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CIPCA 2025

X International Conference on Food Proteins and Colloids

16th - 18th June 2025

Universidade Católica Portuguesa - Porto

CIPCA 2025
X International Conference on Food Proteins and Colloids
Porto, June 16-18, 2025

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Index

The Mission of CIPCA 2025

The X International Conference on Food Proteins and Colloids (CIPCA2025) is committed to advancing the frontiers of food protein and colloid science by fostering collaboration across disciplines and sectors. Our mission is to provide a dynamic platform where researchers, industry professionals, and young scientists from around the world can share their knowledge, exchange innovative ideas, and co-create solutions to address the pressing challenges of the food industry.

Through interdisciplinary dialogue, CIPCA2025 seeks to inspire new partnerships and stimulate research that links fundamental science with real-world applications. By exploring the complex interactions of proteins, peptides, polysaccharides, and colloidal systems, the conference aims to highlight their relevance in improving food quality, safety, health, sustainability, and innovation.

Rooted in its strong tradition since the first edition in 1998, CIPCA has grown into a globally recognized event, especially engaging the Iberoamerican scientific community. Building on this legacy, the 2025 edition will continue to serve as a meeting point for excellence, fostering the development of novel ingredients, functional food systems, and sustainable biobased solutions that will shape the future of food.

The Chair of the CIPCA 2025 Organizing Committee,

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Centre for Biotechnology and Fine Chemistry, Universidade Católica Portuguesa, Portugal

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Sandra Borges
Centre for Biotechnology and Fine Chemistry, Universidade Católica Portuguesa, Portugal

Invited speakers

Plenary Speakers

Ana M. R. Pilosof

Professor, Institute of Food Technology & Chemical Processes (ITAPROQ), Faculty of Exact and Natural Sciences, University of Buenos Aires, CONICET, Argentina. Renowned for her pioneering work on protein–polysaccharide interactions, hydrocolloid functionality, and the rheology of complex food systems.

Brijesh K. Tiwari

Principal Research Officer, Teagasc (Irish Agriculture and Food Development Authority), Ireland. Specializes in emerging food-processing technologies—particularly high-pressure and pulsed-light treatments—and their impact on protein structure and safety.

Isidra Recio

Senior Researcher, Institute of Food Science Research (CIAL, CSIC-UAM), Spain. International authority on bioactive peptides from dairy and plant proteins, with extensive work in enzymatic hydrolysis and nutraceutical applications.

Mario M. Martinez

Professor, Food Technology Division, Department of Agricultural Engineering, University of Valladolid, Spain. An expert in cereal chemistry and starch–protein structures, with a long track record in developing sustainable processing methods for plant-based ingredients.

Keynotes Speakers

Ana Carla Kawazoe Sato

Associate Professor, University of Campinas (Unicamp), Brazil. Specializes in functional properties of plant proteins—particularly sorghum and legumes—with emphasis on their use in emulsions and gel systems.

Antonio A. Vicente

Professor, Department of Biological Engineering, University of Minho, Portugal. Leader in edible films, biopolymer formulation, and colloidal engineering, focusing on eco-friendly packaging and functional biopolymer matrices.

Bartosz Sołowiej

Full Professor, Department of Dairy Technology and Functional Foods, Faculty of Food Sciences and Biotechnology, University of Life Sciences in Lublin, Poland. Expert in leveraging protein–polysaccharide interactions to enhance functional properties of dairy and food products, with a focus on developing health-promoting formulations (e.g., sports nutrition bars, fortified dairy desserts) and studying rheological and textural attributes of novel food systems.

Cristobal Noe Aguilar Gonzalez

Professor, Bioprocesses & Bioproducts Research Group, Food Research Department, School of Chemistry, Universidad Autónoma de Coahuila, Mexico. Leader in solid-state fermentation and bioconversion of agro-wastes into high-value protein ingredients and bioactive compounds.

Diana Oliveira

Senior Scientist, PFx Biotech, Portugal. Drives R&D on bioactive peptides and alternative proteins, with a track record of translating lab-scale protein innovations into commercial ingredients.

Isabel Sousa

Researcher, LEAF – Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, University of Lisbon, Portugal. Expert in sustainable extraction of plant proteins, protein–polysaccharide interactions, and upcycling of agro-resources.

Lorenzo Pastrana

Senior Researcher, International Iberian Nanotechnology Laboratory (INL), Portugal. Pioneers nano-encapsulation and smart delivery systems for food proteins, focusing on improving stability, bioavailability, and sustainability.

Özlem Özmutlu

Professor of Food Engineering, Hochschule Weihenstephan-Triesdorf (HSWT), Germany. Focuses on alternative protein sources, process optimization for novel ingredients, and valorization of plant and microbial proteins.

Conference Programme

16 june 2025

2:00 p.m.

Universidade Católica Porto

Registration

2:30 p.m.

Carvalho Guerra Auditorium

Opening

3:00 p.m.

Carvalho Guerra Auditorium

Interactions Between Colloids and Other Food Components I

Protein digestion and intestinal signalling for satiety - Interactions between colloids and other food components

Prof. Dr. Isidra Recio (Plenary Speaker)

3:30 p.m.

Fermented beverages based on organic cow or goat whey with organic sea buckthorn or rosehip juices as an innovation in sustainable and functional food

Prof. Dr. Bartosz Sołowiej (Keynote Speaker)

3:50 p.m. - 4:45 p.m.

Presentations | Poster-pitch session (Parallel Sessions)

Break

5:00 p.m.

Carvalho Guerra Auditorium

Interactions Between Colloids and Other Food Components II

Glycemic index and other characteristics of bread - Interactions between colloids

Prof. Dr. Isabel Sousa (Keynote Speaker)

5:20 p.m. - 6:15 p.m.

Presentations | Poster-pitch session (Parallel Sessions)

Sunset

17 june 2025

9:00 a.m.

Carvalho Guerra Auditorium

Advances in Colloids Functionality I

Beyond the structural and techno-functional changes of plant proteins: what is the impact on health?" - Advances in colloids functionality

Prof. Dr. Ana Pilosof (Plenary Speaker)

9:30 a.m.

Functional Colloids for Next-Gen Nutrition: Peptides, Proteins, and Fermentation

Dr. Diana Oliveira (Keynote Speaker)

9:50 a.m. - 11:05 a.m.

Presentations | Poster-pitch session (Parallel Sessions)

Break

11:20 a.m.

Reimagining the Mediterranean Plate: Innovations in Alternative Protein Design and Sustainability

Prof. Dr. Özlem Özmutlu (Keynote Speaker)

11:40 a.m. - 12:55 a.m.

Presentations (ProxIMed Special Session) | Poster-pitch session (Parallel Sessions)

Lunch

2:00 p.m.

Carvalho Guerra Auditorium

Novel Colloidal Systems in Food Applications I

Structuring plant-based foods using less refined protein fractions”**-** Novel colloidal systems in food applications

Prof. Dr. Mario M. Martinez (Plenary Speaker)

2:30 p.m.

Nanoscale enhanced functional edible coatings and films

Dr. Lorenzo Pastrana (Keynote Speaker)

2:50 p.m. - 3:50 p.m.

Presentations | Poster-pitch session (Parallel Sessions)

Break

4:10 p.m.

Carvalho Guerra Auditorium

Novel Colloidal Systems in Food Applications II

Engineering plant-based ingredients for food applications

Prof. Ana Carla Sato (Keynote Speaker)

4:30 p.m. - 7:00 p.m.

Presentations | Poster-pitch session (Parallel Sessions)

Conference Dinner

18 june 2025

9:00 a.m.

Carvalho Guerra Auditorium

Emerging Technologies in Food Protein Research I

Novel technologies for extraction of proteins

Prof. Dr. Brijesh K. Tiwari (Plenary Speaker)

9:30 a.m.

Solid-state fermentation for food proteins: innovations, considerations and perspectives

Prof. Dr. Cristobal N. Aguilar (Keynote Speaker)

9:50 a.m. - 10:35 a.m.

Presentations Break

10:50 a.m.

Carvalho Guerra Auditorium

Emerging Technologies in Food Protein Research II

Modulating protein-based systems using electric fields

Prof. Dr. Antonio A. Vicente (Keynote Speaker)

11:10 a.m. - 11:40 a.m.

Presentations

Final considerations

Posters

Colloids for edible films

CIPCA25-31927

Microbial Hydrolysis Of Pectin Removed Apple Pomace Using S. Cerevisiae And K. Marxianus

Deniz Sevim Çabuk - Middle East Technical University, Food Engineering, Ankara, Turkey

Ayşe Sultan Akgun - Middle East Technical University, Biotechnology, Ankara, Turkey

Mecit Halil Öztop - Middle East Technical University, Food Engineering, Ankara, Turkey

Abstract:

This study explored the valorization of pectinremoved apple pomace via microbial hydrolysis, investigating whether coculturing *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* could synergistically boost hydrolytic efficiency, enhance reducingsugar and solubleprotein yields, and identify optimal processing parameters for maximum substrate conversion.

To examine these objectives in detail, apple pomace underwent acid-assisted pectin extraction, and the resulting pectin-removed apple pomace was utilized as a fermentation substrate. Fermentation was investigated using a full factorial design to assess the impact of solid load (5%, 10%, 15% w/v), inoculum volume (1%, 2%, 3% v/v), and fermentation period (1, 2, 3 days) on reducing sugar and protein content throughout the process. The fermentation media, which consisted of pectin-removed apple pomace and distilled water**,** were autoclaved, inoculated with yeast strains, and incubated at 30 °C and 200 rpm for three days, with samples collected daily for analysis. Reducing sugar was quantified using the DNS method, and soluble protein was determined via the Lowry method. ANOVA and Tukey's tests were conducted to identify significant factors influencing the responses.

Statistical analysis showed that reducing sugar change was significantly influenced by solid load and fermentation period, while all three variables affected soluble protein production. A low solid load of 5%, with a 2-day fermentation and 1% inoculum volume, yielded the highest reducing sugar change (49.66%). This value reflects the relative rise in reducing sugar concentration. Conversely, soluble protein peaked at 4.155 g/L under 15% solid load, 1-day fermentation, and 3% inoculum, while reducing sugar change is 11.10 %. The results demonstrate a clear inverse relationship between sugar and protein yields, where enhancing one results in the reduction of the other. Importantly, co-culturing was found to strengthen hydrolytic efficiency and broaden operational flexibility, thereby reinforcing microbial hydrolysis as a viable and sustainable alternative to enzymatic methods.

Collectively, these findings underscore that integrating *S. cerevisiae* and *K. marxianus* for hydrolyzing pectin-free apple pomace offers a sustainable strategy to convert agro-industrial waste into fermentable sugars and proteins. This approach supports circular bioeconomy principles and provides a foundation for developing eco-friendly bioprocesses that can contribute to bioproduct generation from low-value residues.

CIPCA25-64516

Production and evaluation of chitosan films incorporated with grape pomace flour or extracts as active packaging for fresh poultry meat

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Ana Luisa Fernando - MEtRICs, Departamento de Química, Faculdade de Ciências e Tecnologia, FCT, Universidade NOVA de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

Abstract:

Grape pomace is a by-product of the winery industry with significant potential as a raw material for extracting bioactive compounds in a circular economy framework. Grapes are rich in phenolic compounds, and even after wine production, the filtration by-product (grape pomace) retains a portion of the original active biomolecules, particularly those found in the peel. In this study, we explored the potential use of red grape pomace flour and its hydro-alcoholic extract (50% ethanol) incorporated into chitosan films to preserve fresh poultry meat. To produce the films, 1% (v/v film forming dispersion) of hydroalcoholic extract or 1% (m/v film forming dispersion) grape pomace powder were incorporated into chitosan films, 30 % (m/m of chitosan) glycerol was also used as plasticizer. Pure chitosan films were produced, and the films used to wrap the fresh poultry meat. Additionally, meat samples were stored without packaging as a control. The shelf life of the samples was monitored over 14 days of refrigerated storage (4°C), assessing parameters such as pH, titratable acidity, humidity, color, volatile basic nitrogen (VBN), lipid oxidation, and the count of mesophilic, psychrotrophic, and enterobacteriaceae microorganisms. Analyses were conducted on days 0, 3, 7, 10, and 14. The main results indicated that chitosan films incorporated with grape pomace showed significant antimicrobial activity, reducing the counts of mesophilic and psychotrophic microorganisms. Furthermore, there was less variation in the pH and titratable acidity of samples packaged with the biopolymers, suggesting better preservation of meat quality. Total volatile basic nitrogen results showed a lower production of volatile nitrogen compounds in the treated samples, indicating a reduction in protein degradation. Comparatively to unwrapped meat, pure chitosan films proved to protect better the meat sample from the deteriorative processes, however, with the incorporation of grape pomace hydroalcoholic extract or the grape pomace flour this effectiveness was even enhanced. Thus, the films were effective in preserving poultry meat, acting both as antioxidants and antibacterial, and can be considered a promising alternative for active packaging in the food industry.

Acknowledgements: This work was funded by MEtRICs unit through national funds from FCT/MCTES (UID/04077). C.R. acknowledge FCT/MCTES individual PhD research grant (doi.org/10.54499/2020.04441.BD) and V.G.L.S. the individual researcher contract (doi.org/10.54499/2023.09446.CEECIND/CP2836/CT0011).

Keywords: Hydroalcoholic extract, antioxidant, antibacterial, biopolymer.

