation between a-lactalbumin nanotubes and nanospheres<sup>3</sup>. This is conducive to the development and design of environmentally responsive delivery carriers with a variety of physical structures and physicochemical properties. We have developed different types of a-lactalbumin nanotubes for delivering bioactive compounds. For instance, we found that flexible tubular nanoparticles were effective at penetrating mucus, thereby improving the bioavailability and therapeutic efficacy of the administered compounds<sup>4</sup>. In another study, we found that nanotube-based composite microsphere delivery system exhibited excellent а mucoadhesive and mucus-penetrating properties, improving the bioavailability of hydrophobic capsaicin and maintaining the homeostasis of gut microbiota<sup>5</sup>. In addition, we also found that  $Mn^{2+}$  can induce the nanotubes formation bearing an nanozymes function which possessed the anti-rheumatoid arthritis effect with loaded capsaicin<sup>6</sup>. The information addressed in this presentation may provide inspirations for the future development of more advanced nanocarrier systems in functional food applications.

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#### P52. Structuring Ability of Seaweed Soft Particle Suspensions Driven by Polysaccharide Diversity

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Seaweeds are increasingly valued in the food industry due to their rich content of bioactive compounds, proteins, lipids, and dietary fibres, which offer health benefits and functional properties. Their low environmental impact, requiring no arable land, freshwater, or pesticides for cultivation, makes seaweeds a sustainable alternative to traditional food sources. Unlike traditional approaches focusing on extracting specific hydrocolloids, this study emphasises using whole seaweed to create particles through processing, leveraging their structural potential and reducing side streams from seaweed-based ingredients.

While suspensions of plant polysaccharide particles have been extensively studied<sup>1,2</sup>, there is still limited knowledge about the potential of seaweed-derived particles<sup>3</sup>. Different seaweed species—red, brown, and green—contain distinct polysaccharides, such as porphyran, alginate, and ulvan, respectively, located in their cell wall and extracellular matrix. We investigated the rheological behaviour of aqueous suspensions of seaweed soft particles, with a focus on their application as rheology modifiers. Rheological measurements revealed that these suspensions exhibit versatile flow behaviours and viscoelastic responses, depending on processing methods and polysaccharide composition. Confocal Scanning Laser Microscopy (CSLM) provided insights into the particles microstructure, including size, shape and cell wall integrity, which are critical for controlling suspension properties. Additionally, small-angle X-ray scattering (SAXS) enabled a nanoscale analysis offering a deeper understanding of the impact of processing on seaweed structure.

Our results highlight the importance of species selection in optimising seaweed for specific industrial applications. Furthermore, using seaweed particles, rather than focusing solely on isolated seaweed hydrocolloids, offers new opportunities for the development of innovative, clean-label ingredients. This work bridges the gap between fundamental structural studies and practical applications, supporting the sustainable use of marine resources to meet the growing demand for environmentally friendly and healthier products.

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### P53. Microfluidic fabrication of thiolated hyaluronic acid-alginate microsphere with dual adhesion function for colon-targeted codelivery probiotics and postbiotics

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The health benefits of oral probiotics are significantly hindered by their low survival rate in harsh stomach conditions and short retention time in intestine. Herein, we developed a novel microsphere with dual adhesion to bacterial and intestinal mucosa using thiolated hyaluronic acid (HA-SH) and sodium alginate (SA) for colonic targeted delivery of probiotics. This dual-network microsphere was prepared in microfluidics platform through an ingenious combination of photoinduced disulfide crosslinking and ionic crosslinking, achieving uniform particle size  $(257 \pm 20.5 \mu m)$  and high encapsulation efficiency (82.1%). Owing to the protective effects of the microsphere, the embedded probiotics exhibited high survival rates (8.69 log CFU/mL) in simulated gastric fluid and a high release rate (80.2%) in the intestine. Moreover, the adhesion tests demonstrated that the grafting of thiol groups into the microspheres significantly enhanced both bacterial adhesion rate (53.9%) and mucosal adhesion rate (74.33%). In DSS-induced colitis mice, the microsphere embedded with probiotics and postbiotics demonstrated superior therapeutic efficacy by downregulating proinflammatory cytokines, repairing the intestinal barrier, and remodelling the gut microbiome. Overall, this microsphere offers a promising strategy to enhance the vitality, colonization, and health benefits of oral probiotics.

## P54. Algal protein-based 3D-printed fish-analogs as a new approach for sustainable seafood

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Rising global demand for animal-products exceeds human-population growth. This unsustainable trend causes harmful ecological effects. Overfishing causes extinction of aquatic animals and a dangerous biodiversity loss harming aquatic ecosystems. Hence, replacing animal-based food, particularly beef and fish, with sustainable alternatives is an urgent vital global mission. Analogs of animal-based products include plant-based, tissueculture-based and fermentation-based products. Fish analogs have mainly been based on plant-protein, fungi, tissue-culture, but to our knowledge, fish analogs made of algae, particularly macroalgae, as the major component and protein-source have not been reported. 3D-food-printing is a fast-developing technology, enabling formation of complex three-dimensional structures with various heterogeneous topologies and tailorable compositions. Herein, we report the co-extraction of proteins and polysaccharides from the red marine-macroalgae Gracilaria cornea, and using the extract in injection-based 3Dprinting to form prototypes of salmon-fillet. Two bioinks were used: a red bioink dyed with microalgal-astaxanthin, for the muscle tissue, and a white bioink dyed with CaCO3, for the 'connective' tissue. Algal proteins have excellent nutritional amino-acid composition, and the co-extraction with agar facilitates 3D-printing thanks to its pseudoplastic and gelling properties. This study highlights macroalgae as an exciting natural raw-material for fish analogs towards sustainable seafood production, thereby decreasing harm to ocean fisheries.

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### P55. Chickpea Aquafaba as a Novel Stabiliser for Chili Oleoresin Encapsulation: A Study on Optimisation, Rheological Behaviour and Microstructure

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Aquafaba, a byproduct generated from the cooking or canning of raw chickpeas, is often undervalued as a food processing waste stream. In previous work, we have addressed the formation of these nanoparticles during cooking and shown their potential technofunctionality as encapsulation or emulsification agents for bioactive compounds, such as capsaicin, the primary alkaloid in chili peppers.<sup>1</sup> While our research has demonstrated the potential of aquafaba in facilitating capsaicin encapsulation, the instability of these emulsions during storage underscored the necessity for further optimisation of the formulation<sup>1</sup>. In light of these findings, this study aims to optimise stable chili oleoresinin-water emulsions stabilised with British Kabuli chickpea aquafaba, moreover examining their rheological behaviour and morphological characteristics as colloidal systems. To this end, the optimised formulations of chili oleoresin-in-water emulsions stabilised by aquafaba were investigated using Response Surface Methodology (RSM). The components of emulsion formulations, including aquafaba (%), oil (%, oleoresin: MCT, 1:1 (w/w)) and water (%) were optimised through the mixture design approach of RSM to minimise the instability index of emulsions, as measured by a centrifugation/laser scanning device (LUMiSizer). Based on the fitted model of the variables at play, the most stable formulations (overall desirability value  $\geq$  0.96) were selected for their microstructural (via CLSM and Cryo-SEM) and rheological properties analysis. Additionally, the particle size and zeta potential of the emulsions were monitored over 21 days. The formulations which showed the highest stability (i.e. lower instability index values) had an aquafaba content in the range of 13.85 to 15.0%, oil content between 1.0 and 6.64%, and water content from 78.36 to 85.15%. These emulsions also showed the capacity to gel after freshly prepared upon quiescent standing at 25 °C, as noted from small-deformation oscillatory rheological evidence, namely a steep increase of the G' over the G' modulus, and gel-like frequency dependence of both moduli after gelation. Also, the complex viscosity ( $\eta^*$ ) significantly increased with aquafaba contents. Upon shearing and breakup of the gel structure and subsequent standing in the rheometer, the emulsions gelled again. This reversible behaviour was diagnostic of physical interactions underpinning the structure of the gel network. Cryo-SEM images revealed the presence of raspberry-like surface topology at the surface of the oil droplets, consistent with our hypothesis of Pickering emulsion stabilisation by aquafaba nanoparticles. Our results provide compelling evidence that aquafaba has the capacity to be used as a wall material for capsaicin and chili oleoresins encapsulation, leading to gelled emulsions. Ongoing studies are seeking to address the bioactivity, bioavailability, cytotoxicity and sensory properties of these systems, and to the full realisation of these systems in the future formulation of functional foods.

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### P56. Synthesis and Characterization of Bacterial Cellulose–Alginate Composites: Influence of Mannuronic and Guluronic Acid Ratios on Hydrogel Structure.

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Bacterial cellulose (BC), synthesized by *Acetobacter* species, is a versatile polysaccharide with exceptional mechanical strength, thermal stability, high crystallinity, and barrier properties against water vapor, oxygen, and ultraviolet radiation. These features make BC a promising candidate for applications in food, pharmaceutical, and biomedical sectors<sup>1</sup>. To enhance its functionality, this study employed *in-situ* modification by incorporating alginate, a polysaccharide derived from brown seaweed, into the BC culture medium (HS). Alginate, comprising  $\beta$ -D-mannuronic acid (M) and  $\alpha$ -L-guluronic acid (G), exhibits physicochemical properties influenced by its M/G ratio and block distribution, which impact behaviors such as gelation and mechanical performance<sup>2</sup>.

BC-alginate hydrogels were synthesized by integrating alginates with varying M/G ratios during BC biosynthesis. Advanced characterization techniques were used to analyze these composites. Fourier-transform infrared spectroscopy with attenuated total reflectance (ATR-FTIR) identified molecular interactions, small-angle X-ray scattering (SAXS) provided nanoscale structural information, and scanning electron microscopy (SEM) facilitated surface morphology examination. Mechanical and rheological properties were assessed through uniaxial compression tests and small-amplitude oscillatory rheology.