CIPCA25-67015

Lobosphaera sp. as a source of protein and bioactive compounds to produce biodegradable films

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Rui M.S.C. Morais - Universidade Católica Portuguesa

Abstract:

Nowadays, there is a great variety of food packages, such as conventional glass, paperboard, aluminum, and plastics. In some cases, these could be replaced by more environmentally friendly packaging, such as biodegradable film coatings made from biological sources. These materials, meant to extend the shelf life of perishable food products, need to protect these products physically, chemically, and biologically. In this work, a microalga *Lobosphaera sp*. was used to extract protein and bioactive compounds to produce biodegradable films with antioxidant and antimicrobial properties. The bioactive compounds were extracted with a hydroethanolic solution 90% (50 °C, 120 rpm, 2 h, repeated twice), and the protein was extracted with water (50 °C, 2 h), followed by the use of cellulase, and protease at adequate pH and temperature (50 and 40 °C respectively, for 2 h each), followed by centrifugation and freeze-drying. Casting was the method used to elaborate the films. Their antioxidant activity (ABTS, DPPH) and physical properties were determined. The film with 2% alginate, 0.5% protein-rich extract, and 0.25% bioactive-rich extract presented an ABTS of 451.06 ±14.68 and a DPPH of 212.81 ±39.12 μM TE/mg film, higher than the control 3% alginate that had an ABTS 120.15 ±6.81 and a DPPH 85.97 ±3.19 μM TE/mg film. The color parameters of this film were L* = 54.06 ±7.80, hue = -87.0 ±0.6°, and chroma = 17.49 ±2.67. Its thickness was 0.076 ±0.011 mm, its water vapor permeability (WVP) was 16.04 ±0.98 (g.mm.m-2.day-1.kPa-1), and its solubility was 100% in water. Incorporating the bioactive-rich extract into the film formulation resulted in significantly higher ABTS and DPPH values and a lower WVP when compared with 2% alginate and 0.5% protein-rich extract without the bioactive-rich extract. Therefore, *Lobosphaera sp*. is a good source of bioactive and nutrient compounds to produce biodegradable films. These films can be used in active packaging, which extends the shelf life of food by scavenging or emitting various compounds, avoiding the addition of antioxidants and antimicrobials directly to the food product and allowing controlled release during storage.

Acknowledgments: This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through Project UIDB/50016/2020. Valter Martins acknowledges FCT for the PhD research grant to with reference UI/BD/152825/2022.

Keywords: Lobosphaera, films, protein, bioactives, antioxidant.

Emerging technologies in food protein research

CIPCA25-33400

Enhanced in vitro protein digestibility through the addition of proteases to soybean feed

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Martinez-Sanz Marta - Instituto de Investigación en Ciencias de la Alimentación, CIAL (CSIC-UAM, CEI UAM+CSIC), C. Nicolás Cabrera, 9, 28049 Madrid, Spain

Isidra Recio - Instituto de Investigación en Ciencias de la Alimentación, CIAL (CSIC-UAM, CEI UAM+CSIC), C. Nicolás Cabrera, 9, 28049 Madrid, Spain

Abstract:

Protein digestibility is important in the assessment of protein nutritional quality, with efficient protein digestion being crucial to maintain health and well-being. Despite being a rich source of protein, soybean feeds contain several antinutritional factors such as trypsin inhibitors and allergens such as glycinin and β-conglycinin. A proposed strategy to improve soybean protein digestibility could be supplementation with exogenous proteases. However, research is needed to evaluate the efficacy of proteases and to transfer the findings into practical recommendations for feed formulation. In light of this background, the aim of this work was to assess the impact of protease supplementation in soybean feed on *in vitro* protein digestibility and protein cleavage. The standardized INFOGEST static *in vitro* digestion method was applied to compare the *in vitro* digestibility of a control soybean feed with two soybean feeds enriched with proteases (Protease I and II). The obtained results demonstrated significantly higher protein digestibility in both protease-enriched soybean feeds, as determined by total amino group analysis using ortho-phthalaldehyde (OPA), and total amino acid analysis. Peptide analysis through nanoLC MS/MS of the soybean digests revealed higher peptide release in soybean supplemented with both additives, which was consistent with its higher digestibility. In addition, peptides in regions not hydrolysed in the control sample were found in soybean with additional proteases, including peptides from Trypsin inhibitor A. The peptide fraction analysis also indicated distinct enzyme specificity across protein families (β-conglycinin *vs* glycinin). In conclusion, the results confirmed that the enrichment of soybean feeds with proteases improves their digestibility. Consequently, this finding could serve as a seed to improve the digestibility of food and feed substrates, and thus, enhance the protein quality required to meet nutritional needs.

Keywords: in_vitro_digestibility, protein quality, soybean, proteases

CIPCA25-57723

Optimizing protein digestion and bioavailability in cow milk-based products using Protease S53

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Abstract:

In the stomach, proteins are broken down into smaller peptides and amino acids, which are then absorbed in the small intestine. This initial phase not only enhances nutrient absorption but also reduces the burden on the pancreas, helping to prevent common digestive issues such as bloating and gas. The use of specific enzymes to enhance protein digestibility and support gut health is therefore of great importance. This study investigates the application of protease S53 (formerly known as P24), part of the sedolisin or serine-carboxyl peptidase family. This enzyme is produced through precision fermentation by the U.S.-based company Digestiva.
Protease P24 is active in acidic environments, making it particularly suitable for use in the stomach, where the pH is naturally low. It has been shown to enhance protein digestibility and bioavailability, thereby improving the nutritional quality of foods while supporting more sustainable and eco-friendly dietary choices. The primary goal of this research is to develop a dietary supplement that leverages P24 to improve protein digestion and maximize nutrient absorption.

In this study, P24 was tested on a consumer product based on 4% fat cow milk. The product underwent in vitro digestion using the Infogest model, with a 1:100 enzyme-to-substrate ratio, accounting for both protein content and matrix effects. The results showed that increasing the enzyme concentration did not significantly enhance protein hydrolysis, indicating that the milk matrix itself may limit further protein breakdown. Across all enzyme levels, a consistent 15% degree of hydrolysis (DH) was observed during the gastric phase, highlighting the stabilizing effect of the dairy matrix. Additionally, batch-to-batch variability in DH% was noted, emphasizing the need for consistent formulation in enzymatic digestion studies.

Distinct patterns in the release of free amino acids and peptides were observed during the gastric and intestinal phases. These release profiles suggest that the milk matrix influences both the rate and extent of amino acid release, which is critical for effective nutrient uptake. Peptide molecular weight (MW) analysis revealed a higher proportion of smaller peptides, which are generally more permeable and bioavailable to intestinal cells.

To further evaluate absorption, the study measured permeation across Caco-2 cells, a widely used model for intestinal absorption. Results confirmed that smaller peptides showed higher permeation rates, supporting the bioavailability potential of the P24-digested product. Interestingly, changes in enzyme load did not significantly affect peptide permeability, reinforcing the conclusion that the milk matrix, rather than enzyme concentration, plays a dominant role in digestion and absorption dynamics.

In conclusion, the 4% fat cow milk product containing P24 exhibits a distinct digestion profile characterized by matrix-stabilized hydrolysis and the generation of small, absorbable peptides. The study highlights the potential of protease S53 to enhance protein digestion and nutrient absorption, making it a promising candidate for use in functional foods and dietary supplements. These findings contribute to a better understanding of how dairy-based matrices interact with enzymes and influence digestive health, paving the way for more effective nutritional strategies.

Keywords: Milk Protein; Digestion; Bioavailability

CIPCA25-81512

Identification and quantification of amino acids by high performance liquid chromatography and fluorescence detector (HPLC-FLD) of ultrasound-extracted tarwi protein

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Abstract:

Tarwi (*Lupinus mutabilis* Sweet) is an Andean legume with a high protein content (41 to 51 percent). Proteins have a high nutritional value; they also have techno-functional properties since they can be ingredients of food products and help to establish the structure and final properties of the food. Protein isolates and concentrates can be extracted from tarwi by isoelectric precipitation extraction (a commonly used technique), but research indicates that tarwi protein, extracted by the common technique, has lower functional characteristics (such as solubility and rheological properties) compared to soy protein. In this research, it was proposed to apply ultrasound technology in the extraction of proteins from debittered and defatted tarwi flour and its effect on the amino acid content in the protein concentrate obtained by ultrasound. The identification and quantification of amino acids was carried out using the technique of High-Performance Liquid Chromatography and Fluorescence Detector (HPLC-FLD), present in proteins extracted by isoelectric precipitation that went through an ultrasound process before (treatment 1) and after (treatment 2) of extraction; in addition to a control treatment, without sonicating, (treatment 3). Treatment 1 consisted of the flour being sonicated before protein extraction by isoelectric precipitation with a 20 kHz 500 W Vibra Cell VC-505 ultrasonic processor with a 12.5 mm probe at energy density levels of 500 J/mL for a time of 5 minutes of sonication. The sample was kept in an ice bath to maintain a stable temperature range of 10 to 15 °C during sonication. Treatment 2 refers to the same sonication process but after isoelectric precipitation of the protein. In the 3 treatments, the presence of the essential amino acids was observed: histidine (His), threonine (Thr), valine (Val), methionine (Met), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), lysine (Lys) and tryptophan (Trp); and non-essential amino acids: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glutamine (Gln), glycine (Gly), arginine (Arg), alanine (Ala), tyrosine (Tyr) and asparagine (Asn). The presence of essential amino acids, including methionine (sulfur amino acid), is an indicator that tarwi protein is important for body growth and development, so it could make it an excellent substitute for animal protein.

Keywords: Tarwi, ultrasound, isoelectric precipitation, aminoacids

Food colloids and health (bioactive peptides, allergenicity)

CIPCA25-12773

Chia mucilage reduces lipid absorption: how does it act?

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Abstract:

Chia mucilage (CM) is a plant-based hydrocolloid with thickening and stabilizing properties. Its emulsifying capacity have been evaluated in various food matrices. However, its relevance extends beyond emulsion stabilization, as its impact on digestive health positions it as a functional ingredient with the potential to modulate lipids absorption. The objective of this study was to evaluate the effect of CM on lipid digestion. Oil-in-water emulsions (80/20) were formulated with different CM concentrations (0.1-1%), and their droplet size, stability, and behavior during simulated gastroduodenal digestion in vitro were evaluated. For this purpose, two types of mucilage were selected (CM A: high viscosity; CM B: low viscosity).

Emulsions formulated with higher CM concentrations showed significant lower oil droplet size and higher stability (> 3 months). Additionally, emulsions formulated with CM A showed a higher stability than emulsions formulated with CM B, which could be attributed to the higher viscosity of CM A. Subsequently, the kinetics and extend of lipolysis were measured by quantifying the free fatty acids released during in vitro duodenal digestion. The results revealed that CM-stabilized emulsions released significantly fewer free fatty acids (10-15%) compared to a control emulsion stabilized with protein (100%). To understand the reason of this high reduction, the existence of interactions between CM and bile salts (5-15 mM) were evaluated by determination of particle size and solution turbidity of CM:BS mixtures. The higher particle size and turbidity of CM:BS mixtures compared to pure CM and BS confirmed the interaction between them. This interaction could prevent the efficient migration of BS to the lipid interphase, thereby reducing their capacity to facilitate lipolysis.

The interest in CM as an emulsifier responds to the growing demand for sustainable and environmentally friendly solutions in the food industry, making it a viable alternative to synthetic or animal-derived emulsifiers, in line with trends toward responsible consumption of plant-based products. These findings highlight the potential of chia mucilage as a valuable ingredient in the development of functional foods aimed at controlling lipid absorption. Its ability to stabilize emulsions and modulate lipid digestion positions chia mucilage as a key ingredient in the formulation of healthy products, offering a natural alternative for those seeking to improve their lipid profile through diet.

Keywords: chia mucilage, lipid-digestion, bile salts

CIPCA25-27377

Enhancing enzymatic hydrolysis of chickpea and lentil protein for biopeptide production.

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Abstract:

The incorporation of plant-based protein sources into our diet presents an important challenge. In particular, hydrolysates emerge as novel source proteins. Legumes are being extensively studied for their health-promoting properties and remarkable sustainability. One of the key challenges in the food industry is the extraction and purification of bioactive peptides. These peptides, consisting of 2 to 20 amino acids with a molecular weight below 4000 Da, exhibit enhanced biological activity. They are known for their antioxidant, antihypertensive, antidiabetic and antimicrobial activities, among others.

This study aimed to investigate the effect of hydrolysis time on chickpea and lentil protein using Alcalase® 2.4L, and the antioxidant activity of the final hydrolysate. Chickpea and lentil flours provided by InProteins, S.L. were dissolved in an alkaline solution (pH 10.5) and precipitated at their isoelectric point (pH 4.3). Hydrolysis was conducted in an orbital shaker for two hours under the following conditions: a liquid-to-solid ratio of 50 g/L, Alcalase® 2.4L at a concentration of 6 mL/L, a temperature of 50°C, and pH 8. The volume was recorded to determine the degree of hydrolysis (DH) following the method proposed by Ghribi et al. (2015). Protein quantification was performed using the Lowry method. Protein profiles were analyzed by SDS-PAGE on a discontinuous buffered system with a 12% polyacrylamide separation gel. Electrophoresis was conducted and gels were stained with Coomassie Brilliant Blue. Following hydrolysis, an aliquot was taken to assess antioxidant activity using the DPPH test, following the methodology proposed by Xu et al. (2020).

Firstly, protein from chickpea and lentil flours was solubilized in alkaline water for 1h, assisted by ultrasounds. This process resulted in final protein concentrations of 2.08 g/L for lentil and 1.93 g/L for chickpea, corresponding to extraction yields of 41.10% and 36.66%, respectively. Following isoelectric separation, the protein isolate underwent enzymatic hydrolysis. The degree of hydrolysis (DH) was most pronounced during the first 30 minutes, reaching 62% and 70% of the total DH for chickpea and lentil, respectively. After two hours of hydrolysis, the final DH values were 24.98% for chickpea and 26.25% for lentil. The DH value for chickpea aligns with findings from Xu et al. (2020), who reported a similar DH to 25%. Protein hydrolysis in the 50–250 kDa range was confirmed by SDS-PAGE analysis, as no protein bands were detected after 30 minutes of hydrolysis. After hydrolysis, antioxidant activity was evaluated using the DPPH method, resulting in inhibition percentages of 8.82% and 13.87% for chickpea and for lentil. Xu et al. (2020) reported higher DPPH activity of 25% after hydrolyzing chickpea flour with Alcalase for three hours, suggesting that extended hydrolysis might enhance the release of antioxidant peptides

The findings of this study demonstrate that enzymatic hydrolysis was efficient within a short time, suggesting that one hour may be sufficient to produce hydrolysates for functional food development. While the hydrolysate exhibited slight antioxidant activity, further optimization of hydrolysis process and research to isolate and identify peptides from legume hydrolysates are ongoing.

Keywords: Alcalase, antioxidant activity, plant protein

CIPCA25-36668

Granulometric distribution and level of selected proteins in whey protein concentrate WPC 80

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Abstract:

Whey products, including whey protein concentrates (WPC), are used as food ingredients as well as in the cosmetic and pharmaceutical industries. Whey proteins are characterized by a wide range of functional properties, such as the ability to bind water, thicken and gel, and also facilitate the formation and stabilization of foams and emulsions. The most important whey protein stabilizing the emulsion is β - lactoglobulin (60% of the whey protein fraction), followed by α - lactalbumin (20%) and bovine serum albumin (3%). Whey protein can potentially be used as a component of various types of colloidal particles, and in terms of stability, it can be a suitable alternative to adding fat to food. The aim of the study was to assess the granulometric distribution of solid particle size and the level of selected proteins in WPC 80 whey protein concentrates. 18 samples of WPC 80 type powders were tested. The level of lysozyme, β-lactoglobulin, α-lactalbumin, lactoferrin and amyloid A was determined using immunoenzymatic ELISA methods. Granulometric distribution of the size of pneumatically dispersed solid particles was performed on a HELOS laser diffractometer with a RODOS Vibri attachment. Whey protein is the main source of bioactive peptides and has antihyperlipidemic, antioxidant and antihypertensive effects. α-lactalbumin has antiviral properties, while milk enzymes, i.e. lysozyme, together with lactoferrin, have antibacterial effects. Our studies have shown that the level of lysozyme in the tested WPC 80 samples was in the range of 0.29-0.32 mg/100 g of product, β-lactoglobulin in the range of 55.9-56.7 g/100 g, α-lactalbumin 18.2-18.76 g/100 g, lactoferrin 69-71 mg/100 g, while the level of amyloid A was below the limit of quantification. The largest share in all tested powders was in the range of 100 to 200 μm. This share constituted over 1/3 of all particles and ranged from 33.59% to 46.59%. The second largest fraction was from 200 to 350 μm. This share ranged from 11.84% to 30.54%. Particles from both of these ranges, i.e. from 100 to 350 μm, constituted over 50% in all tested powders. It should be emphasized that in WPC80 there were no particles larger than 500 μm, but about 2.5% were particles below 10 μm. Due to the high content of valuable whey proteins in WPC80 powder, it can be classified as a functional food. In addition, a large share of particles with a size of 100-350 μm indicates that the colloidal solution obtained after dissolving the powder will be stable.

The research was supported by funds from the project: the task entitled “Research network of life science universities for the development of the Polish dairy sector— Research project” financed under the targeted subsidy of the Minister of Science and Higher Education (Warsaw, Poland; no. MEiN/2023/DPI/2862).

Keywords: β-lactoglobulin, lactoferrin, lysozyme, particle size

CIPCA25-49468

Impact of simulated digestion on the bioactivity of red lentil and quinoa protein hydrolysate

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Abstract:

The development of nutraceutical formulations enriched with bioactive peptides has gained considerable interest in the scientific community. Studies have shown that peptide hydrolysates from seeds can be rich in bioactive peptides with different properties. However, to consider these ingredients in nutraceutical formulations, it is necessary to ascertain whether these properties remain unaltered after digestion. This study aimed to evaluate the bioactive potential of two peptide hydrolysates obtained from quinoa and red lentil flours before and after simulated digestion. Firstly, the hydrolysates were subjected to simulated gastrointestinal digestion using the INFOGEST 2.0 protocol. A characterization analysis was to identify differences between the native hydrolysates and their digested counterparts. The molecular weight profile was determined using HPSEC, the protein content quantified by BCA assay, and the peptide sequences identified through UHPLC-MS, followed by *in silico*analysis. The potential antioxidant activity (measured by ORAC and ABTS assays) and the potential antihypertensive effect (assessed via ACE inhibition assay) of the hydrolysates and their digested forms were evaluated. The findings suggest that the red lentil (RLPH) and quinoa (QPH) hydrolysates (with a protein content of 120 mg BSA Equivalent/ g dry weight of sample and 91 mg BSA Equivalent / g dry weight of sample, respectively) showed good potential antioxidant and antihypertensive effects. The ABTS assay revealed that RLPH had the highest scavenging activity (60 µmol Trolox Equivalent/ g dry weight of sample), while the QPH showed the strongest antioxidant activity via ORAC assay (389 µmol Trolox Equivalent/ g dry weight of sample). Both samples exhibited noteworthy ACE-inhibitory activity (267 and 217 IC50 µg BSA Equivalent/ mL values for the RLPH and QPH, respectively). However, simulated digestion led to peptide degradation, resulting in a marked reduction in bioactivity. To address this, further assessments are underway to explore the cytotoxic and bioactive properties of these hydrolysates, including DPP-IV inhibition activity, GLP-1 secretion study, and prebiotic effect. In conclusion, these hydrolysates show potential as high-value ingredients. However, further stabilization strategies are needed to ensure the stability of the peptides throughout human digestion.

Keywords: Peptides, ACE inhibitor, Antioxidants, INFOGEST

CIPCA25-56130

Evaluation of selected cow milk bioactive peptides depending on the breed and feeding season

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Abstract:

The study aimed to obtain as much new information as possible regarding whether breed and diet influence the variability of bioactive peptides (BPA) level in cow's milk. The research material consisted of cows' milk collected from cows from 9 conventional farms in eastern and south-eastern Poland. Milk for testing was collected in two feeding seasons: summer (June-September 2024) and winter (October 2024-January 2025), from Holstein-Friesian (HF) and Simmental (SIM) dairy cow breeds. In each feeding season, milk was collected three times, at the same time from each farm (at the beginning of the study period, during it, and at the end of each feeding season). Cows were fed with silage and corn silage (CS, S) or silage (S) or Total Mixed Ration (TMR). Milk from the summer season HF cows contained more of some valuable BPAs than SIM milk. A beneficial increase in the levels of casokinin (HF: 93.79-114.63 ng/ml; SIM: 73.21-89.35 ng/ml), colostrinin (HF: 354.29-433.03 ng/ml; SIM: 321.53-392.99 ng/ml) was observed in milk from HF cows than SIM cows breed milk. In the winter season, milk from HF cows contained more casokinin (HF: 69.01-84.43 ng/ml; SIM: 50.79-62.08 ng/ml), immunoglobulins (HF: 3804.15-4649.51 µg/ml; SIM: 2947.24-3602.18 µg.ml) and caseinomacropeptide (HF: 342.23-418.28 ng/ml; SIM: 289.14-353.39 ng/ml). In turn, feeding silage and corn silage (CS, S) in the summer season more beneficially increased the level of some BPA, compared to feeding in the TMR system and with silage (S) only. This concerned a favourable increase in the levels of colostrinin (CS, S: 368.94-450.92 ng/ml; TMR: 301.07-367.97; S: 343.74-420.12 ng/ml), mucin (CS, S: 282.15-344.85 ng/ml; TMR: 202.01-246.91 ng/ml; S: 228.37-418.28 ng/ml) and ceruloplasmin (CS, S: 2302.48-2814.14 ng/ml; TMR: 724.17-885.09 ng/ml; S: 1315.64-1608.00 ng/ml:). In winter season feeding cows with silage and corn silage caused an increased content of colostrinin (CS, S: 421.14-514.72 ng/ml; TMR: 329.87-403.17 ng/ml; S: 264.22-322.94 ng/ml), caseinomacropeptide (CS, S: 1272.65-1555.46 ng/ml; TMR: 204.62-250.089 ng/ml; S: 324.78-396.96 ng/ml) and lactokinin (CS, S: 387.74-473.90 µg/ml; TMR: 362.82-443.44 µg/ml; S: 202.34-247.30 µg/ml) in milk compared to feeding in the TMR system and silage. A higher content of beneficial BPAs means that the milk of HF cows will have stronger neuroprotective and anti-cancer properties, strengthening the immune system and helping fight obesity. regarding the feeding season, milk from the summer season has better health properties, especially from cows fed with silage and corn silage. Higher BPA content may result from a higher share of roughage in the rations, especially silage, but a lower share of legume feed. Moreover, increasing the share of starch-rich feed in rations, such as cereals and corn silage, negatively affects the biological value of cow's milk and reduces the level of BPA in milk.

The research was supported by funds from the project: the task entitled “Research network of life science universities for the development of the Polish dairy sector— Research project” financed under the targeted subsidy of the Minister of Science and Higher Education (Warsaw, Poland; no. MEiN/2023/DPI/2862).

Keywords: breed, feeding, season, milk

Food colloids as vehicles for bioactives: digestibility and bioavailability

CIPCA25-17462

Bioaccessibility and Bioavailability of Bioactive Compounds from Mushroom Biomass Following in vitro Gastrointestinal Digestion

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Abstract:

Mushrooms represent a promising source of bioactive compounds for functional food applications due to their rich nutritional profile, containing α-glucans, β-glucans, lectins, and other bioactive macromolecules. These macromolecules exhibit numerous health-promoting properties, such as prebiotic, immunomodulatory, anti-inflammatory, and antioxidant activities. While previous research has primarily focused on polysaccharide fractions from mushroom fruiting bodies1,2, this study explores the biochemical potential of complete mushroom biomass (MB). The present study investigated the bioaccessibility and bioavailability of bioactive compounds present in *Trametes versicolor* (TV), *Hericium erinaceus* (HE), and *Pleurotus ostreatus* (PO) species, following simulated gastrointestinal digestion (GID), with a special focus on polysaccharide and protein fractions.

MB was biochemically characterized and subjected to *in vitro* GID using the standardized INFOGEST protocol. Dialysis membranes (3.5 kDa) were used to mimic the passage throughout the duodenum and jejunum and predict the colon-available and serum-available fractions. Additionally, a Transwell assay with co-culture of Caco-2 and HT29-MTX cells was also carried out to validate permeability. Chromatographic and enzymatic assays were used to quantify the bioactive molecules throughout GID, while the molecular weight (MW) distribution of proteins and peptides was determined by size exclusion chromatography. Total phenolic compounds (TPC) and antioxidant activity were evaluated through Folin-Ciocalteu, ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid), and FRAP (ferric reducing antioxidant power) assays.

Biochemical characterization revealed that polysaccharides were the most prevalent, varying between 76.15 and 80.45 % of dry weight (DW) without significant differences between species. (1→4)-α-glucans were the most abundant, followed by (1→4)-β-glucans. A wide distribution of MW was found in the polysaccharide fraction, with a higher concentration of polysaccharides over 107 kDa. Protein content ranged from 4.08 to 6.28 % DW in the three species, with TV exhibiting significantly higher content of total and

soluble protein. The three species predominantly comprised low MW proteins and peptides (<3 kDa)3. Other bioactive macromolecules were also identified and quantified, namely, gamma-aminobutyric acid, ergosterol, vitamins, organic acids, and phenolic compounds. Beyond nutritional properties, the biochemical profile of MBs suggests their rich bioactive potential. However, the bioaccessibility and bioavailability after GID are critical for deciphering health benefits.

Following simulated digestion, results revealed that (1→4)-α-glucans and (1→4)-β-glucans were the most prevalent groups in both colon-available and serum-available fractions (28-40 % DW), with results validated by permeability assays using Caco-2/HT29-MTX co-cultures. The colon-available fraction also contained proteins and peptides (<75 kDa) as well as fatty acids (oleic and linoleic acids), suggesting prebiotic activity. In contrast, the serum-available fraction was richer in low MW peptides (<1.2 kDa), amino acids (Tyr, Val, Phe, and Leu), and phenolic compounds (730-863 mg GAE/ 100 g DW). This fraction exhibited substantial antioxidant capacity using different methods (e.g., FRAP: 177-305 mg ISHE/ 100 g DW), indicating potential for systemic bioactivity.

These findings demonstrate that MB represents a valuable source of bioactive compounds after GID. Both colon-available and serum-available fractions showed potential for human health, expanding perspectives in the nutraceutical field and highlighting opportunities for future research into mushroom-based functional foods.

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Keywords: Mushroom biomass; Bioactive macromolecules; Gastrointestinal

CIPCA25-36170

Stability of potato starch-based bigels for curcumin delivery

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Abstract:

Bigels are semi-solid systems composed of hydrogel and oleogel phases, gaining attention in food applications as texture modifiers and carriers for both hydrophilic and hydrophobic bioactive compounds. In this study, curcumin, a hydrophobic bioactive with known anti-inflammatory and antimicrobial properties, was incorporated into potato starch-based bigels. The formulations included BG1 (5% curcumin), BG2 (2.5% curcumin), and BG3 (0% curcumin). The bigels were prepared using soybean oil and glycerol monostearate as the oleogel, along with potato starch as the hydrogel, maintaining a 60:40 oleogel-to-hydrogel ratio. The stability of the curcumin bigels was evaluated under two different storage conditions: room temperature (25°C) and refrigerated temperature (5°C) over a 60-day period. The samples were characterized by mechanical properties, color, rheology, confocal microscopy, and curcumin degradation. The results revealed that storage temperature significantly influenced the mechanical properties of the bigels. Lower temperatures (5 °C) increased the elasticity of the bigels, with BG1 exhibiting stronger structuring compared to BG2 and BG3. Structural analysis indicated that the phase distribution of the bigels depended on both the temperature and the formulation, which affected the overall gel network. Temperature sweep analysis showed that bigels containing higher curcumin concentrations exhibited stronger structuring at elevated temperatures (above 65°C), with the storage modulus (G') and the loss modulus (G") overlapping. However, upon cooling, the gel network strengthened, with G' dominating, suggesting that higher curcumin content contributes to a more robust network. Frequency sweep analysis confirmed that all bigels displayed slight frequency dependence, maintaining a gel-like plateau at higher frequencies. Confocal microscopy demonstrated uniform oleogel phase distribution within the bigel matrix. Curcumin stability was influenced by concentration and storage conditions. At 5°C, BG1 and BG2 retained over 80% of initial curcumin content after 60 days, indicating refrigeration slowed degradation. At 25°C, curcumin degradation was accelerated due to hydrolysis and oxidation. Notably, BG1 showed slightly faster degradation than BG2, suggesting that higher curcumin concentrations might enhance molecular interactions, making it more susceptible to degradation. Color analysis revealed that storage at 5°C resulted in higher L* values, indicating increased brightness and more stable color. For BG1 and BG2, there was a noticeable increase in L* over time, with BG1 showing a small shift of the yellowish at 5°C. BG3 exhibited a more stable color, with smaller fluctuations in a* and b*, suggesting it was less affected by temperature changes. BG2 and BG3 exhibited greater firmness, cohesiveness, and adhesiveness, especially at 5°C, indicating a more stable gel network. Firmness and cohesiveness increased until day 45, followed by a slight decline at day 60, possibly due to structural rearrangements. Stickiness increased at 25°C, indicating partial destabilization, while adhesiveness decreased, particularly at higher temperatures. BG1 exhibited the highest instability, especially at 25°C after extended storage. In conclusion, potato starch-based bigels show promise as carriers for curcumin, with refrigeration at 5°C being the optimal condition for maintaining curcumin stability and mechanical integrity over time. These findings highlight the importance of temperature control in bigel-based formulations, especially when a longer shelf life is required.