The results demonstrated that the interaction between BC and alginate led to modifications in the structure and properties of the composite, with these changes being dependent on the M/G ratio. Variations in the M/G ratio appeared to influence the mechanical and rheological characteristics of the hydrogels, suggesting a role of the M/G distribution in determining the final material properties.

This research highlights the potential of BC–alginate hydrogels as customizable biomaterials, offering valuable insights into their structural interactions and functional properties.

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# P57. Insights into the ability of microalgal proteins to promote the survivability of *Lacticaseibacillus rhamnosus* GG during processing, storage and *in vitro* digestion

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Structurally engineered xero-carriers (anhydrobiotics) are widely employed in the development of probiotic supplements<sup>1</sup>. To fulfill their basic function, these carriers must maintain the biological activity of living cells when exposed to common physicochemical stressors such as highly acidic conditions, elevated temperatures, varying ionic strengths, exposure to water vapor, oxygen, and bile salts, etc<sup>2</sup>. Milk proteins are considered as the golden standard for protecting the biological functions of various probiotic bacteria, including Lactobacilli and Bifidobacteria, but face limitations due to dietary restrictions, allergies, cultural factors, and environmental concerns<sup>3</sup>. Microalgal proteins, such as those extracted from *arthrospira platensis* (spirulina) and *chlorella vulgaris*, offer a promising substitute due to their high protein content (60-70%), abundance of bioactive compounds (e.g., phycocyanins, carotenoids, polyphenols, and chlorophylls), high biological value, and environmentally sustainable nature<sup>4</sup>.

In the present work the impact of protein isolate obtained from spirulina and chlorella on the survival rate of *Lacticaseibacillus rhamnosus* GG (LGG) during lyophilization, storage and *in vitro* digestion was investigated. Different approaches in structuring the powder precursors i.e., solution or hydrogel prepared via indirect acidification, were assessed. The microstructural, physicochemical and thermal properties of the powders were determined. Accelerated storage trials at different temperatures (T = 4, 20, 37 °C) and water activities ( $a_w = 0.11$  and 0.54/0.75) were conducted for modelling the LGG cell inactivation kinetics. Moreover, the biological activity of LGG as associated to the colloidal changes of the delivery systems were analysed under simulated in-vitro digestion conditions (INFOGEST 2.0). Matrix disintegration, peptic cleavage during *in vitro* gastrointestinal transit were analysed using confocal laser scanning microscopy, SDS-PAGE combined with the OPA assay, respectively. Finally, the adhesion properties of LGG to a mucus-secreting co-culture intestinal cell line model (Caco-2/HT-29) were evaluated.

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# P58. Oat Protein Concentrate Produced via Dry Fractionation as a Potential Food Ingredient

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The global food production system faces challenges related to resource inefficiency and environmental impact, necessitating more sustainable approaches. Plant-based proteins are increasingly recognized as viable alternatives to animal proteins due to their reduced environmental footprint. However, conventional wet extraction methods for plant proteins are resource-intensive and can degrade the structural and functional integrity of proteins, limiting their nutritional and application potential. In contrast, dry fractionation methods, such as the combination of milling and air classification, offer a more sustainable alternative by eliminating water use and reducing energy consumption while preserving proteins in their native state<sup>1</sup>.

Oats, primarily valued for their beta-glucan and starch content, possess a protein fraction with a superior digestibility score among cereals (DIASS of 77%). Despite this, oat protein remains underutilized due to techno-functional challenges such as limited solubility, and poor gelling and foaming capacity<sup>2</sup>. These properties could vary if the proteins remain in their native state through dry extraction methods. However, the similarity in particle size between oat starch and protein presents an additional challenge to achieving efficient separation using dry fractionation techniques.

Our work aims to refine the dry fractionation process to efficiently produce oat protein concentrates with fewer steps, improving feasibility and scalability. By analyzing the composition and key techno-functional properties of the resulting fractions, this work provides valuable insights into their potential applications in food formulations. This research contributes to the growing body of knowledge on sustainable food processing and highlights the potential of oats as a dual-purpose crop for starch and protein-based ingredients. By optimizing dry fractionation processes, this work addresses key challenges in sustainable food ingredient development while supporting the shift towards a resource-efficient food system.

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# P59. WavePure®, a label-friendly seaweed powder for food, feed, and cosmetic applications: Challenges and Opportunities.

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The negative consumer perception of highly transformed additives and ingredients push the whole industry to rethink its traditional approach and to pivot towards the development of low-processed additives and ingredients<sup>1</sup>. In parallel with this trend, a better valorisation of the marine biomass seems to be essential to overcome the future challenges of the humanity regarding the demography and the preservation of arable land for agriculture.

In this context Cargill launched WavePure®, a brand of label-friendly seaweed powders which are produced without chemical modification from *Gracilaria, Kappaphycus,* and/or *Eucheuma Denticulatum*. Developing such ingredients combining consumers' expectations, customers' requests and regulatory constraints opens promising opportunities but required also to overcome several challenges such as color, taste, etc.

Initially the seaweed powders that Cargill developed and for which it was awarded with a patent<sup>2</sup> via WavePure® brand were focused in dairy and dairy alternative categories due to the great body and consistent mouthfeel that they bring. However, the seaweed powders can be also interesting in other categories than dairy: beverage, glazing, water-jelly (with and without sugar), etc. Today, WavePure® brand is growing offering great texture that we can transpose in multiple categories in food, feed, and cosmetic applications.

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### **P60.** Influence of pH on freeze structured plant proteins

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Meat analogues have a poorer texture and mouthfeel than animal meat, in particular whole cuts. This prevents consumers from switching to a more sustainable, healthier, plant-based diet. The unsatisfactory texture properties of plant-based meats can be attributed to a fibrous texture that does not resemble the hierarchical fibrous texture of the animal muscle. Structure is linked to water holding capacity and succulency, and both product attributes are typically compromised in the case of plant-based meat analogues. While extrusion is the commercial technology of choice, it does not create the desirable, hierarchical, fibrous structures.

In this research freeze-structuring is investigated as an alternative approach. Although this technology is not novel, it needs to be further developed to outweigh, ideally overcome, the challenges of scale-up. In brief, elongated or needle-like ice crystals are directionally grown through a protein slurry and parallel structures form between the ice crystals. Melted structures are then held in place through gelation and intermolecular bond formation of the hydrocolloids present.

To meet nutritional targets of meat analogues, the use of polysaccharides should be minimised and protein maximised. Polysaccharides are relied upon for their contributions to strength and texture. To minimise polysaccharide use, their functionality should be optimised; plant protein contributions to texture should be exploited to offset the need for them. For texture enhancement, plant proteins must be able to make intermolecular bonds (which does not occur in their native state). Freeze structuring requires free water in the slurry for ice crystals to grow. To balance maximising protein content and water availability, pretreatments should improve protein solubility.

To assess the hypothesis that the denaturation state of the protein relates to the final texture, mixtures of plant protein and sodium alginate were non- and uni-directionally frozen followed by texture analysis. Non-pH adjusted protein, with all other conditions unchanged, resulted in a disordered not self-supporting final composite material. Whereas alkaline treatment led to a highly structured and firm composite. Non-directionally frozen samples had a random and disordered structure whereas uni-directionally frozen samples were aligned and anisotropic. The impact of freezing on texture will be explored. Relationships between the choice and effects of protein pre-treatment, sample composition, and processing protocol will be discussed, as well as impacts on final material properties.

# P61. The effect of polyvinyl alcohol (PVA) on swelling behaviour of chitosan (CS) aerogel

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CS aerogels with their low density, high surface area, and high porosity, are suitable for food applications e.g. delivering nutrients to the gastrointestinal tract (GIT). To ensure their structural integrity and controlled nutrients release in the GIT, the swelling behaviour and structural changes during digestion must be understood. This study investigated the effect of polyvinyl alcohol (PVA) concentrations (0-5 wt.%) on the functionalities of CS aerogels as nutrient carriers. CS aerogels were characterized by shrinkage ratio after freeze-drying, mechanical properties, microstructure, and swelling ratio in water, HCI solutions (pH 1.5–4.5), and simulated digestive fluids (with or without pepsin, pancreatin, and bile salts). SEM images showed large pores and dense structures with 5 wt.% of PVA, caused by the coexistence of enriched and depleted precursor phases before gelation. This resulted in stress concentration during freeze-drying, leading to pores collapse and increased shrinkage (22.49%-65.99%). Bulk density increased from 0.09 to 0.24 g/cm<sup>3</sup>, and Young's modulus significantly increased from 1.94 MPa to 10.5 MPa when adding 5 wt.% of PVA. Significant swelling of CS-PVA aerogels occurs within 1 hour in water, PVA addition decreased the swelling ratio of CS aerogels from 600% to 136% after 1 hour of soaking. At pH 1.5, pure CS and CS-PVA (0.5 wt.%) aerogels dissolved within 1 and 2 hours respectively, while higher PVA concentrations prevented dissolution. At pH  $\geq$ 2.5, the swelling behaviour of aerogels was similar to that in water. During in-vitro digestion, metal cations were expected to form coordinate complexes with active unprotonated amino and hydroxyl groups of CS-PVA aerogels to strengthen crosslinking and decrease liquid uptake. Pepsin, pancreatin, and bile salts reduced the surface tension of the liquid media from 69.74 mN/m to 31.83 mN/m, affecting digestive fluids absorption. In conclusion, PVA improved the structural stability of CS-based aerogel in water and acidic solutions which is important for their role as a nutrient carrier. Future research will focus on in-vitro digestion of CS aerogels under more realistic conditions.
# P62. Intrinsic viscosity of exopolysaccharides: determination methods and functionality estimation

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The intrinsic viscosity  $[\eta]$  is commonly used to describe the behaviour of hydrocolloids in aqueous solution and reflects interactions of single molecules with the respective solvent at infinite dilution. Therefore, [ŋ] characterises the ability of a hydrocolloid to enhance viscosity, and, especially for microbial exopolysaccharides, it is known to serve as an estimation for their functionality in fermented food systems (e.g., contribution to stiffness in milk gels<sup>1</sup>). Intrinsic viscosity is affected by properties of the hydrocolloid (e.g., molecular mass, conformation, chemical structure, charge), the solvent (e.g., pH, ionic strength), and the environment (e.g., temperature). To determine  $[\eta]$  of hydrocolloids – solvent pairs, viscosities of a hydrocolloid concentration series are measured with capillary viscometers or rheometers and the reduced specific viscosity extrapolated to a concentration of zero. On the other hand, it is possible to determine relative viscosity directly from one single measurement with differential viscometers<sup>2</sup>. The aim of this study was to evaluate three different methods of  $[\eta]$  determination, using (I) concentration series and single point estimations from a rolling ball viscometer, (II) concentration-dependent flow curves from a rheometer and (III) size exclusion chromatography with coupled refractive index and viscosity detectors (SEC-RI/IV). Different commercial and fermentatively produced homo- and hetero-exopolysaccharides from lactic acid bacteria served as hydrocolloids for this study.