Keywords: Bioactive; Mixed gel; Curcuma; Rheology

CIPCA25-61925

Peptide-Based Nanosystems for Non-Alcoholic Fatty Liver Disease Treatment

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Abstract:

Cardiometabolic diseases, including diabetes, cardiovascular disease, and NAFLD, are a growing global health concern. Obesity, NAFLD, and type 2 diabetes form an interconnected triad that increases morbidity and premature mortality. The rising prevalence of NAFLD and its complications underscores the need for innovative treatments to address these related conditions effectively.

One of the primary targets in NAFLD treatment is reducing lipid accumulation in the liver. While pharmaceutical interventions exist, their adverse side effects have led to a growing interest in alternative, more natural therapeutic agents. Natural products have gained prominence in this context due to their ability to reduce hepatic steatosis through multiple mechanisms. These include decreasing lipogenesis and oxidative stress, enhancing β-oxidation and insulin sensitivity, and inhibiting inflammatory pathways [1]. Their reduced side effect profile compared to conventional treatments makes them an attractive option for long-term management of NAFLD.

Among natural product sources, fish by-products stand out as a promising and underutilized resource. Rich in bioactive lipids and proteins, these by-products have demonstrated significant potential in managing NAFLD and associated conditions such as type 2 diabetes.

This study aimed to develop peptide-loaded nanosystems (nanoliposomes and hybrid nanoparticles), for the treatment of NAFLD.

Nanoliposomes were produced by combining lipid component (lecithin), <3 kDa peptide fraction and surfactant followed by ultrasonication [2]. Hybrid nanoparticles were obtained through an emulsification process, where an organic phase containing lecithin and polyethylene glycol was added to an aqueous phase containing chitosan nanoparticles [2] [3]. The nanosystems were characterized for their physicochemical properties and stability. Their biological potential was assessed through cytotoxicity evaluation, cellular uptake studies using a hepatic steatosis model.

The nanoliposomes exhibited advantageous physicochemical characteristics, with a particle size below 90 nm and a polydispersity index between 0.2 and 0.3, indicating a consistent size distribution. The zeta potential ranged from -40 to -50 mV, suggesting stable colloidal properties [2] [4]. Encapsulation efficiency reached approximately 70%, ensuring effective peptide retention in the formulations. These findings highlight the potential of peptide-loaded nanoliposomes and hybrid nanoparticles as promising delivery systems for NAFLD treatment, offering improved stability, bioavailability, and therapeutic efficacy.

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Keywords: NAFLD; Peptide; Nanoliposomes; Hybrid nanoparticles

CIPCA25-67592

Synergistic Antifungal Effects of Fermented Plant Extract-Based Nanoemulsions: A Green Alternative to Chemical Fungicides

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Abstract:

Increasing resistance of phytopathogens to conventional fungicides necessitates the development of eco-friendly and sustainable alternatives. In this study, we explored the synergistic antifungal efficacy of nanoemulsions formulated from fermented plant extracts and presented a novel green solution for plant disease management. Solid-state fermentation (SSF) has been employed to enhance the bioavailability and potency of bioactive compounds in plant extracts, followed by encapsulation into nanoemulsions to improve stability, bioactivity, and targeted delivery. The physicochemical properties of the formulated nanoemulsions, including droplet size, polydispersity index, and ζ-potential, were characterized to optimize their antifungal performance. In vitro and in vivo assays against common phytopathogens demonstrated superior antifungal activity compared with unfermented extracts and conventional fungicides, which was attributed to the synergistic interactions between fermentation-enhanced bioactives and the nanoemulsion delivery system. This study highlights the potential of fermented plant extract-based nanoemulsions as viable and biodegradable alternatives to synthetic fungicides, contributing to sustainable agriculture and reducing environmental toxicity.

Keywords: Phytopathogens Mexican Oregano, Green Chemistry

CIPCA25-68534

Water-in-oil high internal phase emulsions as delivery systems for antioxidant compounds: Structural and rheological insights

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Abstract:

Water-in-oil (W/O) systems with a dispersed fraction of 74% (w/w) or above are classified as high internal phase emulsions (HIPEs). These colloidal systems have attracted interest due to their potential to reduce the saturated fat content in food products, reducing caloric intake and the possible incidence of cardiovascular problems. In addition to these features, a key advantage of W/O HIPEs is their high water content which enables the encapsulation of hydrophilic bioactive compounds. To investigate the role of W/O HIPEs as delivery systems for antioxidant compounds, two bioactive compounds—catechins and *β*-carotene—were selected for incorporation, individually and in combination. Using a conventional dripping process, W/O HIPEs containing 80% (w/w) aqueous dispersed phase with a fixed concentration of polyglycerol polyricinoleate and sunflower wax, each at 0.5% (w/w), solubilized in the continuous sunflower oil phase. The bioactive compounds were incorporated as follows: *β*-carotene at concentrations of 0.01–0.02% (w/w) solubilized in the continuous phase, and catechins at 0.135–0.270% (w/w) solubilized in the dispersed phase. Additionally, blends with the highest concentration of both bioactive compounds were tested. The W/O HIPEs were analyzed for droplet size distribution, rheological properties, and stability. The results show that all formulations presented small droplets with homogeneous size distribution. The W/O HIPE control and the formulation containing 0.01% *β*-carotene showed the smallest mean diameters of 3.67 ± 0.24 μm and 3.94 ±0.35 μm, respectively. Conversely, the W/O HIPE incorporating both bioactive compounds exhibited the largest mean droplet diameter at 7.72 ±0.47 μm. After 30 days of storage, coalescence was observed in all emulsions, which resulted in larger droplet sizes. The highest mean droplet diameter observed was 36.83 ±1.74 μm in W/O HIPE containing 0.270% (w/w) catechins. Regarding the rheological behavior, the W/O HIPEs with bioactive compounds (individually) at different concentrations presented higher viscosity than the control sample. Conversely, after 30 days, a structural rearrangement and viscosity reduction were observed in all formulations, along with a decrease in thixotropic behavior over time. However, the emulsions did not show phase separation after 30 days of storage at 5°C and 23°C, maintaining a gel-like rheological behavior ($G' > G''$). This characteristic may favor a controlled release of the bioactive compound. In conclusion, our findings indicate that the incorporation of bioactive compounds does not alter the stability of W/O HIPEs. Furthermore, this study contributes to a deeper understanding of the functionality of antioxidants and their influence on the structuring of W/O HIPEs, providing valuable insights for future food applications.

CIPCA25-71424

Enhancing the bioactive potential of defatted rice bran: enzymatic hydrolysis and bioaccessibility of protein hydrolysates

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Abstract:

In recent years, interest in the circular economy has grown as a paradigm for promoting sustainable food production and minimizing waste in the production chain. In this context, rice bran, a byproduct of rice processing (*Oryza sativa*), stands out due to its high nutritional value, low cost, and wide availability. This study aimed to evaluate the bioactive properties and bioaccessibility of protein hydrolysates derived from a concentrate (DRBC) enriched with protein and total dietary fiber (TDF), obtained from defatted rice bran (DRB) powder. The enzymatic hydrolysis of DRBC was carried out using 1.0 mL L^{-1} of protease (Protease from *Bacillus licheniformis*, Subtilisin A, ≥2.4 U g^{-1}, P4860) at 50 °C, with stirring at 150 rpm and pH 8.0. The hydrolysis reaction was stopped at different time intervals (5 to 120 min) to assess changes in the properties of the hydrolysates. The study of bioaccessibility, conducted through *in vitro* gastrointestinal simulation following the standardized INFOGEST 2.0 protocol, revealed a significant increase in the antioxidant and antihypertensive capacities of DRBC and its hydrolysates, especially in the hydrolysate obtained after 120 minutes of protease hydrolysis. Therefore, enzymatic hydrolysis of DRBC stands out as an effective strategy to enhance its bioactive properties by releasing bioactive peptides without altering the total polyphenol content. In the analysis of the bioaccessible fraction, ferulic acid and gallic acid were identified and quantified (HPLC-DAD). An increase in the content of ferulic acid in the bioaccessible fraction was observed, while gallic acid showed a decrease. The stability of ferulic acid during the *in vitro* gastrointestinal digestion simulation may be attributed to a protective effect provided by the TDF to which it is bound. These findings suggest that both acids have the potential to be absorbed and metabolized after digestion, allowing them to exert their effects throughout the body. Additionally, these findings support the potential of the hydrolysates as ingredients in functional foods, making them a promising strategy for valorizing DRB.

Keywords: enzymatic hydrolysis, proteins, bioaccessibility, bioactive

CIPCA25-84807

Double pickering emulsion stabilized by whey protein isolate microgel

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Abstract:

Multilayer water/oil/water emulsions enable water-soluble compounds to be entrapped in a colloidal system, providing enhanced protection and stability. The study of Pickering emulsions stabilized with microgels as substitutes for traditional surfactants has demonstrated improved stability against coalescence. Whey protein isolate (WPI) exhibits excellent solubility and emulsifying and interfacial tension reduction properties, due to its hydrophilic and hydrophobic regions, making it a suitable ingredient for emulsion stabilization. In this context, the present study aimed to investigate the use of different protein concentrations during the microgel formation process and their application in multiple emulsions containing ferrous sulfate and ascorbic acid as active compounds, with a view toward future microencapsulation via spray drying. Three microgels, designated M4, M6, and M8, were obtained through thermal processing of aqueous solutions containing 4%, 6%, and 8% (w/w) WPI, respectively. Subsequently, nine emulsions were produced by adding each microgel at 4%, 6%, and 8% (w/w) to the aqueous phase. Emulsion stability was assessed via creaming index and compared to emulsions stabilized by raw WPI at the same concentrations. Four key findings were highlighted regarding stability: (i) all emulsions formulated with microgels exhibited greater stability compared to those containing raw WPI, a result attributed to the characteristic ζ-potential below -30 mV of WPI microgels; (ii) higher microgel concentrations promoted greater emulsion stability, with emulsions containing 4% (w/w) of any microgel exhibiting lower creaming values (58.95% after 48 hours), while those with 8% (w/w) in the aqueous phase reached 72.73%; (iii) protein concentration during microgel production influenced the physical stability of the emulsion, but did not necessarily ensure increased stability as concentration increased, as emulsions containing M6 showed lower creaming values compared to those produced with other microgels; and, (iv) although emulsions formulated with M4 and M8 demonstrated better stability after 48 hours, the M6 emulsion remained intact (100% creaming index) for the first six hours of analysis due its lower ζ-potential (-38.67 mV). This value indicates a repulsion which prevents aggregation and ensures better initial stability, making it suitable for feeding into the spray dryer for microencapsulation. These findings suggest that incorporating any microgel at 8% (w/w) in the aqueous phase leads to emulsions with enhanced stability compared to those formulated with raw WPI. Future reasearch will focus on evaluating the impact of these formulations on the protection and digestibility of the compounds after microencapsulation.

Keywords: Coalescence; Stability; W/o/w; Creaming; Colloids

Interactions between biopolymers and their impact on structure

CIPCA25-38307

Hydrogel systems of sulphated exopolysaccharide from Porphyridium cruentum ionically crosslinked for skin wound healing applications

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Abstract:

Hydrogels have gained prominence in biomedical research, in particular in applications related with wound healing and regeneration, due to their versatile properties, such as easily adaptable shape, water-holding capacity, or their adjustable degradation rates. Natural origin hydrogels are gaining traction, for their useful properties when compared to synthetic counterparts, as they are often biocompatible, can exhibit some degree of bioactivity, and are usually biodegradable. This means they are locally absorbed, eliminating the danger of damaging the wound during its removal, while also providing antibacterial or antioxidant properties that may enhance wound healing (1). Within the group of natural materials, marine algae and their metabolites have been widely recognized for their bioactive properties with applications in various industries, such as pharmaceutical, biomedical, cosmetical, and nutraceutical (2,3) The red unicellular microalgae from the genus Porphyridium (Porphyridiales, Rhodophyta) is a natural source for a variety of interesting bioactive compounds, including several pigments (such as carotenoids), phycoerythrin, oligosaccharides, phycobiliproteins, and sulfated exopolysaccharides (EPS) (4,5). These polysaccharides have unique rheological properties in aqueous media, making them highly attractive for developing hydrogel systems. They can have tailored viscosity, presenting non-Newtonian fluid behaviour (6). EPS have also shown to possess several biological properties of high interest as therapeutic agents, such as anti-bacterial, antiviral, immunomodulatory, and antioxidant activities (6). In this work, EPS from *P. cruentum* were biochemically and physically characterized for their potential as bioactive molecules and building blocks for the development of a new biomaterial platform. Here, we address the healing and regeneration of complex wounds as potential application.

The rheological behavior of hydrogels formed from different aqueous solutions of EPS (0.5, 1.5, 2,5 wt% in 0.1M NaOH) in the presence of divalent and trivalent metal ions (M2+ and M3+) was measured and compared to gel-cation systems of alginate, a well-characterized polymer. Samples were assayed for their post-gelling properties using a rheometer with a flat-plate geometry. Frequency sweep tests were conducted at a low strain of 0.5%. Bacterial growth inhibition assay was done via the drop-plate method, against *Staphylococcus aureus* after a 24h incubation period. Biocompatibility was assayed via an indirect contact assay using Human Dermal Fibroblasts (hDF).

EPS formulations were able to form gels in the presence of Ce3+, Fe2+, Ca2+, Mg2+, and Cu2+. Polymer and crosslinker concentration, as well as crosslinker nature, had a significant effect on gel formation, and post-gelling properties. Higher polymer concentrations (1.5 and 2.5%) led to the formation of stiffer gels. Only Ce3+ and Ca2+ were able to induce gel formation even under standard conditions and led to strong and very homogeneous gels, and for this reason they were selected for further studies. Both Ce3+ and Ca2+-EPS hydrogel formulations had a very important bactericidal effect on *S. aureus*, and no colonies were detected in the agar plates after 24h incubation period. Moreover, all formulations used revealed to be biocompatible after indirect contact assay. This biomaterial platform holds further potential beyond wound healing, that can extend to broader tissue engineering applications, by offering biocompatibility, antibacterial properties, and tunable mechanics.

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Keywords: exopolysaccharide, hydrogel, wound healing

CIPCA25-42257

Egg-Free Light Mayonnaise Stabilized by Lupin Protein–Proanthocyanidin Complexes: Rheological, Tribological, and In Vitro Digestibility Studies

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Abstract:

Oil-in-water (O/W) emulsions stabilized by plant protein–phenolic compound complexes offer significant potential for creating light egg-free mayonnaise. In this study, complexes of lupin protein and grape seed extract were used as stabilizers to develop mayonnaise-like emulsions. The effects of acetic acid (pH), sodium chloride (NaCl), and sucrose — key ingredients in commercial mayonnaise — were investigated. Rheological and tribological analyses were performed to assess the influence of these ingredients on the bulk and surface properties of the emulsions. Based on droplet size distribution, viscosity, thixotropic recovery, and microstructure analysis, an optimal formulation containing 350 mmol NaCl and 4 wt% sucrose was identified, yielding an egg-free emulsion with physicochemical properties comparable to commercial light mayonnaise. Additionally, the produced emulsion exhibited *in vitro* digestibility similar to the commercial product. The bioaccessibility of phenolic compounds from grape seed extract was also confirmed, highlighting the potential of lupin protein–phenolic compound complexes for developing functional egg-free mayonnaise alternatives.

Keywords: Digestibility, Rheology, Tribology, Plant protein

CIPCA25-64116

Impact of Process Conditions on the Mechanical and Rheological Properties of Pea Protein Hydrogels

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Abstract:

Global population growth and consumer demand for healthier lifestyles have led to an increased demand for plant-based foods. However, the complex structure of animal proteins, as well as their functional and sensory properties, are difficult to be imitated by globular plant proteins. In this sense, plant protein hydrogels in foods can be explored in different aspects to produce customized textures, structures and techno-functional properties. However, as the structure of foods has a crucial impact on many of their sensory, nutritional and functional properties, understanding the impact of different processes on rheological and mechanical properties is very relevant. The present work aims to evaluate the impact of different processes – heat-set and cold-set (enzymatic and acidification) - on the mechanical and rheological properties of pea protein hydrogels. For this purpose, protein gels (15% w/w) were produced from an initial heat treatment (90 °C for 30 min), followed by cooling in an ice bath. For enzymatic gels, transglutaminase (TG) was added at 20 U/g protein, and for acidification, 2% (w/w) glucono-delta-lactone (GDL) was used to gradually reduce the pH. The thermal and GDL gels were formed at 25 °C and the transglutaminase gel at 45 °C (optimal enzyme temperature) for 4 h, and all were kept refrigerated for 48 h for subsequent analyses. The gels were characterized by confocal microscopy, electrophoresis, rheology (oscillatory over 4 h), compression, and water holding capacity. The prior heat treatment applied to the gels increased the surface hydrophobicity by 28%, unfolding the protein chains and ensuring better enzymatic performance and strengthening intermolecular interactions. The gel with only heat treatment proved to be self-sustainable, more continuous and uniform compared to the GDL and TG gels, which presented regions of greater protein agglomeration and gaps, according to the results visualized in confocal microscopy. The enzymatic gel, which was more granular, presented greater water holding capacity, rupture stress and storage modulus (G'). GDL gels (2% w/w) were developed to reach the isoelectric point of pea protein (pH 4.5) after 48h, allowing greater interaction between the chains and generating a strong gel. Therefore, the gel expelled more water, presenting the lowest water holding capacity. The GDL gels presented intermediate G', stress and strain at rupture between the treatments and a higher Young modulus. The electrophoretic profile of the proteins revealed the presence of high molecular weight groups in all treatments. Although they had the same protein content, the different preparation methods resulted in gels with different structures. Understanding the impact of the conformation of protein chains on different processes is of utmost importance, as they generate relevant impacts on the sensory and nutritional properties of foods.

Keywords: hydrogels, vegetable protein, structure, processing

CIPCA25-68551

Role of dietary fibres in modulating the gastrointestinal digestion of food proteins

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Abstract:

The increasing demand for food, combined with rising environmental concerns, are reshaping the global food system, leading to a growing interest in alternative, non-animal protein sources such as seaweeds. Despite their nutritional potential, seaweeds remain underutilized in the food industry, mainly due to their relatively low protein digestibility, which is associated with the intricate structure and composition of their cell walls, rich in non-digestible polysaccharides (i.e. dietary fibres). Therefore, a deeper understanding of the role of seaweed dietary fibres in the gastrointestinal digestion of proteins is crucial for developing strategies to enhance protein extraction and digestibility.

This work investigated the impact of several polysaccharides commonly present in seaweeds, such as agar, alginate, carrageenan, and cellulose, on the gastrointestinal digestion of casein, a widely recognized food protein. All samples were subjected to *in vitro*gastrointestinal digestion following the standardized Infogest protocol [1]. The resulting digestion products were subsequently analysed for mass and protein distribution, rheological properties, microstructure and amino acid composition. Moreover, the effect of fibres on the nanostructural arrangement of the digestion products was examined using advanced small angle X-ray scattering techniques (SAXS). Our results showed that alginate notably reduced protein digestibility, followed by carrageenan. In contrast, agar only decreased casein digestibility by a maximum of 17%. Furthermore, altering the agar/casein ratios did not result in any significant changes in protein digestibility. Additionally, small amounts of solubilised polysaccharides appeared to prevent the assembly of the released peptides into bile salts mixed micelles.

In conclusion, this work underscores the role of dietary fibres in modulating protein digestion. These findings have potential implications for the digestibility and bioavailability of proteins from seaweeds and other alternative protein sources.

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Keywords: Protein-digestibility, dietary fibres, protein-polysaccharide interactions

CIPCA25-72905

Comparison of techno-functional properties of RuBisCO proteins extracted from water lentil, alfalfa and sugar beet leaf

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Abstract:

There is an increasing demand for incorporating nutritious components into our daily diets. In addition to plant-based proteins extracted from seeds and legumes, novel sources such as leaf proteins have gained significant attention due to their rich amino acid profile, high protein yield compared to other plant-based proteins. One of the most important leaf protein is RuBisCO, also an enzyme responsible for carbon dioxide fixation during photosynthesis. It is also the most abundant protein on earth, made up of half of the total soluble protein found in leaves and green plants.

The objective of this study is to compare the techno-functional properties such as solubility, total protein content, amino acid profile and foaming properties of RuBisCO proteins which are extracted from different leave sources such as sugar beet, alfalfa and water lentil to assess their potential usage in food formulations for achieving the highest quality in the industry. The crude protein content (%) was determined through Kjeldahl Method. The solubility of proteins (%) was expressed as the proportion of soluble protein content, which was determined by Lowry Assay, to crude protein content. The foaming capacity and stability were also determined by making emulsions through homogenization, followed by the calculation of volume changes.

The solubility of sugar beet leaf (SBL) RuBisCO was found to be the lowest compared to other proteins, at 43.75%. All RuBisCO proteins show a low trend of crude protein content; however, one of them shows great solubility which is water lentil (WL) RuBisCO. WL RuBisCO protein's ability to solubilize in water is high although it's total crude protein content is only 60.17%. The crude protein content and solubility of RuBisCO proteins also varied significantly. WL RuBisCO protein has a crude protein content of 60.17% and a solubility of 91.46% which are the highest crude protein and solubility values among all. SBL RuBisCO has a crude protein content of 57.91%, but its solubility is the lowest at 43.75%. In contrast, alfalfa RuBisCO showed the lowest crude protein content which is 49.01% with a solubility of 67.10%. The SBL RuBisCO has the highest amounts of L-Alanine, L-Serine, L-Valine, L-Isoleucine, Glutamine and L-Glutamic Acids among all, which are 9.32%, 7.94%, 7.84%, 5.11%, 10.89% and 12.08% (% of total amino acids), respectively. On the other hand, alfalfa RuBisCO has the highest L-Leucine with a value of 10.62%. The highest L-Aspartic Acid and L-Arginine values belong to WL RuBisCO which are 29.07% and 17.65%, respectively. The foaming capacity of SBL RuBisCO is 84.19% which is the highest than that of alfalfa RuBisCO and WL RuBisCO. The foaming stability of SBL RuBisCO also has the highest value among other RuBisCO proteins.

In conclusion, comparing techno-functional properties of novel sources of leaf proteins is crucial for revealing their potential usage in the food industry. Identifying the properties of each RuBisCO protein that demonstrate the best performance will contribute to the production of high quality food products in the future.

Keywords: amino acid profile, RuBisCO

Interfacial Engineering

CIPCA25-56246

Correlation between protein association and techno-functional performance in black bean protein

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Abstract:

Foods owe their characteristic texture to the techno-functional properties provided by the proteins present, for example, a gelled, foamed or emulsified structure (breads, sausages, desserts, smoothies, etc.). Therefore, it is interesting to evaluate possible new sources of vegetable protein as an alternative to animal proteins to provide the industry with new options for the development of vegan/vegetarian foods and also contribute to sustainable production. One of the possible sources is the black bean (*Phaseolus vulgaris* L.), one of the most frequently consumed legumes, which contain 20–30% protein and are an important source of bioactive components that contribute to human health.

The aim of this work was to evaluate and understand the foaming and emulsifying properties of black bean protein (BBP) in order to improve the use of this protein as a potential ingredient for the formulation of plant-based food. Solubility, particle size, air-water and oil-water interfacial properties, foaming and emulsifying properties of BBP at concentrations 1–2% w/w and pH 3–7 were evaluated. Solubility of BBP was maximum at pH 3 and 7, and minimum near to its pI (4.2). Related with these results, the highest particle size was observed at pH 4 and 5, and the lowest at pH far from pI: pH 3, 6 and 7. BBP solutions at pH 7 showed higher foam overrun (FO) than at pH 3 and 5. Contrary, the highest foam stability was observed at pH 5. These foaming properties can be explained by the interfacial behaviour, since higher surface pressure was obtained for pH 7 and 3, while a significant higher elastic modulus (E) was obtained at pH 5 (66 mN/m vs 19 and 23 mN/m for pH 3 and 7, respectively), indicating the formation of a much more elastic interfacial film, which would allow to stabilize more efficiently the foams at pH 5. On the other hand, emulsions prepared at pH 7 showed a slight lower droplet size and a higher stability than emulsions prepared at pH 3. These results are also in agreement with the oil/water interfacial behaviour. Finally, a correlation between air-water interfacial and foaming properties, and between oil-water interfacial and emulsifying properties was done, and it highlighted the relevance of proteins interactions at the interface to stabilize dispersed systems and the relation of it with the particle size in the bulk.

From the results of this work, it was concluded that BBP foaming and emulsifying properties can be modulated by pH, and it can be explained by the interactions both at the interface and in the bulk. This knowledge could be used for the design of stable foam or emulsion plant-based foods at different pH.

Keywords: black-bean proteins, techno-functional properties, interactions

CIPCA25-67312

Effect of high pressure homogeneization and pH on the foaming properties of commercial pea protein

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Abstract:

Commercial pea protein is widely recognized as a sustainable alternative to animal proteins. However, like most plant-based proteins, it exhibits inferior techno-functional properties, mainly because denaturation and aggregation during industrial processing. In particular, the improvement of its foaming properties remains largely unexplored. The aim of this research was to study the impact of high-pressure homogeneization (HPH) and pH on the solubility, particle size, and rheological behavior of commercial pea protein to elucidate their influence on foam formation and stability.

Commercial pea protein (5% w/w) was processed in a high-pressure homogenizer (HPH) at 1000 Bar (PandaPLUS, GEA, Germany) at room temperature, and then pH was adjusted to 5 (1000 Bar-pH 5) and 6.5 (1000 Bar-pH 6.5). Rheological properties (Anton Paar Rheometer, Germany), particle size distributions (Malvern Panalytical Ltd, UK) and soluble fraction (% of the initial mass of the protein isolate present in the supernatant) were performed for each sample. Foam properties were evaluated by whipping solutions at room temperature in a graduated tube for 3 min with Griffin & George stirrer at 200 rpm. Protease treatment (15 min at 40°C, E/S 1/10) was applied to the sample processed at 1000 Bar- pH 5. The enzymatically treated sample was then mixed with the non hydrolyzed sample (1000 Bar–pH 5) at different ratios (50:50 and 25:75) to evaluate their combined effect on foaming.

Despite HPH treatment significantly increased solubility (from 16% to 85%) and reduced particle size (from 35.8 μm to 1 μm), no improvement in foaming properties was observed (overrun decreased from 220% to 200% with low stability). When the HPH-treated solution (1000 Bar) was adjusted to pH 5, solubility decreased (down to 9%), leading to the formation of protein aggregates (~8 μm). Under these conditions, the foam was stable to collapse and drainage, due to its viscoelastic behavior and particles adsorption, but foaming capacity decreased (overrun reduced to 140%).

When combining the 1000 Bar–pH 5 solution with the protease-treated solution, foaming properties were significantly enhanced, particularly at a 25:75 ratio. This effective combination of green technologies optimized the foaming capacity of pea protein isolate, increasing overrun to 250% and improving foam stability by 70% as compared to the original commercial protein.