To allow correct calculations from SEC-RI/IV measurements, the purity and the refractive index increment dn/dc of all samples were determined. For commercial homo-exopolysaccharides, e.g. dextran (molecular mass approx.  $10^5$  Da), all three determination methods showed a good agreement of [ $\eta$ ] (0.040 – 0.049 mL/mg). With increasing molecular mass (>  $10^6$  Da) and higher (intrinsic) viscosity, a broader variation occurred (dextran from *Weissella confusa* DSM20194: 0.059 – 0.156 mL/mg; hetero-exopolysaccharide from *Streptococcus thermophilus* DGCC7919: 0.294 – 0.343 mL/mg), presumably as a result of shear tinning effects in the capillary of the rolling ball viscometer.

In ongoing experiments, selected exopolysaccharides are analysed for  $[\eta]$  before and after enzymatic modification of the molecular structure (e.g., degree of branching, position of branching). Our findings contribute to the establishment of structure – functionality relationships for exopolysaccharides and allow a targeted synthesis of polymers with a defined functionality.

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### P63. Influence of annealing and crosslinking of PVA films on gallic acid release kinetics and antioxidant properties.

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Polyvinyl alcohol (PVA) coatings are a sustainable and eco-friendly solution that offers excellent oxygen barrier properties<sup>1</sup> and is safe for food contact packaging materials and can be formulated to actively protect food<sup>2</sup>. However, improvements are required due to their high sensitivity to water and poor mechanical properties. Crosslinking with citric acid, a natural chemical crosslinking agent, combined with annealing, can address these limitations<sup>3</sup>. This study aimed to investigate the effects of crosslinking and annealing on the antioxidant properties and release behavior of gallic acid. Films were composed of 78.4% (w/w) PVA, 16.6% citric acid (cross-linker) and 5% gallic acid (antioxidant) and obtained from film-forming solution cast and dried for 24h at room temperature. Films were then annealed in oven at 100°C for 30 minutes. Kinetics of release of gallic acid were conducted in food simulant according to UE/10/2011 normalized test <sup>4</sup>. Samples were periodically assayed and analyzed by UV-VIS spectrometry. From the kinetics and the analytical solution of the 2nd Fick's law proposed by Crank. (1975)<sup>5</sup>, the apparent diffusion coefficient of this antioxidant was calculated. The Radical Scavenging Activity (RSA) of films was measured using the DPPH• test <sup>6</sup>.

Results indicate a competitive interaction between the crosslinker and the antioxidant. Notably, the timing of antioxidant addition after introducing the crosslinker significantly influenced the properties and release rate. The findings demonstrate that incorporating a crosslinker and applying annealing greatly enhance the release rate of gallic acid. The interactions involved between GA and biopolymer chains are able to control the release mechanism and thus it should be considered for an optimized antioxidant activity. The diffusion coefficient (D) of GA was calculated from the release kinetics and was higher when films were annealing or incorporated citric acid. The structural analysis by FTIR confirms the assumptions of intermolecular interaction between PVA, citric acid and GA when films was annealing and could explain the results of the release properties.

In conclusion, this study provides valuable insights into optimizing the antioxidant properties of PVA coatings for potential food packaging applications.

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### P64. Understanding pea protein-polysaccharide interactions under acidic conditions

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Molecular interactions between polysaccharides and proteins are critical to understanding technological functionalities, as for example, colloidal suspension stability in acidic beverages. Quantifying such interactions is a challenge, and it requires a holistic approach, linking the molecular properties with studies under relevant conditions. Recently, water-soluble polysaccharides extracted from legume side streams have shown potential to have value-added as functional ingredients, suggesting their utilization as stabilizers in acidic beverages<sup>1,2</sup>. However, the mechanism by which these hydrocolloids interact and stabilize plant proteins in acidic medium needs to be fully understood. The objective of this work was to carry out a detailed investigation of pea protein-polysaccharide interactions. The work was conducted using both industrial and lab-extracted pea protein isolates (PPI and lab-PPI), and different globular protein fractions, namely albumins, legumins and vicilins. A comparison between high methoxyl pectin (HMP) and water-soluble pea polysaccharide (PPS) was also performed due to the widespread use of HMP in acid protein beverages.

Thermodynamic parameters were derived using isothermal titration calorimetry (ITC) at selected pH values. Protein-polysaccharide mixtures were then evaluated at different mixing ratios in a pH range from 1.5 to 8, and their turbidity changes were measured by a spectrometer at 600 nm. In the case of industrial PPI, homogenization was critical for interactions to occur with HMP or PPS, supporting our published results on complex formation with PPI and soluble pea polysaccharide<sup>3</sup>. Lab-PPI, on the other hand, interacted with polysaccharides and formed soluble complexes near the protein isoelectric point, without the aid of homogenization. Specific interactions occurred in the 3.5-5.0 pH range for albumins, legumins, and vicilins with both PPS and HMP. Cryo-electron microscopy confirmed the formation of protein-polysaccharide complexes.

In conclusion, this study demonstrates the importance of a better understanding of the supramolecular structure of PPI and its impact of processing history and composition on the formation of soluble complexes at acidic pH. A better understanding of how each globular protein fraction, with its distinct molecular structure, interacts with polysaccharides is critical for the successful development of stable, appealing, and more sustainable acidic drinks, and for the optimal utilization of a side stream from legume production.

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#### P65. Water dispersible edible films based on cellulose microfibrils

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Edible films are constructed of cellulose microfibrils (CMFs) and xanthan gum (XG). To control the aggregation of CMFs when dried, water soluble polysaccharides are added. Water insoluble dietary fibre comprising cellulose, hemicellulose and pectin was used as a source of CMFs. The dispersions with different ratios between of XG and CMF are obtained by homogenisation. The dispersions were made into films by solvent casting and evaporation under vacuum. The resulting films' thickness ranged from 22 to 32 micrometre and had a density ranging from 1.1 to 1.6 g/mL. The film thickness increased with increasing CMF/XG ratio, while the density inversely scaled with this ratio. Uni-axial mechanical testing was performed to obtain tensile strength (TS), elongation at break (EAB) and Young's modulus (E). Films with higher CMF/XG ratios had higher TS and E, but lower EAB than films with a low CMF/XG ratio. Glycerol was found to be an effective plasticizer, reducing TS and E, but increasing the EAB of all films. Dispersibility of the films was obtained by measuring the viscosity in water. Films became less dispersible when the CMF/XG ratio increased and at low CMF/XG ratios the rheology of the dispersions was fully recoverable to the one of the initial dispersions. Our result show that these films could be used as a delivery system for beneficial ingredients in food products.

### P66. Functional and physicochemical properties of chia seed mucilage extracted using an innovative extraction method

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seed mucilage (CSM) novel, eco-green, sustainable Chia is а and edible hydrocolloid derived from chia, offering a promising alternative to commercial hydrocolloids. This study explores the application of centrifugal force and drying methods to compare the techno-functionalities of CSM obtained through a novel, faster, and scalable extraction approach as opposed to traditional methods. Liquid mucilage extracted using a modified cream separator is subjected to freeze-drying (CSMF) and oven-drying (CSMO). The efficiency of this innovative extraction method is compared to the traditional dry fractionation approach, known for its complexity and time consumption. Remarkably, the modified unit significantly (p < 0.05) reduces mucilage separation time (4.06 ± 1.28 min), opening doors for future commercialization. CSMF obtained through cream separator and freeze-drying, exhibits superior colour properties. Additionally, it demonstrates superior performance compared to the traditional approach in terms of functional properties such as water-holding capacity (WHC), solubility, and particle size. Furthermore, the advantages of the faster extraction approach are reinforced through charge analysis, Fourier Transform Infrared spectra (FTIR), thermal transitions, and rheological properties. The microstructure analysis of CSM highlights the influence of drying techniques on its morphology. This study not only introduces a novel method for mucilage extraction but also validates the findings through comparison with existing literature on CSM.

## P67. Comparison of the impact of various natural cross-linkers for gelatin coatings on their functional properties.

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PLA (polylactic acid) is a biomaterial for packaging applications derived from renewable resources, mainly corn, manioc and sugarcane starches. It is increasingly used in packaging for its environmental benefits. It's a renewable biopolymer, biodegradable, safe, transparent, but also, and unfortunately, rather expensive, sensitive to moisture and heat, compostable only in industrial units, difficult to recycle and with poor barrier properties <sup>1</sup>. Applying thin layers to PLA-based films or packaging reduces gas transfer, especially oxygen, increases mechanical strength and reduces noise in use, enhances compostability, and reduces additive migration <sup>2</sup>. Gelatin, the 3rd biopolymer of natural origin available, inexpensive, easily modifiable while remaining compatible with use in food contact, easily derived from waste from the seafood industry, is being considered for PLA film coating by many authors <sup>3,4</sup>. Indeed, oxygen permeability of PLA films could be reduced up to 600 times <sup>5</sup>, and allowed also to provide bioactive and or responsive properties to packaging materials <sup>6</sup>.