Keywords: Pea protein, foams, high-pressure, protease

New processes and ingredients

CIPCA25-15555

Production of fish protein hydrolysates and gelatine from industrial Cape hake by-products

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Abstract:

In the present work, a set of enzymatic, chemical and physical processes, under a biorefinery approach, were applied to valorise industrial Cape hake by-products (trimmings, heads, viscera, skins and frames) aimed to produce high-value protein substrates, fish protein hydrolysates-FPH and gelatine, interesting ingredients for multiple applications. The volume of Cape hake processed by Galician food companies reached 265,172 tn in 2020, from which 50-60% are by-products. This kind of substrates generated from filleting of Cape hake species (a mixture of *Merluccius capensis* and *M. paradoxus*), and captured in the Namibia coasts (Southeast Atlantic Ocean), were kindly supplied by Nueva Pescanova S.L. (Redondela, Spain). Enzymatic hydrolysis were performed in 5L-jacketed reactor by utilizing Novozym 37071 (Nordisk, Denmark), under the following conditions: 0.1% (v/w) of protease, solid:liquid ratio of 1:1, 60ºC, pH-control at 8.2, agitation of 250 rpm, and 3 h of hydrolysis. After digestion, hydrolysates were filtered to eliminate clean bones, centrifuged to recover oils, quickly heated to enzyme deactivation and freeze-drying. Gelatine was extracted from skins using sequential steps of: 1) acid-alkalis washing treatments, 2) soft thermal aqueous extraction, 3) purification by ultrafiltration and 4) final oven-drying. FPH and gelatine were analysed by proximate composition, amino acid content, average molecular weight of protein and peptides and heavy metal presence. Other determinations were *in vitro* digestibility of FPH and gel strength for gelatine.

Dry FPH production yields were different depending on the waste substrate: 110 g FPH/kg of fresh frames, 122 g FPH/kg head, 148 g FPH/kg viscera, 130 g FPH/kg skins and 150 g of FPH/kg trimmings. Their proximate composition (g/100 g FPH) was ranging 6-10% of moisture, 13-16% of ashes, 4-6% of total lipids (22% in the case of viscera-FPH) and 72-75% of total protein (55% in viscera-FPH). Additionally, 90 g oil (rich in omega-3)/kg viscera were recovered. In terms of nutritionals, the *in vitro* digestibility was always larger than 90%, all amino acids were included in the hydrolysates (with glutamic acid, glycine and aspartic acid as predominants), and the percentage of essential amino acids was higher than 39%. The average molecular weight of peptides was of 2.7 kDa in viscera-FPH and in the interval of 1.20-1.37 kDa for the other FPHs. The presence of Pb and Cd was lower than 0.02 ppm in almost all hydrolysates (Cd: 0.2 ppm in head-FPH and 0.77 ppm in viscera-FPH), and Hg did not exceed 0.14 ppm.

Gelatine was only extracted from skin hake, obtaining a production yield of 2% (w/w of fresh skin) and with a composition, in dry terms, of 3.7%, 5.9%, 3.4% and 87.8% of moisture, ash, total fat and total protein, respectively. The content of glycine and imino acids (proline and hydroxyproline) was of 22% and

9% + 7%, respectively, being the essential amino acids percentage (28.2%) quite lower than found in the FPH. The gel strenght of hake gelatine was of 62 g-blooms. In conclusion, the present work highligths the validity of the sustainable operations proposed for the recovery of valuable protein material, FPH and gelatine, from Cape hake industrial wastes.

Keywords: hake; valorization; FPH; gelatine; biorefinery

CIPCA25-19169

Improved synthesis of plant and bacterial cellulose nanocrystals to reinforce bioactive protein films

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Abstract:

The ability to isolate nanoscale cellulosic structures (1-100 nm) with remarkable properties has renewed interest in cellulose. Cellulose nanocrystals (CNC) are rod-like particles characterized by nanoscale cross-sections and lengths ranging from 100 nm to several microns, primarily consisting of crystalline regions. The objective of this study was to optimize the synthesis of CNC from both plant and bacterial sources and to analyze their functionality in relation to the food industry, particularly as reinforcing agents for protein matrices capable of encapsulating bioactive compounds.

Bacterial CNCs were successfully isolated through acid hydrolysis of dried bacterial nanocellulose (BNC) retrieved from the floating pellicle generated during Kombucha tea production. The influence of the BNC drying method and its concentration on the yield and main characteristics of the CNCs was examined. Plant-based CNCs were synthesized using the same acid hydrolysis process from cellulose isolates extracted from soybean hulls, employing various bleaching methods. The resulting CNCs were characterized using Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), X-ray Diffraction (XRD), and zeta potential measurements. Optimized protocols enabled the production of bacterial CNCs with needle-like morphologies, diameters ranging from 3 to 10 nm, and lengths between 100 and 300 nm, exhibiting 93% crystallinity and a zeta potential of -38 ±3 mV. The plant-derived CNCs obtained after bleaching soybean hulls with NaClO2 displayed similar morphologies but larger dimensions (diameters of 7-10 nm and lengths of 254-620 nm), lower crystallinity (71%), and lower zeta potentials (-68 ±1 mV).

Both types of CNCs were evaluated as reinforcements in soy protein films produced by casting from suspensions of soy protein isolate (SPI, 5% w/v) plasticized with glycerol (20% relative to SPI content) and containing varying amounts of CNC (2, 4, and 8% relative to SPI content) at pH 10.5. The films were characterized in terms of optical properties, water vapor permeability (WVP), mechanical properties, solubility, and contact angle. Both CNCs effectively reinforced the protein films, enhancing tensile strength and Young's modulus, reducing WVP at lower CNC concentrations, and decreasing solubility at higher CNC contents, without significantly altering their appearance. Notably, CNCs derived from soybean hulls exhibited a significantly stronger reinforcing effect compared to bacterial CNCs, likely due to their larger dimensions and increased stability and charge in dispersion.

Films reinforced with plant-based CNCs were further analyzed as carriers for Fe^{2+}. The addition of SO4Fe to the filmogenic dispersions, followed by pH adjustment to 2, promoted cross-linking into films. This was evidenced by reductions in water content, solubility, elongation, as well as an increase in elastic modulus, tensile strength, surface hydrophobicity, opacity and coloration. However, the presence of Fe^{2+} in the films only improved WVP in those containing 4% CNC.

Finally, the diffusion of Fe^{2+} and Fe^{3+} ions from the material into aqueous media was investigated to assess the potential for food fortification through packaging. The presence of CNC influenced the release of iron ions, resulting in a decrease in their release as the concentration of CNC increased. Furthermore, the ratio of Fe^{2+} to Fe^{3+} ions increased at higher CNC concentrations, suggesting a potential protective effect of nanocelluloses against the oxidation of Fe^{2+}.

Keywords: soybean hulls, kombucha tea, encapsulation

CIPCA25-26135

Recombinant Cru1 isoform from Brassica napus oligomerization using proteinase K treatment

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Abstract:

Rapeseed (*Brassica napus*) oil production generates protein-rich waste, often discarded or used as animal feed. Understanding seed storage proteins can enhance plant-based protein applications in human diets. Five different isoforms of the protein cruciferin (Cru) are known, which together make up about 60% of seed storage proteins in rapeseed. Monomeric Cru consists of two disulfide-linked polypeptide chains and assembles into hexamers, which are dimers of trimers. Some post-translational modifications are necessary for its oligomerization.

We expressed and purified Cru1 from *E. coli* as a single polypeptide chain. CryoEM analysis showed that it forms correct trimers, but that those form "misaligned" hexamers, differing from plant-derived (PD) cruciferin. To mimic post-translational modifications, we treated Cru1 with low concentrations of proteinase K. Our goal was to examine how this treatment affects its structure, surface characteristics, and oligomerization.

We monitored mass changes using mass photometry, as well as thermal stability and size using differential scanning fluorimetry (DSF) and dynamic light scattering (DLS), and followed the change in peptide composition with reducing SDS-PAGE.

Proteinase K treatment led to a shift in Cru1 mass, originally appearing as a trimer, as well as to the appearance of new species with peaks at 40 - 50 and 250 - 275 kDa. These species have an average melting temperature (Tm) above the melting temperature of non-treated Cru1, and exhibit a double melting curve transition. Reducing SDS PAGE shows a changing profile of the sample.

The new species observed in mass photometry have peaks at 40 - 50 and 250 - 275 kDa, and appear within the first hour of digestion. The 250 – 275 kDa species plateaus in number of counts after 25 minutes and remains stable for up to a week, being mostly resistant to further digestion. We hypothesize that this species is the correctly “aligned” hexamer.

In SDS PAGE we observe a change in the band profile throughout the experiment, with distinct bands of lower mass emerging over time.

Thermal stability measurements revealed two transitions, which became less distinguishable over time. The treated samples displayed a higher Tm than untreated Cru1, approaching that of PD cruciferin. This suggests that enzymatic treatment induces the formation of thermally stable, properly assembled hexamers.

Proteinase K digestion altered Cru1's mass, thermal stability, and SDS-PAGE profile, suggesting the formation of a possible Cru1 hexamer. Further analysis is needed to confirm its structural and functional properties. Our study demonstrates that the production and characterization of recombinant seed storage proteins can help our understanding of how such proteins assemble and what defines their assembly and stability.

Keywords: Cruciferin, hexamerization, post-translational modification

CIPCA25-27774

Ultrasound-assisted extraction of proteins from *Ulva lacinulata*: Advancing sustainable alternatives to animal-based proteins

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Abstract:

Seaweeds are emerging as sustainable and alternative protein sources due to their high protein and dietary fiber content. However, their complex cell wall structure poses a significant challenge, often leading to low protein extraction yields. This study aimed to enhance protein extraction from the green seaweed *Ulva lactinulata* by employing an ultrasound (US) pre-treatment in combination with a pH-shifting method.The effects of the seaweed's physical state (frozen vs. freeze-dried) and the US pre-treatment durations (1, 5, and 30 minutes) were assessed.

The crude seaweed samples were characterized in terms of gross composition (protein content, lipids, mineral content and carbohydrates). Protein-rich extracts were successfully obtained by a pH shifting process, which involved alkaline extraction followed by acid pH precipitation. Optimal extraction conditions were achieved with solubilization at pH 12 and precipitation at pH 3 Notably, applying a 5-minute ultrasound pre-treatment under these conditions doubled the protein yield to 47%, compared to the yield without pre-treatment. In contrast, when freeze-dried biomass was employed for protein extraction, it was observed that the preservation process caused the collapse of the cell wall, significantly reducing the extraction yield compared to the frozen samples. The extracted fractions consisted mainly of proteins (21–40%) and carbohydrates (29–51%), with ulvans being the dominant polysaccharides. The amino acid profile revealed a high concentration of essential amino acids (37–41%), surpassing other non-animal protein sources. Furthermore, the extracts contained low levels of metals and inorganic compounds, ensuring their safety for human consumption.

The combination of US pre-treatment and pH-shifting proved to be an effective and energy-efficient approach for disrupting cell walls and enhancing protein recovery from *U. lacinulata*. These findings highlight the potential of seaweeds as valuable sources of high-quality alternative proteins, offering novel opportunities for sustainable food production in the industry

Keywords: Proteins, extraction, seaweeds, polysaccharides

CIPCA25-32085

Physico-chemical characteristics of texturized pea protein as meat replacer

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Abstract:

The market for plant-based meat alternatives is growing and therefore the research into different sources of meat replacers such as chickpeas, lentils, legume blends, potato or fungus. Pea is commercially available in large supply and can be texturized to replicate the fibrous structure of the muscle. However, the appearance, composition and properties are highly dependent on the texturization method: steam texturization, dry or low or high- moisture extrusion, thermal extrusion etc. The aim of this work is to study the characteristics of the texturized pea protein in relation to meat properties. Texturised pea protein was supplied by Grupo Dacsa (Valencia, Spain) at different times of production. The proximate composition was evaluated according to AOAC methods: moisture (AOAC 950.46), total fat (AOAC 985.15), ash (AOAC 920.153) and total protein (AOAC 992.15) using 6.25 as conversion factor. Fatty acid composition was determined in the Soxhlet-extracted fat according to the method described by Lurueña-Martínez et al. (2010). The different fatty acids were identified by retention time using a mixture of fatty acid standards and the contents were expressed in g per 100 g of total fatty acid methyl esters. All analyses were performed in triplicate. Textural properties were analysed according to the method proposed by Osen et al. (2014). A square shaped sample (27 x 27 mm) was cut longitudinally (FL) and parallel (FT) in the direction of the fibres using a knife blade and the shear strength was recorded. All determinations were carried out with at least 12 replicates. Colour was measured using a HunterLab MiniScan and L*, a* and b* parameters were determined using a 10° observer and a D65 illuminant. Ten replicates were carried out. The results of the proximate composition analysis revealed that textured pea protein (TPP) was characterised by higher protein (37.5%) and ash (2.9%) than pork and higher total fat values (3.1%) than chicken meat and lower moisture (46.3%) despite the fact that it was processed by high moisture extrusion. The fatty acid profile showed high levels of C18:2 n6 (44.9%), followed by C18:1 (28.4%), C16:0 (13.3%) and C18:3n6 (6.9%) resulting in a profile characterised by high levels of mono- and polyunsaturated fatty acids (28.8 and 52.5% respectively) compared to pork and ruminant meat and even chicken meat. The colour of the TPP showed the following values L*= 57.5, a*=9.2 and b*=35.9. The L* and a* values were similar to those described above for TPP and slightly lower than those obtained for pork and chicken, but the yellow component was significantly higher. Finally, the texture analysis allowed to conclude that the longitudinal and transverse forces were different (27.44 vs 41.41 N) because the textured fibres are arranged longitudinally in the extrusion direction, which confirms that TPP t has a fibrous morphology similar to that of cooked meat. In conclusion, the product tested could replace meat in some applications as it was more yellowish and less moist, but with a better fatty acid profile.