This work aims to optimize the formulation of gelatin-based coatings by comparing the efficacy of various natural chemical agents (di-acids, phenolic compounds, etc.) capable of cross-linking gelatin without heat treatment. Several application techniques (spray coating, meter-bar coating, casting) are also considered. The aim is to reduce the solubility of the coating in aqueous media and, above all, to reduce gas permeability. Understanding the relationships between formulation- and process-induced structural modifications (measured by UV, FTIR, SEM, etc.) and the barrier and water affinity properties of coated PLA films is crucial, and is therefore being investigated. The modifications of molecular structure had non predictable behavior on the functional properties of the coated films.

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#### P68. Formation and characterisation of pectin microgels

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A method for fabricating pectin microgels has been developed to efficiently produce microgels with consistent physical properties, including density, particle size, and uniform morphology. Low methoxy and amidated pectins have been used with calcium cations as the cross-linker at three concentrations (40, 80, and 200 mM) and two different pH values (2.0 and 7.0). The microgels have been characterised for their physical characteristics (yield, density, particle size, particle morphology and surface properties) and arrangement of pectin chains at the nanoscale via transmission electron microscopy (TEM) and smallangle X-ray scattering (SAXS). The fabrication protocol yielded microgels with consistent properties from batch to batch for each pectin type and pH. Microgels prepared at 80 mM and 200 mM calcium showed higher density, smaller particle size, and more uniform morphology than those prepared at 40 mM due to the faster gelation kinetics and higher crosslink density. Contrary to what is frequently reported in the literature, pectin microgels are not interfacially active. TEM revealed a more compact network microstructure at higher calcium concentrations as it enhances junction zone formation. The amidated pectin showed aggregated clusters because the amide group disrupts junction zone linearity. SAXS quantified the structural changes and the impact of crosslinking on modulating microgels' microstructure. In conclusion, this spray gelation method generated pectin microgels with consistent and tunable properties that can be used in a broad range of food, pharmaceutical and biomedical applications.

# P69. Development of plant-based multilayer antimicrobial films for sustainable food packaging

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People are consuming more fresh fruits and vegetables than ever before. However, the susceptibility of fresh produce to microbial contamination presents great challenges to transport and storage. It can be helped by antimicrobial packaging enriched with essential oils, whereas releasing essential oils results in a loss of antimicrobial activity. To program their release behavior, multilayer films were fabricated by alternatively depositing soy protein isolates (SPI) and high methoxy pectin (HMP) on a substrate, using spray coating, and nano-emulsion droplets containing the active component were incorporated in the primary layer. Carvacrol, one of the main components in essential oils from certain herbs, was chosen to model the release behavior in such a system. The surface morphology and 3D structure were imaged via atomic force microscopy (AFM), multiphoton microscopy, and scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS). The release profile was characterized by gas chromatography-mass spectrometry (GC-MS). The transparent films were fabricated using spray coating with the successful formation of the layer-by-layer structure. The structure could delay the release by varying the number of add-on layers while maintaining the preservation efficacy for strawberries at room temperature. This approach could provide a promising alternative to control the release of essential oils in active packaging, thereby protecting fresh produce from microbial damage in an environmentally friendly way.

# **P70.** Physicochemical and Rheological Characteristics of Hybrid Carrageenans Derived from *Betaphycus gelatinus*

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A hybrid sulfated galactan, composed of  $\kappa$ - and  $\beta$ -carrageenans, was isolated from Betaphycus gelatinus (wild and cultivated) through successive cold (25 °C) and hot (95 °C and 110 °C) alkaline extractions, followed by alkali treatment of the extracted polymer to convert any remaining precursor molecules into the primary carrageenan. Both wild and cultivated Betaphycus galactans (native and alkali-treated) were characterized using highperformance size-exclusion chromatography (HP-SEC), high-performance ion-exclusion chromatography (HPICE), high-performance anion-exchange chromatography (HPAEC), FTIR, and NMR spectroscopy to determine molecular weight and structural properties. No significant differences were observed in molecular weight, monosaccharide composition, sulfate content, or structural makeup between the wild and cultivated samples. However, alkali-treated samples showed a reduction in sulfate content compared to their native counterparts. NMR spectra confirmed that during alkali treatment,  $\gamma$ -carrageenan was converted into  $\beta$ -carrageenan through desulfation, explaining the reduction in sulfate groups. Further enzymatic depolymerization was performed using  $\kappa$ -carrageenase 16A from Zobellia galactanivorans to investigate the distribution of  $\kappa$ - and  $\beta$ -carrabiose moieties. Enzyme-resistant and enzyme-sensitive fractions were isolated and characterized by NMR. The enzyme-resistant fraction was rich in  $\beta$ -carrageenans, while the enzymesensitive fraction contained abundant oligo  $\kappa$ - or  $\kappa$ - $\beta$ -carrageenans, suggesting a blockwise distribution of  $\kappa$ - and  $\beta$ -carrageenans in the *Betaphycus gelatinus* polymer. The effect of alkali treatment and the influence of counter ions like K<sup>+</sup>, Ca<sup>2+</sup>, and Ba<sup>2+</sup> on the thermorheological properties of this hybrid galactan gel were studied using dynamic rheometry. Alkali treatment significantly enhanced the viscoelastic properties compared to the native polymer. K+ ions had a specific effect on the gel formation process of both native and alkali-treated hybrid polymers. This study marked the first observation of the strong gelforming ability of *Betaphycus* polymers, particularly in the presence of  $K^+$  ions.

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### P71. Extraction of Cellulose Nanocrystals from Various Bamboo Species for Advancing Packaging Material in Cassava Starch-Based Films

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Cellulose nanocrystals (CNCs) were successfully extracted from various bamboo species, including Thyrsostachys siamesi Gamble, Dendrocalamus sericeus Munro (DSM), Bambusa logispatha, and Bambusa sp., using a chemical-mechanical method. The process began with the pre-treatment of bamboo fibers to remove lignin and hemicellulose, isolating cellulose. Subsequently, the cellulose was hydrolyzed with sulfuric acid under ultrasonication to produce CNCs. Scanning Electron Microscope (SEM) analysis revealed CNCs with diameters (cross-section) ranging from 11 to 375 nm. Among the various bamboo species studied, CNCs derived from DSM exhibited the highest yield of extraction and crystallinity, making DSM the chosen reinforcing material for film fabrication. Plasticized cassava starch-based films were prepared by incorporating varying amounts of DSM-derived CNCs (0%, 4%, 8%, and 12%) and subsequently characterized. The addition of CNCs notably influenced the films' properties. Increased CNC content resulted in reduced water solubility and decreased water vapor permeability (WVP). As CNC content increased from 0% to 12%, water solubility dropped from 53.2% to 8.7%, and WVP decreased from 5.2 to 2.1 g·mm/h·m<sup>2</sup>·kPa. Atomic force microscopy (AFM) investigation revealed a uniform dispersion of CNC particles from the film surface analysis at CNC contents of 4% and 8% However, the highest CNCs content at 12% resulted in more CNCs agglomeration in cassava starch-based film. Mechanical testing revealed that films with 8% CNCs had the highest tensile strength of 4.2 MPa, compared to 1.9 MPa in unreinforced films. Based on the results of this experiment, cassava starch reinforced with CNCs from bamboo film may offer potential as a future packaging material.

Keywords: bamboo; biopolymer; cassava starch; cellulose nanocrystals; composite film; ultrasonication

# P72. Thicker than water: exploring the diverse relatives of *Plantago ovata* to address the narrow hydrocolloid functionality of psyllium in food and the gut

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Psyllium husk (PH), a heteroxylan (HX)-rich material milled from the surface of *Plantago ovata* seeds, is known for its high viscosity and gelling capacity making it a widespread fibre supplement and hydrocolloid in foods. However, PH applications face challenges, including hydration adjustment difficulties and sensory issues like gumminess. These arise from HX's unique rheological and water-holding behaviours, which were previously poorly understood.

Over the past decade, we have employed various plant science strategies to uncover the drivers of PH quality, making significant advances in its development and genetics<sup>1</sup>. However, we have also demonstrated that *P. ovata* has extremely limited genetic variation, resulting in a narrow range of HX properties for tailored applications. Through fractionation and advanced rheological techniques, *P. ovata* HX has been shown to form 'physical gels', with substitutions playing a critical role in network formation and rheological behaviour<sup>2</sup>. With this considered, we have explored dozens of alternative Plantago species and discovered that HX is shared as the most abundant polymer, but importantly their substitution levels, and subsequent rheological and water absorption behaviours, vary widely. Adding whole seed flour from several diverse *Plantago* species to gluten-free bread showed that the texture- and dough rheology-enhancing properties partly independent of HX content and chemistry and were more strongly associated with the proportion of more gel-like (greater G' and lower tan  $\delta$ ) HX fractions<sup>3</sup>. One species that we have named Australian Psyllium (AP) showed particularly potent hydrocolloid behaviour (even compared to PH) so we have employed fractionation and binary starch-HX gel fabrication to probe this further, showing that AP HX and AP HX-starch gels had extremely high resilience under dynamic oscillatory deformation, with flow points and flow transition indices double that of P. ovata HX and HX-starch gels. Applying AP in vivo, these advantages provide more potent dietary fibre functionality, reducing cholesterol levels more than P. ovata in mice fed an obesogenic diet.

Exploring the diverse relatives of *P. ovata*, we show that this is a richer resource for targeted hydrocolloid functionality. Future work will compare AP and *P. ovata* HX chemistry and rheology more deeply to uncover the underlying mechanisms, as well as exploring broader hydrocolloid applications.

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# Flash Flash presentations

## Flash 1. Modeling of extrusion process to reduce allergenicity in snack with greek nuts

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According to World Allergy Organization (WAO) data, almost 250 million records of people globally, are affected by food allergies, especially Infants (5–8%). Up to date literature has highlighted the application of common processing technologies in allergenicity reduction, including thermal, enzymatic, and fermentation treatments<sup>1</sup>, which are not cost-effective, and can potentially degrade final product quality <sup>2</sup>.

In this context, processing technologies can be applied, that induce both thermal and structure modifications in a food matrix, providing a more sustainable solution in food production. Despite food processing, the type of food matrix involved, in terms of structure and allergenicity are also key factors in potential reduction of food allergens.

The extrusion influences allergenicity by modifying protein structures—a high-shear, highpressure, and heat-intensive process—inducing molecular transformations and crosslinking in proteins and polysaccharides, which can impact allergenic potential.

The **purpose of this study was** to evaluate the effect of extrusion on reducing allergenic properties in Greek pistachios and walnuts varieties, by examining changes in protein secondary structures. Three main functions of the extrusion were used (thermal, torque, mixing) for process optimization. The application of different temperatures 100, 120 and 140 °C, various shear stresses 60, 70 and 80 rpm, and moisture content 30%, 35% and 40% were applied to evaluate the expansion ratio, the color and the bulk density and specific mechanic energy of the extrudates. The JMP program was used to minimize the trials and express the best mathematical equitation for the optimization of the quality characteristics of extrudates.

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# Flash 2. Tuning functional properties by starch by a combination of enzymatic treatment and infrared processing

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It is now well acknowledged that reducing the swelling of starch may increase the strength of the starch-based gel whilst limiting the accessibility of digestive enzymes to the starch chains, resulting in a reduction in starch digestibility and therefore reduced calorific gains. Often heat processing is an approach used to achieve such reduction in swelling behaviour of starch. This study investigates for the first time the effect of infrared (IR) thermal treatment (220 °C/3 min/ 30 % moisture) and combination of enzymatic processing (5 h papain+ 3/5/7 h pullulanase) with IR on structural, physicochemical, and functional characteristics of sorghum corneous endosperm starch. The native sample exhibited high swelling power (SP) and solubility index (SI), however lacked the structural stability. IR treatment alone moderately reduced SP by 10.74%, indicating limited starch granule modification. Among the dual enzyme and IR treatments, 5IR (5h pullulanase treatment + IR) showed the most pronounced reduction in SP (20.58%) and SI (47.85%), signifying superior granule integrity due to enzymatic debranching and thermal restructuring. Microscopic analysis revealed the native sample had polygonal granules with smooth surfaces, while IR treatment resulted in central depressions, indicating partial restructuring. Dual treatments, 3IR (3h pullulanase + IR) and 7IR (7h pullulanase + IR), altered granule shape with surface pitting and size reduction. In contrast, 5IR granules exhibited a smooth, compact, and spherical morphology, demonstrating resistance to enzymatic hydrolysis and improved rigidity. Fourier Transform Infrared Spectroscopy (FTIR) spectra highlighted structural superiority of 5IR, with the highest 1047/1022 ratio and low 1022/995 ratio indicating increase in crystalline region and double helices proportion. X-ray diffraction confirmed maximum crystallinity index (62.66%) in 5IR, significantly higher than native (41.24%), IR (53.40%), 3IR (51.03%), and 7IR (44.27%). This increase was attributed to enhanced amylose-amylopectin interactions, chain rearrangements, and IR-induced retrogradation. Pasting parameters, including viscosity, were significantly reduced in 5IR, indicating superior stability compared to other samples. In summary, the novel findings illustrate that a unique combination of enzymatic processing with infrared thermal treatment is a promising processing approach to produce starch with reduced swelling ability, enhanced structural stability, and higher crystallinity. The dually modified starch might make an ideal hydrocolloid for applications requiring resistance against mechanical and thermal degradation with potential applications in a range of starch-based food and beverage formulation.

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### Flash 3. Impact of Heat Treatment on the Molecular Interactions of Pea Protein Fractions

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Plant-based proteins are increasingly being used as alternatives to animal proteins in innovative food products. They often contribute to food structure through protein gelation, which involves the dissociation, unfolding and aggregation of individual protein fractions. Pea protein is usually obtained by precipitation or filtration, which yields a mixture of different globulins, but loses smaller protein fractions like albumins. Currently, most research focusses on pea globulin mixtures, overlooking the structural differences of the individual protein fractions. Aggregation of pea protein is usually facilitated by heat treatment, which exposes functional groups and promotes attractive interactions leading to protein association. This process is strongly dependent on the structural characteristics and molecular properties of the proteins. Therefore, understanding the molecular interactions of untreated and heat-treated individual fractions, as well as their mixtures, is essential for a comprehensive insight into the aggregation and gelation behavior of pea protein mixtures.

To elucidate these relationships, the two globulin fractions vicilin and legumin and an albumin fraction were extracted from a low processed pea flour. Individual protein fractions were either analysed untreated or subjected to a heat treatment at 90 °C prior to analysis or mixing. Molecular weight distributions of untreated/heat-treated protein fractions were analysed by SDS-PAGE. Molecular properties were characterised before and after heat treatment by zeta potential and surface hydrophobicity measurements at pH 7. Determination of intrinsic viscosities of individual protein fractions and their mixtures (untreated/heat-treated) allowed the analysis of solvent/protein and protein/protein interactions by applying Philippoffs rule of additivity<sup>1</sup> and calculation of the Huggins coefficient.

Mixtures of untreated legumin/vicilin and untreated legumin/albumin showed no attractive interactions, likely due to their net negative charge. On the other hand, mixtures of untreated vicilin/albumin showed strong attractive interactions, despite their net negative charge, as has been indicated by a deviation from Philippoffs rule and a high Huggins coefficient. This behaviour is most likely driven by electrostatic interactions between partial charges of vicilin and small albumins. In general, heat treatment caused an increase in surface hydrophobicity and promoted the formation of high molecular weight aggregates as indicated by SDS-PAGE and increased intrinsic viscosity. Legumin und vicilin formed soluble aggregates while albumin aggregates precipitated. Mixtures of individually heat-treated protein fractions showed no pronounced attractive interactions, despite the observed alterations in their molecular properties.

In summary, the interactions between untreated protein fractions are primarly governed by electrostatic interactions. Heat treatment shifts the balance toward attractive hydrophobic interactions; however, electrostatic repulsion remains the dominant factor. These results highlight the complexity of molecular interactions involved in pea protein aggregation and contribute to a more comprehensive understanding of the underlying mechanisms.

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# Flash 4. Driving pea and whey protein hydrolysis towards the development of hybrid beverages with improved foamability

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The development of hybrid foods, in which animal proteins are partially substituted with plant proteins, represents a cutting-edge approach to intensify the utilisation of alternative proteins while limiting the use of animal ones. This approach leverages the strengths of both protein sources representing a strategy to improve the functionality of entirely plant-based food systems and to reduce the environmental footprint of those formulated with animal proteins<sup>1</sup>. Furthermore, these binary systems could also improve sensory and nutritional aspects, catering for the dietary preferences of health-conscious and/or flexitarian consumers, who seek to decrease animal protein intake without eliminating it<sup>2</sup>.

The design of hybrid foods is challenging, particularly because the limited technological properties and undesirable sensory attributes of plant proteins hinder their application as hydrocolloids in food products. Targeted modifications are necessary to unlock the full potential of plant proteins and maximise their functionality. Breaking proteins into fragments of different dimensions through enzymatic hydrolysis offers a promising strategy to steer protein functionalities allowing the development of functional and sustainable food products.

The present study aimed to optimise the enzymatic hydrolysis process to obtain hydrolysates of whey proteins (WPH) and pea proteins (PPH) which were then combined to formulate a hybrid beverage with enhanced foaming capacity. To this aim, proteins were hydrolysed by using Alcalase 2.4L at increasing hydrolysis degree. The samples exhibiting optimal foamability were selected to prepare WPH/PPH blends at different ratios (0:100; 25:75; 50:50; 75:25; 100:0). Foaming ability and stability measurements indicated that the 50:50 ratio exhibited the highest foaming properties. Subsequent analyses were conducted to characterise the interfacial and microstructural properties of the foam, aiming to elucidate the interactions between WPH and PPH within the hybrid system. A tensiometer was employed to measure interfacial tension, providing insights into the surface properties of the foam. Additionally, scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) were utilised to examine foam morphology at microstructural levels.

This work highlights the potential of enzymatic hydrolysis as a tool to steer the functional properties of proteins and the successful strategy of blending animal and plant protein sources for a foaming beverage formulation. By analysing the interactions between WPH and PPH, we aimed to deeply understand the combined and synergistic behaviour in product development.

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### Flash 5. Production of sugar reduced ice cream by incorporation of inulin and low viscous carrot soluble dietary fibre

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In Europe, around 3000 million litres of ice cream were produced in 2022, making ice cream one of the most popular confectionary products. However, since ice cream contains 20 to 30 % of sugar, nutritionists recommend decreasing its intake since high sugar consumption is linked to several non-communicable diseases. Consequently, scientific efforts are conducted to develop new healthy ingredients that partially replace sugar while maintaining the consumer acceptance. In this context, soluble dietary fibre (SDF) is a promising ingredient due to its high nutritional value (prebiotic, prevention of cardiovascular disease). To fortify ice cream with dietary fibre, according to the European Regulation 1924/2006, the dietary fibre content should be at least 1.5 g per 100 kcal of ice cream. Nevertheless, at such high concentrations, most SDF (except inulin) are highly viscous, and their incorporation will negatively affect the ice cream's sensorial properties. Hence, a combination of mechanical and enzymatic treatments have been used, to produce low viscous SDF that could be incorporated into ice cream with minimal impact on sensorial properties.