Keywords: Pea, Texturized Protein

CIPCA25-33912

Physico-chemical and techno-functional characterisation of wheat germ for its application as a protein supplement

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Abstract:

Wheat germ is a fraction of the wheat kernel that is separated during the milling process and, although it has historically been considered a by-product, it represents a highly nutritious part of the grain. Its high protein content, around 25%, makes it an ingredient of great interest to the food industry, especially in the formulation of products with a higher nutritional value. As well as standing out for its protein content, composed mainly of albumins and globulins, it has a good balance of amino acids and is an excellent source of essential minerals and vitamin E. Its lipid fraction is mainly composed of monounsaturated and polyunsaturated fatty acids. However, its stability is a challenge, as it contains enzymes such as lipase and lipoxygenase, which accelerate lipid oxidation, reducing its shelf life and limiting its preservation. For this reason, germs are subjected to stabilisation processes that inactivate their enzymes and prolong their shelf life, including defatting processes, heat treatments or storage under controlled conditions. Some of the industrial applications of germ are the enrichment of the nutritional profile of bakery and pastry products or their inclusion in fermented and functional beverages to improve the protein and mineral content and provide bioactive compounds.

In this research the physicochemical composition and techno-functional properties of 5 wheat germ samples of 4 different commercial brands were analysed. Their proximate composition was studied by analysis of fat, protein, ash and moisture. In addition, the water activity, colour and pH properties of the samples and their techno-functional behaviour were characterised by analysing water and oil retention capacity, swelling capacity, foaming capacity, foam stability, emulsion activity and stability and gelling capacity. The correlations between their composition and techno-functional properties were studied through a correlation matrix and a GH biplot was applied to identify the variables that most influence the discrimination between different commercial brands.

The protein contents among the different germs analysed showed values between 26.27 and 31.24g/100g dw. All composition parameters analysed showed differences between commercial brands as did the physico-chemical properties.Analysis of the significant correlations between the physico-chemical parameters of the germ and its functional properties revealed a negative correlation between the protein content and the properties of foaming capacity, foam stability and emulsifying activity and stability of the emulsion formed. In addition, the protein content showed a positive correlation with the gelling capacity of the germ when tested at pH 5, 7 and 8. Fat content showed negative correlations with all techno-functional properties except for water holding capacity and gelling capacity, for which a positive correlation was shown. The L* and b* parameters of the CieLab space showed significant correlations with all the techno- functional parameters, except for the oil retention capacity for the colour parameter b*. The results obtained show the great diversity existing within the marketed germs, which may condition their viability for their application in food formulation.

Keywords: Wheat germ, Physico-chemical, Techno-funtional,

CIPCA25-36904

Techno-functional and structural characterisation of alternative protein mixtures

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Abstract:

Alternative proteins are a promising market in the food sector since they provide sustainable solutions to the currently consumed proteins. However, poor sensory or techno-functional properties constitute hindrances to the success of these protein products [1,2]. Therefore, the objectives of this work were to map the main techno-functional properties of two alternative protein sources (i.e., lupin beans and edible house crickets) and improve them by mixing those proteins in different ratios.

Lupin protein concentrate (L) and house cricket (*Acheta domesticus*) flours (whole -WC- and defatted -DC-) were used. All samples were tested for main technological characteristics using standard methods. Afterwards, samples were mixed (insect/lupin) in 1:1, 1:3, and 1:9 ratios, and their solubility, foaming capacity and stability, emulsifying and gelling capacities, and water/oil absorption capacities (WAC/OAC) were measured. Structural characterisation was performed (DLS, Particle Size Distribution, SEM) to understand how the different proteins interact.

There was no gel/emulsion formation, while solubility showed a similar pattern: a drastic reduction in mildly acidic environments. Nevertheless, WC/DC solubility increased significantly when mixed, and insect inclusion did not majorly affect the solubility of L (until 50% of WC was added). Foaming exhibited a similar trend, mainly when DC was used, though no relevant foaming stabilities were revealed. There was a significant increase in WAC with insect flour addition. DLS seemed to indicate protein structural stability even when mixed (more negative zeta potential), and overall, there was an increase in particle size with the increasing incorporation of insect flour. SEM showed particles with different shapes and sizes, some smoother/more porous (L) and some irregular/rougher (WC).

There was a clear interaction between WC/DC and L, which appeared to be synergistic, with improvements being detected on several functional properties. Various treatments can be applied to further increase functionality, and thereby formulate food products with these novel, alternative and more sustainable ingredients.

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Keywords: Alternative proteins, Techno-functional properties

CIPCA25-40113

Novel Processing Approaches for Regional Plant-Based Texturizing Ingredients for Food Applications

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Abstract:

Interdisciplinary research at HSWT (University of Applied Sciences Weihenstephan-Triesdorf) and partnerships with industry stakeholders enable the research project *RegioTexFood*, fostering green technologies for resource-efficient processes aligned with sustainability goals. The project aims to optimize food supply chains by developing regionally adapted cultivation concepts for novel raw materials used in hydrocolloids, proteins, and fibers. Various cultivation systems are considered, including open-field and indoor vertical farming systems. In addition, underutilized plants are introduced as sustainable sources of regional texturizing ingredients, such as proteins, hydrocolloids, and fibers used as thickeners, stabilizers, or protective films in food applications. Sustainable "green technologies" like pulsed electric fields (PEF), high-frequency ultrasound, and high-pressure homogenization are used to enhance solubility and yield, ensuring resource-efficient processing.

A previously completed and published research titled "Impact of Various Extraction Technologies on Protein and Chlorophyll Yield from Stinging Nettle" at the Institute of Food Technology at HSWT optimized protein extraction from stinging nettles using PEF-pretreated samples provided by industry partner Elea Technology GmbH. This led to a significantly increased protein yield and a much shorter extraction time. In addition, the combination of PEF treatment and ultrasound at HSWT led to a more effective decolorization of the samples compared to untreated samples. Current research at HSWT (Institute of Food Technology) also investigates protein extraction from indigenously grown duckweed in indoor vertical and greenhouse farming using PEF treatment, with initial results indicating a significantly improved protein yield. Based on these achievements, the project *RegioTexFood* sees great potential in identifying plants with high texturizing ingredients, the improvement of regional cultivation of plant proteins and hydrocolloids through the use of optimally adapted indoor vertical farming systems. The subsequent phases of the project include the development of environmentally friendly and sustainable extraction processes for texturizing ingredients, the investigation of the properties of plant fibers, the scaling up of production, and the development of prototypes.

The overarching objective of the project is to ensure the effective utilization of extracted fibers, hydrocolloids and proteins within a food matrix, thereby facilitating the execution of successful application trials on industrial level. Extraction methods will be developed from lab to pilot scale with industry collaboration. In order to enable the completion of the food value chain, the food startup Happy Ocean Foods GmbH contributes to the field through product application tests for fish alternatives. The close cooperation between research and industry offers the opportunity to benefit from the synergistic expertise of the partners and is beneficial for the development of industrial solutions and the translation of research results into market applications.

Keywords: hydrocolloids, texturizers, ultrasound, PEF

CIPCA25-40519

Developing a high-protein fruit puree using co-products from agro-food processing

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Abstract:

In the last years, we have been witnessing an increase in the challenges of the global food system due to population growth, resource shortage and environmental problems. The linear economic model is contributing to waste generation and it is not considered sustainable. Circular economy, on the other hand, is based on closed-loop systems that simultaneously minimize environmental impact and still have economic value. Agro-food byproducts often have valuable nutrients and bioactive compounds that can be incorporated into novel food products, thus contributing to the circular economy. In addition, consumer demand for protein-rich foods continues to increase and this fact has to be taken into consideration when developing new food products.

In this study, a high-protein fruit puree was developed using primarily agro-food byproducts. Apple pomace, a fiber-rich residue from juice production, was selected as the main matrix component due to its content of dietary fiber, micronutrients, and phenolic compounds. To enhance the protein content, rice okara (a byproduct of rice milk production) was incorporated. Additional ingredients included lemon juice (as a natural preservative and flavor enhancer), xanthan gum (as a stabilizer), and water. Several formulations were prepared varying the proportions of the main components, and the samples were subjected to physicochemical (pH, °Brix, water activity), nutritional (proximate composition including protein content), and sensory analyses (flavor, texture, appearance). The formulation was optimized to achieve a balanced nutritional profile while preserving favorable sensory attributes. Subsequently, a second formulation phase was carried out to explore the potential for further protein enhancement. In this stage, the proportion of rice okara was increased, and banana was introduced as a natural sweetener and flavoring agent to compensate for potential sensory alterations. The results confirmed that it is possible to significantly improve the protein content without negatively affecting the sensory acceptability of the final product.

This work demonstrates that, by valorizing by-products, we have created a food product with low environmental impact while meeting the consumer demand for protein-rich foods.

Keywords: Agro-food; Byproducts; Protein; Puree

CIPCA25-42056

Unlocking the potential of trás-os-montes' natural matrices for innovative next-gen products

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Abstract:

The growing concern about the adoption of health-conscious habits emphasizes a balanced and diverse diet to ensure adequate intake of essential nutrients, such as fiber, proteins, vitamins, and minerals, as well as bioactive compounds with potential health benefits. At the same time, the development and commercialization of new bio-based food to replace conventional products of animal origin (such as dairy and meat products) have increased. This growth can be attributed to the rising of consumer interest, whether stemming from metabolic, allergic, cultural, traditional, or religious factors. Although soy dominates plant-based products, consumer interest in other meat and milk substitutes suggests a growing appreciation for diverse crops, enabling resource diversification and new market opportunities. The Trás-os-Montes region, located in an upland area in the northeast of Portugal, boasts a rich culinary tradition that reflects its natural environment and historical influences, highlighting the importance of connection with the land and respect for natural cycles. Renowned for its unique ingredients and distinctive products, such as meat products, cheese, nuts, legumes, fruits, vegetables, and mushrooms, this region plays a crucial role in Portuguese cuisine and, by extension, in the diets of many other countries. With the growing demand for sustainable and nutritious alternatives to animal-based foods, the local ingredients from Trás-os-Montes could offer promising, nutritious and delicious options for bio-based protein alternatives, that cater to both individuals seeking a healthier diet or those opting to reduce their consumption of animal-based products. In this sense, this study aims to evaluate the potential of key local crops, including nuts (*Corylus avellana* L., *Juglans regia* L. and *Castanea* spp.), legumes (*Lupinus albus* L., *Phaseolus vulgaris* L., *Cicer arietinum* L. and *Vicia faba* L.), and mushrooms (*Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm., *Agaricus bisporus* (J.E.Lange) Imbach and *Lentinula edodes* (Berk.) Pegler), to develop innovative, value-added, and healthy food products. These alternative sources of proteins will be integrated into the diet through their incorporation into analogues of traditional foods, such as bio-based versions of smoked meats (e.g., chouriço and alheira) and dairy-free cheeses, while also exploring the valorization of bio-residues generated in the production chain. This initiative seeks to foster economic opportunities for local producers, promote sustainable food innovation, and enhance the visibility and appeal of Trás-os-Montes.

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Keywords: Natural Matrices; Protein; Healthy food

CIPCA25-46254

Pracaxi oil as a modulator of cocoa butter crystallization: Anti-crystallizing effect

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Abstract:

O uso de modificadores de cristalização lipídica, como óleos vegetais, tem se mostrado uma estratégia eficaz para controlar o comportamento de (re)cristalização da manteiga de cacau (CB) em produtos alimentícios. Essa abordagem auxilia no processo de temperagem e melhora atributos sensoriais como brilho, cremosidade e crocância, além de melhorar a estabilidade de armazenamento de chocolates e derivados. Nesse contexto, compostos ricos em ácidos graxos de cadeia muito longa (AGCVL), como o ácido behênico, têm sido relatados como aceleradores de cristalização e estabilizadores polimórficos para CB. No entanto, tais compostos raramente são encontrados em matrizes lipídicas naturais. Assim, o óleo de pracaxi amazônico (PO), um óleo amazônico rico em ácidos behênico e lignocérico, surge como uma alternativa promissora para atuar como modificador da cristalização de CB. Nesse sentido, este estudo teve como objetivo investigar o impacto do PO nos processos de nucleação e cristalização de CB, bem como nas características microestruturais e formas polimórficas resultantes do processo de temperagem. As misturas CB:PO foram preparadas nas proporções (p/p) de 97,5:2,5, 95:5, 90:10, 85:15 e 70:30. Os processos de têmpera e cristalização foram estudados usando testes reológicos oscilatórios, com uma varredura de temperatura de 50 °C a 17,5 °C (a 2 °C/min), seguido por um período isotérmico a 17,5 °C por 10.000 segundos (primeiro e segundo estágios de têmpera). As formas polimórficas foram inferidas a partir do comportamento de fusão sob uma varredura de temperatura de 2 °C/min. A microestrutura foi avaliada por microscopia de luz polarizada após 24 horas a 20 °C para CB e misturas 90:10 e 70:30. Os resultados mostraram que a adição de PO atrasou a cristalização do CB. Este efeito foi mais evidente na mistura 70:30, onde o tempo de indução foi significativamente maior do que em outras misturas. A avaliação do efeito do PO na formação de formas polimórficas foi realizada pela diminuição abrupta nos valores de G' com aumentos de temperatura. A forma cristalina β_2 (V) é crucial para a estabilidade e atributos sensoriais desejáveis em produtos baseados em CB. Com exceção da mistura 70:30, todas as amostras apresentaram uma única etapa de fusão (26–32 °C), indicando a presença de cristais β'1 (IV) (instável) e β_2 (V) (estável). A mistura 70:30 apresentou duas quedas de G' abaixo de 30 °C, indicando apenas formas instáveis. Esperava-se que os VLCFAs em PO atuassem como sementes, acelerando a cristalização nas misturas, mas a heterogeneidade dos triglicerídeos (TAGs) e a presença de cadeias muito longas provavelmente resultaram em competição entre moléculas de TAG, retardando o crescimento do cristal e aumentando o tempo para o alinhamento da cadeia, dificultando a formação de formas estáveis. A mistura 90:10 exibiu uma microestrutura semelhante à do CB puro, enquanto a mistura 70:30 mostrou uma formação de cristal diferente, provavelmente devido ao efeito de maiores concentrações de PO. Assim, concluiu-se que o PO apresentou um efeito anticristalização,embora blends com até 10% de PO não tenham afetado a microestrutura ou a formação de formas polimórficas em comparação ao CB puro. O fracionamento de PO, obtendo uma fração rica em VLCFAs, pode ser uma alternativa para obter o efeito de aceleração do processo de cristalização em produtos à base de CB.