This study aimed to partially replace the sugar content of ice cream with two types of soluble dietary fibre, inulin or low viscous carrot SDF and study the impact on the microstructure, rheological behaviour and sensorial properties. For this purpose, low viscous carrot SDF (mainly pectic hydrolysates) was produced by high-pressure homogenisation followed by enzymatic hydrolysis and a subsequent separation by centrifugation. The viscosity and oil droplet size distribution of the premix (*i.e.* all homogenised ingredients before freezing) and melted ice cream were monitored. The hardened ice cream was characterised by conducting oscillatory thermal rheometry (OTR), the melting behaviour was studied by DSC and sensorial analysis (ISO 8587:2006).

Although inulin and carrot SDF showed a similar viscosity in solution, premix enriched with carrot SDF showed higher viscosity (~1.5-fold) than the inulin-premix and control. The difference might be due to the formation of aggregates driven by ionic bridges or electrostatic interactions between the pectic hydrolysates in carrot SDF and milk proteins. Also, the larger size of the oil droplets observed in the carrot SDF-ice cream may have increased the viscosity. Additionally, carrot SDF-ice cream showed higher values for both the storage and loss modulus in the OTR, indicating increased structural rigidity, i.e. a firmer/more solid texture. Finally, carrot SDF-ice cream had a lower melting temperature indicating that the fibre enrichment led to a more concentrated serum phase.

Despite these differences in microstructure, sensory analysis revealed no significant differences in homogeneity, creaminess, and sweetness characteristics among the tested ice cream samples. Overall, the results of this work demonstrate the potential for incorporating tailor-made soluble dietary fibre into ice cream while maintaining the characteristic product properties and without adversely affecting sensory attributes.

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# Flash 6. Preparation of dietary fibre from soybean hulls and its application as fat replacer in low-fat ice cream

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Dietary fibres can be used as fat replacers in ice cream due to their contributions to viscosity and gel-forming ability. In this study, we investigated the effect of agricultural byproduct soybean hull dietary fiber (SHDF) on the physical, rheological, sensory and physiological properties of low-fat ice cream. The structural and functional properties of SHDF before and after modification were characterized. Then the SHDF was added to the low-fat formulation, and its effect on the rheological, structural elements, and sensory properties of the low-fat ice cream was assessed. Finally, the health effects of long-term consumption of SHDF were evaluated using mouse model. The results showed that compared with crude SHDF, modified SHDF possesses desirable fibrous characteristics, leading to a higher water-holding capacity, oil-holding capacity and swelling capacity, which facilitate its homogeneous dispersion in the aqueous phase. The incorporation of SHDF in low-fat ice cream formulations significantly improved the viscosity and solid-like behavior of the ice cream mix. Furthermore, the presence of SHDF was found to effectively reduce the ice content but enhance the melt resistance of the low-fat ice cream. Long-term feeding of SHDF-enriched low-fat ice cream to mice demonstrated its positive effects on body weight regulation, which is related to the ability of SHDF to promote intestinal peristalsis and modulate the intestinal microbiota. These outcomes suggest that SHDF could be a promising fat alternative in the production of low-fat ice cream, offering a healthier option without compromising on texture and quality.

#### Flash 7. Development of Novel Bigels Fortified with Carrot Pomace

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In this study, a novel biphasic system, termed "bigel", was developed by mixing of two distinct gel networks: a hydrogel (water-based phase) and an oleogel (oil-based phase). These phases are combined to form a cohesive material, wherein one gel phase is typically dispersed within the other. The structure of a bigel allows for unique properties that are unattainable in standalone hydrogels or oleogels. To enhance the properties of bigel, carrot pomace, an important source of the bioactive compounds-including carotenoids, vitamins, minerals, and dietary fibers was incorporated. The hydrogel was prepared by heating plantbased protein concentrate with distilled water and gellan gum to 90°C, while oleogel was formulated by heating vegetable oil, lecithin, and carrot pomace powder to 70°C. The oleogel phase was then added dropwise into the hydrogel phase at a ratio of 40:60 while maintaining a high temperature (70°C) with continuous stirring. After homogenization, the bigel samples were cooled down to room temperature and allowed to solidify in the fridge for 1.5 h. The visual appearance, color properties, texture and rheological behavior, and gel stability (oil-binding capacity) of the bigels were evaluated. The results revealed that variations in the type of oil and the concentration of carrot pomace influenced the final properties of the bigels. This study highlights the potential of bigels as a structured gel system, offering opportunities for the incorporation of functional ingredients for innovative food applications. Additionally, the addition of carrot pomace is a sustainable approach to enhance the properties of prepared samples.

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### Flash 8. Fostering the protein transition by hybrid food structures: the case study of gels made by milk whey and pea proteins

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Nowadays, the use of plant sources is highly demanded to pursue the current protein transition. However, the whole substitution of animal proteins with plant ones in food formulations is often challenging due to the reduced technological and sensory properties of plant-based ingredients. In the attempt to address these challenges, the design of hybrid food structures by mixing animal and protein sources could represent a promising compromise<sup>1,2</sup>. However, this strategy is still in an early stage requiring an in-depth understanding of the supramolecular organisation of mixed protein structures in generating food structures, such as hydrogel gels.

Based on these considerations, this work aimed at studying hybrid gels formed by proteins from milk whey (WPI) and pea (PPC). To this purpose, heat-induced gels at pH 7 were prepared at 18% (w/w) total protein content, selected as the concentration at which both proteins exhibited gelling properties and varying the ratio between animal and plant sources (from 0:100 to 100:0). To in deep understand the structures formed between WPI and PPC, gels were characterised in terms of rheological properties, hardness, water holding capacity (WHC), and confocal microscopy. Molecular forces involved in the gelation process were investigated by rheological measurements in the presence of dissociation reagents.

PPC alone (WPI:PPC 0:100) formed a self-standing weak gel whose network was formed by spheroidal protein-rich domains whilst WPI gel (WPI:PPC 100:0) was characterised by a homogeneous fine structure. The progressive addition of WPI to PPC contributed to the network reinforcement as confirmed by the increase of storage and loss moduli. A net-like WPI network was formed among the globular-like PPC domains when PPC was the predominant protein. The inverse behaviour occurred in WPI dominant gels in which WPI formed the primary structure with PPC globules acting as fillers. Blending WPI and PPC at the same concentration (WPI:PPC 50:50) was a special case. The homogeneous structure was interrupted by WPI-PPC flocs and aggregates intimately connected, resulting in an interpenetrated structure mainly characterised by disulfide bridges and to a lesser extent by hydrogen bonds and hydrophobic interactions. The formation of WPI and PPC network also inhibited syneresis, as confirmed by the rise in WHC values.

The milk whey-pea protein mixtures could be used to prepare hybrid gels with different technological properties by regulating the ratio between WPI and PPC opening new opportunities in the design of protein-rich foods. The insights gained in this study could contribute to the rational design of hybrid gel foods.

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### Flash 9. Towards an understanding of the structuring mechanisms of 3D printed flour-based matrices

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3D food printing has been more and more developed in the world for the past 20 years and is used on a wide variety of foods. However, if it seems relatively easy to print a food matrix, there is still a lack of understanding regarding the effects of its rheological and structural characteristics on its capacity to be extruded, shaped according to a model and to maintain its shape after printing. The objective of this work was to investigate the impact of the composition in starch, fibers and proteins of 6 different flours on the rheological and structural properties of flour-based matrices and their printing quality. Wheat, rye, rice, kidney bean, lupin and chia seed flours were used to formulate matrices with a constant dry matter content, obtained through a mechanical and thermomechanical process. These matrices were characterized through a multiscale approach, using texturometry, rheometry, differential scanning calorimetry (DSC) and microscopy, and the pictures of the printed products were analysed through image analysis. The results show the link between the biochemical composition of the flours, the rheological properties of the matrices and their printing quality. More specifically, there is a significant correlation between the stickiness of the matrices and their printing quality, the latter also being highly correlated to the starch composition of the flours and to their hydration level at room temperature (in relation to their water holding capacity). This highlights the importance of a sufficient hydration of the flours to obtain extrudable and well printed matrices and underlines the fact that their printing quality probably results from a combination of factors. We thus formulated hypotheses about the structuring mechanisms of the matrices studied, and now possess hints to further investigate them.

#### Flash 10. Interaction networks of ulvan-based mixed systems

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In contributing to provide broader applicability of ulvan, our current study evaluated the rheological behaviour in mixed ulvan-carrageenan (UL/KC), ulvan-funoran (UL/FUN) and ulvan-gelatin (UL/GLT) systems. Ulvan as known, is a heterogeneous anionic polysaccharide that generally forms weak soft gels which is mostly enhanced by the addition of boric acid <sup>1,2</sup>. The aim of the current study however, was to analyze the interaction mechanisms of these ulvan-based systems by testing the influence of different mixing ratios, pH conditions and cations for prospective food-related applications.

For this, four different fractions of ulvan isolated from Ulva lactuca under either hot or cold conditions and precipitated using either NaCl or ethanol were used as UL samples. It was observed that, the uronic acid and sulfate composition of ulvan specifically influence the rheological behaviour of ulvan systems. In native ulvan fractions with no added salts, ethanol-precipitated ulvans formed relatively viscous solutions than NaCl-precipitated fractions. Adding 0.12 M CaCl<sub>2</sub> to ulvan however, increased the complex viscosity (n) of NaCl-precipitated fractions which contained higher sulfate content. A similar effect was observed in mixed UL/GLT systems where using porcine gelatin, yielded up to ~1500 mPa.s viscous solutions. The differences in the recorded complex viscosity and storage modulus (G') of UL/GLT samples during the cooling and reheating stages rather suggested levels of aggregation at lower temperatures. As expected of gelatin, the influence of isoelectric point (ISP) was evident in the different tested pH ranges. UL/GLT systems prepared using gelatin at pH < 7 reported values of G' higher than at pH > 7. In UL/KC systems, complex viscosity ranging up to ~3000 mPa.s was recorded. Under these mixing conditions, the viscoelastic behaviour of UL/KC decreased with decreasing volume ratios of KC. Additionally, increasing amounts of KC increased the melting temperature (T<sub>m</sub>) in UL/KC. This simply indicated a dependency of this network on the aggregation of the molecular chains of KC. In UL/FUN systems, the influence of Ba<sup>2+</sup> ions which is known to induce gelation in funoran recorded G' (4°C) between ~522 -618 Pa. Inasmuch as divalent cations like Ca<sup>2+</sup> and Ba<sup>2+</sup> showed levels of rheological influence in ulvan systems by bridging the charged units of the molecular chains, the induced impact in the tested binary systems is dominantly attributed to present co-solutes rather than the non-gelling isolated ulvan.

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# Flash 11. Influence of molecular weight on the anti-adhesion bioactivity of exopolysaccharides from *Leuconostoc mesenteroides* against enterotoxigenic *Escherichia coli*

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Enterotoxigenic Escherichia coli (ETEC) is a bacterial pathogen that can cause diarrhoea in mammals by adhering to the intestinal epithelial surface followed by toxin production. To access the intestinal surface, ETEC needs to bind to the intestinal mucus layer and degrade it<sup>1</sup>. Therefore, inhibition of ETEC adhesion to mucin can prevent the incidence of ETECmediated diarrhoea. Certain polysaccharides can act as receptor analogues by adhering to the fimbrial adhesin of ETEC, preventing them from adhering to mucin. Two strains of lactic acid bacteria (LAB) from tempeh-related sources have been previously isolated and identified as Leuconostoc mesenteroides WA and L. mesenteroides WN<sup>2</sup>. These strains can produce exopolysaccharides (EPS) that can act as receptor analogue against ETEC F4. The EPSs were identified as dextran (a-1,6-linked glucan) and levan ( $\beta$ -2,6-linked fructan). L. mesenteroides WA produced more levan than dextran while L. mesenteroides WN produced more dextran than levan (both at 4:1 ratio). Here, we studied the bioactivity of these EPSs to inhibit ETEC F4 adhesion to porcine mucin, compared the bioactivity of EPSs produced in liquid medium and soy flour, and investigated the role of molecular weight (Mw) distribution of these EPSs on anti-adhesion bioactivity. The capability of EPS to inhibit ETEC F4 adhesion to porcine mucin was measured by measuring the growth curve of bacteria that adhered to the mucin layer in the presence of EPS. None of the EPS samples (extracted from LAB culture in liquid medium) showed adhesion inhibition but instead increased ETEC F4 adhesion by 150–300% compared to the buffer control. However, when the LAB strains were grown in soy flour substrate, the water-soluble EPS fraction obtained after fermentation reduced ETEC F4 adhesion with porcine mucin to 15-25% compared to the buffer control. The EPS extract from liquid medium had a Mw distribution of 650-1000 kDa while EPS from fermented soy flour had a more diverse Mw distribution of 20-1000 kDa. To determine if the bioactivity in water soluble extract from soy flour was caused by soyderived small Mw molecules or the EPS, the fraction was further fractionated based on Mw (cutoff: 3 kDa). Anti-adhesion bioactivity was observed in the fraction with Mw >3 kDa while absent in the low Mw fraction. Our experiment indicates that EPS with a broad Mw distribution can inhibit ETEC F4 adhesion to mucin, while EPS with higher but narrower Mw lacks such bioactivity.

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#### Flash 12. Generic behavior of pulse proteins in making foam

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Pulse proteins are complex mixtures of legumins, (con)vicilins and albumins . These protein components have different physicochemical properties (e.g., size, charge, surface hydrophobicity, thermal stability) <sup>1</sup>, and have shown different behavior in the stabilization of air-water interfaces and foam as observed for globulins and albumins from rapeseed, Bambara groundnut, pea and mung bean <sup>2-4</sup>. Many studies have investigated the foaming properties of pulse proteins primarily at a macroscopic level <sup>5-8</sup>, but the interfacial stabilization mechanisms are much less explored. The specific roles of each of these pulse protein components in air-water interface and foam stabilization are therefore still not fully understood.

Here, we extensively characterized the molecular, air-water interfacial and foaming properties of the whole protein extracts and the individual globulin and albumin fractions from lentil, faba bean and chickpea, and we managed to link these properties through correlation analysis. We showed that the thermal denaturation enthalpy is a key parameter highly influencing the foaming properties of pulse proteins. Lower denaturation enthalpy of pulse proteins appears to be linked to higher foamability and higher foam stability. This is possibly related to a higher conformational flexibility and/or smaller particle size that allow for a faster adsorption at air-water interfaces and a faster or higher degree of structural rearrangement at air-water interfaces that increases the interfacial network stiffness. Fast adsorption and stiff interfaces are conducive to foamability and foam stability, respectively. Low denaturation enthalpy is a feature of pulse albumins, and they have the highest foamabilities and foam stabilities compared to the whole protein extract and globulin fractions. For globulin-rich pulse proteins, vicilins and convicilins seem conducive to foam stability by forming stiff interfaces with a network structure with high connectivity, which increase the interfacial resistance to large deformation and enhance foam stability. High content of vicilins and convicilins also tends to induce high surface charge and promotes foam stability by increasing electrostatic repulsion forces between air bubbles. Legumins tend to reduce foamability since they tend to have low surface hydrophobicity and adsorb slowly at air-water interfaces, and they seem to disrupt the interfacial structure by increasing interfacial structural heterogeneity and decreasing the length of protein threads in the network structure. These findings provide deeper insights in the role of pulse globulins and albumins in air-water interface and foam stabilization. The proposed key parameters will benefit the predictability of the interfacial and foaming behavior of pulse proteins.

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# Flash 13. Exploitation of protein-pectin-polyphenol interactions for stabilization of reduced-oil white bean aquafaba vegan mayonnaise

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Aquafaba, a rich source of surface-active soluble proteins and saponins, has become an effective egg substitute in vegan mayonnaise formulations. However, reduced-oil versions of aquafaba mayonnaise often suffer from low emulsion stability and compromised textural/rheological properties. This study aimed to enhance the emulsion stability, rheological, and textural properties of reduced-oil vegan mayonnaise using a combination of citrus pectin (CP) and grape seed extract (GSE). The control reduced-oil mayonnaise (M60) demonstrated the lowest emulsion stability while mayonnaise with 1% (w/v) CP (M60-CP) showed moderate emulsion stability, and mayonnaise with 1% CP and 0.5% GSE (w/v) (M60-CP-GSE) showed the highest emulsion stability. Back extrusion tests revealed that M60-CP-GSE exhibited significantly higher firmness, consistency, and viscosity indexes than M60 and M60-CP. These findings proved the significant contribution of GSE in improved emulsion stability, and textural/rheological properties of aquafaba mayonnaise owing to its ability to interact with hydrocolloids. Zeta potential measurements also showed that the addition of CP increased electronegative charges of oil droplets suggesting roles of repulsive forces formed by pectin carboxyl groups in emulsion stability. Fluorescence microscopy images confirmed that the oil droplets in M60 were coalesced and prone to phase separation, while M60-CP and M60-CP-GSE showed more uniformly packed smaller oil droplets distributed within the mayonnaise matrix. Although the addition of GSE imparted a brownish-red hue to M60-CP-GSE, it provided a reduced-oil vegan mayonnaise with improved textural and rheological properties, emulsion stability, and antioxidant content. This work is one of the first examples of exploiting phenolic interactions to obtain functional emulsion-based vegan food.

### Flash 14. Valorizing agricultural waste: Utilizing corn plant leftover to grow yeast biomass, as a potential source of sustainable protein

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Rising concerns about sustainability, food security, human health, and animal welfare, drive the shift to sustainable protein alternatives. We studied upcycling post-harvest leftover corn leaves and stalks to grow yeast. Yeast biomass is a great source of proteins and other nutrients, useful for developing protein-rich alternatives for animal-based products. After harvest, we shredded the plant, dried it, then chemically and enzymatically hydrolyzed cellulose and hemicellulose, and obtained ~3.5% sugar in the substrate. Next, we used the substrate to grow biomass of a GRAS yeast strain, Yarrowia lipolytica, capable of utilizing also pentoses from hemicellulose, and obtained ~1 gram of yeast biomass using 15~ grams )dry mass( of corn waste. The yeast was separated from the substrate, freeze dried and analyzed for composition. The combination of acid-then-enzymatic pretreatment to the shredded corn plant biomass resulted in the highest yeast protein yield per dry corn plant biomass (28 g/kg), compared to hot water (1.8 g/kg), acid (9.7 g/kg), or enzymes (8.6 g/kg) pretreatments. While further optimization would be required to facilitate practical application, the process shows promise of converting agricultural waste into an alternative source for novel food proteins. In the future, when available land and water resources become scarce and expensive, animal agriculture inevitably diminished, and resource utilization and waste valorization become imperative, the process presented here is likely to become economically and environmentally rewarding.

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Shachar Heppner and Yoav D. Livney\*, Valorizing agricultural waste: Utilizing corn plant leftover to grow yeast biomass, as a potential source of sustainable protein, Future Foods <a href="https://doi.org/10.1016/j.fufo.2024.100523">https://doi.org/10.1016/j.fufo.2024.100523</a>

# Flash 15. Microstructural and Diffusive Characterization of Calcium Alginate Hydrogels

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Hydrogels are three-dimensional polymeric networks that contain a significant amount of water, which co-determines their structure. Natural hydrogels such as calcium-alginate have been extensively used in food and drug applications and claimed to provide control over bioactive compound release to the surrounding digestive fluids<sup>1</sup>. Few studies focus on hydrogel mesh properties and for those studies that consider this, it is measured when the hydrogel is freshly prepared. Yet, alginate hydrogels respond to environmental changes, as occur in the gastrointestinal tract, affecting their mesh size and diffusion into and from the hydrogel network.

In this study, we systematically characterized microstructural changes of alginate hydrogel microcapsules and corresponding diffusion properties under different pH and ionic strength conditions. Hydrogel mesh size was estimated based on swelling ratio and storage modulus. The kinetics of diffusion within the hydrogel was studied using fluorescence microscopy, by conducting one-dimensional diffusion experiments of tetramethylrhodamine (TRITC) dextran within a hydrogel-filled capillary tube. The experimental data were modelled in COMSOL<sup>2</sup>, using a finite element analyzer that enables simulation of solute diffusion, to find diffusion coefficients throughout the hydrogel.

The calculated mesh size of alginate hydrogel (5% w/v) increased with decreasing pH, from  $5.7 \pm 0.2$  nm to  $10.5 \pm 0.4$  nm. In response, the diffusion coefficient of TRITC-dextran (40 kDa) increased from  $1.3 \pm 0.05 \cdot 10^{-10}$  m<sup>2</sup>/s to  $1.9 \pm 0.08 \cdot 10^{-10}$  m<sup>2</sup>/s, respectively. An increase in mesh size and solute diffusivity was also observed at elevated ionic strength. These findings provide valuable information for the correlation between hydrogel mesh size and solute diffusivity under digestive conditions, which are essential for, amongst others, intestinal targeted delivery systems.

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# Flash 16. Utilisation of pectin from fermented cabbage waste in jam production

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Wastes from vegetables and fruits are acknowledged as a serious worldwide problem that affects the long-term viability of the food supply system. These by-products and wastes mostly emerge during or after processing. Volarisation of pectin from these wastes is therefore crucial for sustainability and minimising environmental impacts. Besides, this can lead to the obtainment of value-added products in the food and pharmaceutical industry. This study aimed to obtain pectin from fermented white cabbage waste sourced from a local factory and this study also describes the production of quince jam using low methoxyl pectin from fermented white cabbage waste for the first time. Microwave-assisted extraction was performed to extract pectin from fermented white cabbage waste. Response surface methodology was used to optimise pectin yield using Box- Behnken Design. The highest pectin yield (22.53%) was observed under optimal conditions (microwave power of 539W, time of 116s and pH of 1.5). The physicochemical analysis showed that the fermented cabbage pectin (FCP) was rich in galacturonic acid (% 81.28), but it had a low degree of esterification (23.89%) with a total phenolic content of 2.87  $\pm$  0.28 mg GAE/g pectin. The structural characteristics of pectin were confirmed using FTIR and NMR spectrums, while SEM determined morphological properties.

Furthermore, FCP indicated high water holding capacity  $(8.03 \pm 3.24)$  and low oil holding capacity  $(0.90 \pm 0.06)$ . Regarding emulsion properties, FCP demonstrated high emulsion activity and stability at 4°C. After this structural and physicochemical characterisation, the potential application of FCP was evaluated in quince low-sugar jam production.

# Flash 17. Hydrocolloids for enhanced gelation and colloidal stability of precision fermentation-derived β-lactoglobulin

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Dairy proteins have long been appreciated for their techno-functional and nutritional properties. With precision fermentation already established as a viable technology for ingredient production, there is growing interest in producing recombinant analogues of dairy proteins. This approach allows for the development of isolates containing specific protein variants, which can be bioequivalent to their milk-derived counterparts, or engineered for enhanced functionality. The functionality of such recombinant dairy protein isolate is primarily determined by the protein's molecular characteristics and the isolate's purity. This study explores the use of various hydrocolloid - protein combinations to improve heat-set gelation behaviour and colloidal stability in beverage systems. Fermentation-derived  $\beta$ -lactoglobulin (BLG) was combined with  $\kappa$ -carrageenan, high methoxyl pectin (HMP), low acyl gellan gum (LAGG) and locust bean gum (LBG) at different ratios and the gelation behaviour and gel properties of these combinations were evaluated. Texture analysis revealed that hydrocolloids could work synergistically or antagonistically with BLG in terms of gel hardness, depending on their concentration, and related to that, the occurrence and type of phase separation. Additionally, small and large amplitude oscillatory shear (SAOS & LAOS) rheology was used to characterize specific hydrocolloid -BLG combinations. Significant differences were observed under non-linear deformation, indicating the formation of distinct gel network structures. The role of hydrocolloids in model protein beverages was also examined. Consistent with other dairy protein ingredients, and despite of some physicochemical differences with milk-derived BLG, it was confirmed that HMP could improve the heat stability of fermentation-derived BLG at acidic pH conditions. Our findings demonstrate that selecting the right hydrocolloid and concentration can unlock specific gelation functionalities or stability at challenging pH conditions where  $\beta$ -lactoglobulin tends to aggregate during UHT processing. These insights may guide formulators in developing animal-free dairy foods with tailored textures and inspire academics to explore hydrocolloids in food texture design.

### Flash 18. Chitosan-Genipin Immobilisation System for *Alcalase*: Targeted Modifications in Sodium Caseinate Hydrolysate

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Food proteins are essential, high-quality nutrients with functional properties<sup>1</sup>. Protein hydrolysates, characterised by peptides of lower molecular weight and higher bioactivity, improve nutrient absorption, elevate the nutritional value of proteins<sup>2</sup> and enhance functional properties<sup>3</sup>. Alcalase, a versatile protease, is extensively used. It can cleave a wide range of amino acid sites, and efficiently produces diverse bioactive peptides. Nonetheless, it faces challenges like autolysis, enzyme incorporation into food, potential allergenicity, and high operational costs due to limited reusability<sup>2,4</sup>. To address these issues, and potentially change the specificity and selectivity of the protease, immobilisation systems for Alcalase, utilising chitosan-genipin beads as a support matrix were investigated. Systems varying active enzyme loads called as high (HEC), medium (MEC), or low (LEC) enzyme coverage were assessed. The hydrolysates produced by both the immobilised and free enzymes (FE) were characterised based on the TCA-solubility index, degree of hydrolysis (OPA), peptides molecular weight profile (SEC), and bioactivate peptide identification (LC-MS). The solubility index varied from 56.3  $\pm$  1.0% (LEC) to 76.7  $\pm$  0.8% (HEC), while FE presented 70.7  $\pm$  0.7%. The degree of hydrolysis ranged from 10.96  $\pm$  0.3% (LEC) to 15.3  $\pm$  0.57% (HEC), and FE exhibited  $13.63 \pm 0.45\%$ . HEC within the immobilisation systems significantly increased both the TCA-solubility index and DH percentages and showed substantially higher measurements than FE, indicating enhanced catalytic efficiency and stability due to immobilisation. The immobilisation matrix's optimised microenvironment, reduced feedback inhibition, prevented autolysis, and protected the enzyme from denaturation and degradation, ensuring prolonged activity<sup>5</sup>. In terms of size distribution profile, the peptides produced were represented in three distinct zones. The proportion of smaller peptides ( $\leq$  730 Da) was highest across all treatments, comprising 50.5% (FE) and ranging from 51.6% (HEC) to 40.1% (LEC). Intermediate-sized peptides (between 730 and 1,500 Da) remained around 27.8% across treatments, while larger peptides ( $\geq$  1,500 Da) accounted for 21.1% (FE), 20.5% (HEC), 26.1% (MEC), and 32.3% (LEC). LCMS provided comprehensive data on peptide sequences and revealed that Alcalase immobilisation significantly altered its specificity and cleavage preferences. For instance, HEC showed a specificity for cleaving peptide bonds next to lysine, methionine, leucine, and tryptophan, while LEC had a higher specificity for lysine, leucine, glutamine, phenylalanine and serine. Unique peptides were also generated by specific treatments. In conclusion, Alcalase immobilisation systems improved enzyme stability, leading to a higher proportion of smaller peptides and distinct peptide profiles. It also altered the enzyme-specific cleavage preferences. Lastly, this approach effectively addresses challenges like autolysis and denaturation, while enabling enzyme reuse.

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### Flash 19. Consumer Acceptance of Resveratrol-Loaded Crackers and Cookies Aiming at Functional Food Development.

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Micro-nano encapsulation can play an important role in flavour masking of bioactives, thus proving fundamental in the development of functional food ingredients. The development of functional foods using emulsified resveratrol, was the main goal of this work, via the assessment of the impact of resveratrol addition and its consumer acceptance. High-speed homogenization/ultrasonication was used to produce the resveratrol-loaded emulsions. Functional snacks (crackers and cookies) were developed by using 4 mg of resveratrol/g were with either resveratrol-loaded emulsions or free resveratrol. Results showed an increase in dough elasticity and a decrease in dough consistency due to the incorporation of emulsified resveratrol. Reference and non-encapsulated samples had small visual differences regarding colour. Cookies and crackers loaded with resveratrol-emulsion displayed textural differences, with a decrease in hardness for the cookies, and an increase in hardness for the crackers.

To assess the organoleptic impact of the addition of resveratrol on the cookies and crackers, over one hundred volunteers participated in a sensory analysis. The reference samples were the best-rated samples for both products, followed by the emulsion-loaded sample and the unencapsulated resveratrol-loaded sample. A sharp increase in bitterness was seen in the unencapsulated resveratrol-loaded and emulsion-loaded samples when compared to the reference, especially for the unencapsulated resveratrol-loaded resveratrol-loaded resveratrol-loaded products. Thus, it was possible to obtain a small positive impact of the encapsulation of resveratrol versus the unencapsulated resveratrol. Still, resveratrol's impact is still felt and further progress needs to be achieved to obtain higher consumer acceptance of resveratrol-loaded functional products.