Keywords: Amazonian oil; Pentaclethra macroloba.

CIPCA25-47282

Plant-based protein sources: Phytate content and its effect on mineral bioavailability

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Abstract:

Phytic acid/phytates are forms of phosphorus storage in plants and are primarily found in seeds, grains, legumes, and nuts. Phytates can form complexes with minerals and proteins, which can affect the bioavailability of these nutrients. This is particularly relevant in plant-based products like cereals and legumes, which are important protein sources in vegetarian and vegan diets. The consumption of phytic acid varies widely between countries: low consumption levels (200-350 mg/day) in Western diets low in plant-based foods; moderate consumption (500-800 mg/day) in Western diets that include legumes, cereals, and their derivatives; and consumption exceeding 1000 mg/day in diets based on non-fortified plant-based foods (vegetarian diets) or with a high intake of foods rich in InsP6. In developing countries, phytate consumption can reach more than 2000 mg daily.

Therefore, the presence of phytates in plant-based protein-rich foods may limit the availability of certain essential minerals, which should be considered when evaluating nutritional value in unbalanced diets or vulnerable populations. A large proportion of the foods currently available are products made from cereal flours/gluten, soy, and pea proteins, which leads to reduced absorption of essential micronutrients such as iron and zinc due to the presence of phytates. In this context, this investigation analyzed the nutritional profile and estimated the mineral bioavailability in foods made from plant proteins currently available, such as meat substitutes and protein-enriched cereals. A selection of at least 12 products made from plant-based proteins such as soy, pea, wheat gluten, and others were considered. These were typically presented as follows: i) burgers, sausages, meatballs, nuggets, or deli meats; ii) high-protein cereal products such as bread, bars, cookies, and/or pasta. The commercial food selected were characterized in terms of: moisture, dietary fibre, starch, lipids, protein, and phytic acid by HPLC. The Fe and Zn concentrations were determined using ICP-MS. The contribution to the DRV/AI of Fe, Zn, proteins, and dietary fibre, as indicated by the EFSA, was evaluated for different population groups, considering gender (children, women of childbearing age, pregnant women, adults, and the elderly). The presence of phytates and their effect on mineral bioavailability were analyzed using the phytates/minerals molar ratios.

The results indicated that there is a strong inhibition of mineral bioavailability due to the high phytate content in plant-based protein products. To mitigate this effect, it is necessary to implement strategies such as fortifying these products with minerals or using processing techniques such as soaking, sprouting, or fermentation, which can reduce the phytate content. These interventions would improve mineral bioavailability and ensure that individuals consuming plant-based protein products receive adequate intake of essential micronutrients for health.

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Keywords: Plant-based protein; Phytates; Mineral bioavailability

CIPCA25-49951

Characterisation of chickpea (*Cicer arietinum*) hulls obtained as a by-product of chickpea flour production.

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Abstract:

In recent years, the processing of legumes into flour for human consumption has increased significantly. Legume flours are a source of vegetable protein that is not only high in protein, but also contains resistant starch, fibre and bioactive compounds such as polyphenols and phytochemicals, which contribute to the prevention of chronic diseases such as diabetes and cardiovascular diseases. However, its composition also includes the presence of anti-nutritional factors, such as phytic acid and protease inhibitors, which affect the bioavailability of certain nutrients, making it necessary to use appropriate transformation processes to obtain legume flours that minimise the presence of these undesirable factors.

The process of obtaining flour from legumes results in different types of milled fractions, such as cotyledons, embryo shafts and seed coats. The cotyledon, also known as 'dhal', which is obtained after dehulling these legumes, is what is marketed as legume flour. The seed coatings, also called hulls, account for 24-36% of the weight of the original legume. This represents an important amount of by-product or co-product that could be revalued. Recent studies have shown that the hulls of some legumes have a high phenolic content, and a higher antioxidant activity compared to the shelled seed. These properties suggest a potential use for human consumption through the production of functional foods and supplements. Therefore, the characterisation of these by-products, as well as the study of the anti-inflammatory and antioxidant properties associated with the polyphenols present in them, opens up an interesting line of research.

In this study the by-product obtained in the dehulling process by abrasion at industrial level of chickpeas, prior to their transformation into flour, have been analysed. Its proximate composition was studied analysing its content in carbohydrates, starch, fat, protein, fibre and sugars. The results showed that this by-product is an important source of protein with more than 26 g/100g dw, and contains an important concentration of carbohydrates, mainly starch (32 g/100g dw) and fibre (26g /100g dw). The nutritional composition of this product is similar to that of the raw product. As it is obtained by an abrasion method, without soaking, no losses of soluble compounds are observed. It seems that the distribution of the nutritional composition is uniformly distributed throughout the chickpea. In addition, the phenolic content of the hulls has been analysed. The results show a concentration of 0.8 mg/g of total phenols and 1.3 mg/g of total flavonoids.

The results of the present study showed that chickpea hulls are potential by-products as a source of protein, dietary fibre and phenols that could be used to produce high value-added food products. However, complementary studies of anti-nutritional compounds should be carried out on these by-products.

Keywords: chickpea hulls, byproduct

CIPCA25-50128

Exploring alternative protein sources for bread production: impact on total proteins and free amino acid profile measured by high-resolution mass spectrometry

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Abstract:

The increasing demand for nutrient-dense and sustainable food products has driven the exploration of alternative protein sources. While pea flour has been widely investigated for its nutritional benefits, alga flour remains an underexplored ingredient**,** despite its high protein content, bioactive compounds, and environmental sustainability. Although total protein content is commonly investigated in enriched products, the composition of free amino acids (FAAs) has been less studied. FAAs play a crucial role in both nutritional value and sensory properties, contributing to protein bioavailability, metabolic functions, and precursor roles in neurotransmitter synthesis while also influencing bread flavor, aroma, and textural characteristics through Maillard reactions. Thus, this study evaluated the effects of incorporating alga flour (Honey *Chlorella vulgaris*) and pea protein isolate (PPI) into bread formulations by measuring total protein content and FAAs profiles in the raw ingredients and the final bread products. One control formulation was prepared with the following ingredients per 100 g of flour: 60 g of wheat flour T65, 20 g of semi-whole wheat flour T80, 20 g of whole wheat flour T150, 65 g of water, 1.5 g of salt, and 0.33 g of dry yeast (*Saccharomyces cerevisiae*). In this base formulation, 5% of wheat T65 was replaced with alga flour, or 10% was substituted with PPI. The samples were analyzed for total protein content using the Kjeldahl method. Additionally, FAAs were determined using liquid chromatography and high-resolution mass spectrometry (LC-Orbitrap) after extraction with acetonitrile:water solution (50:50 v/v). The protein content of alga flour was 31.1±0,3 g/100 g, while PPI contained 52.02±0.06 g/100 g of total protein. The control bread had 7.34±0.20 g/100 g dry weight (dw), whereas alga-enriched bread contained 9.03±0.09 g/100 g dw, and PPI bread had 10.08±0.06 g/100 g dw. These values classify the enriched bread formulations as a source of protein, highlighting the potential of these alternative ingredients for improving the protein content of bakery products. The amino acids ARG, ASN, GLN, GLU, HIS, PRO, TRP, TYR, LEU, ILE, THR, PHE, SER, ALA, and LYS were detected in PPI, alga flour, and all bread formulations. Compared to PPI, alga flour exhibited a higher concentration of FAAs, particularly PRO, ARG, LEU, ILE, PHE, and ALA. Furthermore, bread enriched with alga flour showed elevated levels of PRO and ARG and increased content of total FAAs compared to both control and PPI bread. Thus, incorporating alga in bread formulations presents a promising alternative for producing protein-enriched baked goods, and may offer advantages over traditionally used ingredients, such as PPI.

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Keywords: alga protein; pea protein; orbitrap

CIPCA25-51155

Andiroba oil as a potential structuring ingredient

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Abstract:

Andiroba oil is obtained from the seeds of andiroba fruit (*Carapa guianensis*), native to the Amazon rainforest. In Brazil, the oil is used by the local population of the states of Pará and Amazonas due to its therapeutic, cosmetic and insecticidal properties, which are related to the presence of bioactive compounds, such as limonoids. Other bioactive compounds found in andiroba oil include phytosterols, which in addition to having anti-inflammatory and antioxidant properties, may have the ability to structure lipids. Considering the potential of andiroba oil for the formulation of creams for cosmetic applications, it is important to understand the impact of mixing this Amazonian oil with other lipids of commercial/industrial relevance, such as palm olein. This study investigated the influence of andiroba oil composition on the physical and rheological properties of blends containing palm olein was investigated. The presence of bioactive/minor compounds in andiroba oil was evaluated by Gas Chromatography/Mass Spectrometry (GC-MS). Palm olein and blends containing 10 and 20% (w/w) of andiroba oil were characterized for solid fat content (SFC%) by Nuclear Magnetic Resonance (RMN); cold stability at 7 °C; and rheological properties, by oscillatory rheology, through a time sweep at 7 °C (before analysis, the samples were conditioned at 50 °C). The predominant class of bioactive compounds identified in andiroba oil were phytosterols, of which lanosterol and β-sitosterol stand out, representing approximately 21% and 19%, respectively, of the unsaponifiable fraction of andiroba oil. The SFC% of the samples at 7 °C progressively decreased from 45.5 ± 0.5 % to 33.9 ±0.3 % as the proportion of andiroba oil in the blends increased from 0 to 20%. In contrast, the higher the andiroba oil content, the lower the cold stability of the samples, since pure palm olein remained clear for a longer period (at least 1 hour) compared to the blends (clear for about 10-30 minutes). Storage modulus (G') of palm olein was much lower (490 Pa) than that of blends containing 10 and 20% (w/w) of andiroba oil (6427 and 19253 Pa, respectively). These results indicate that andiroba oil acted as a structuring agent, since the blends were much stronger/harder than pure palm olein. Such structuring cannot be explained by lipid crystallization, considering that the SFC% of the blends was lower compared to palm olein. Instead, it is hypothesized that the phytosterols in andiroba oil, especially lanosterol, formed a colloidal network that entrapped the lipid phase, thus producing an oleogel. In conclusion, this study provides valuable information for the development/design of new cosmetic ingredients and formulations, based on Amazonian andiroba oil, with tuned/variable physical and rheological properties.

Keywords: Phytosterols, Rheology, Oleogel, Chromatography

CIPCA25-55802

High-resolution mass spectrometry for free amino acids determination in bee pollen

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Abstract:

Bee pollen is a highly nutritious natural product, widely recognized for its rich content of proteins and free amino acids (FAAs), which play a crucial role in human and bee nutrition. Amino acids contribute to the biological activity of bee pollen, influencing its antioxidant and antimicrobial properties. Their composition varies depending on the botanical and geographical origin of the pollen, making it essential to accurately characterize its amino acid profile. Advancements in high-resolution mass spectrometry (HRMS) and chromatographic techniques have significantly improved the identification and quantification of amino acids in bee pollen. The aim of this study was to determine the profile and concentration of FAAs in bee pollen samples through liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) with orbitrap detection. For that, samples of commercial multifloral bee pollen from the northeast region of Portugal, collected in 2024, were analyzed. The FAAs were obtained after extracting with a water-acetonitrile solution, to which N-acetyl-L-tyrosine was added (as an internal standard). The amino acids chromatographic separation was accomplished on a bioZen Glycan column (Phenomenex, 100 mm×2.1 mm id, 2.6 µm column) at 40 °C, using 10 mM ammonium formate (A) and ammonium formate 10 mM:acetonitrile (10:90 v/v, B) as mobile phase in a 12 minutes gradient elution. The gradient was from 0-5% B in 2 minutes, 5-50% B in 5 minutes, maintained 50% B for 1 minute, then return to 0% B in 0.1 min, with reequilibration time of 4 minutes. The MS detection was performed in positive mode using an Orbitrap mass spectrometer. For qualitative analysis, full scan (60,000 resolution in the 70-300 m/z range) was used, while selected ion monitoring (SIM) mode was applied for quantitiative analyses. FAAs were identified based on their accurate mass, comparison of their retention time with that of authentic standards, and MS/MS experiments. In total, 20 amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, lysine, serine, threonine, valine, methionine, proline, isoleucine, leucine, phenylalanine, tryptophan and tyrosine) were assessed. The developed chromatographic method proved to be highly accurate and effectively overcame the experimental challenges associated with conventional liquid chromatography. The study highlighted the presence of essential and non-essential amino acids in bee pollen, emphasizing their nutritional significance, which can have significant role for its application in functional foods, dietary supplements, and apitherapy. Additionally, amino acid profiling can serve as a biomarker for pollen authentication and quality control in the food and pharmaceutical industries.

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Keywords: Orbitrap; HILIC separation; chromatography; MS

CIPCA25-59768

Applicability of lentil varieties grown in Spain in the production of protein-rich fermented milks

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Abstract:

According to the Codex Alimentarius, food fortification is the addition of one or more essential nutrients to a food with the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups. The advantages of fortification over other methods of correcting nutritional deficiencies is that it does not require the development of new dietary habits, as the added nutrient is incorporated into foods widely consumed by the target population.

Lentils are rich in protein and can therefore be used for protein fortification of foods. Furthermore, their incorporation into other foods allows new probiotic and prebiotic products to be developed, since they are a source of oligosaccharides such as inulin and raffinose. The use of lentils in the form of flours is the simplest way to incorporate lentils as an ingredient in other preparations. These flours retain most of the functional properties of their sources, so their incorporation into different food vehicles is currently a field undergoing extensive development. Previous experience in the application of lentil flour in the production of yoghurts has given good results in nutritional, technological and sensory terms.

Spain is the second largest producer of lentils in the European Union, but the flour from lentils grown in Spain has been little studied.

This research has evaluated the feasibility of incorporating flour made from lentils grown in Spain for the production of fermented milk. For this purpose, the suitability of the three most widely cultivated lentil varieties in Spain, namely: Castellana, Armuña and Pardina, has been tested. Their influence on the fermentation process as well as the quality of the final product throughout its shelf life has been evaluated. Their pH, acidity, total solids, and fatty acids have been studied. In addition, the water retention capacity, the tendency to syneresis and the texture by measuring its hardness, cohesiveness, consistency and viscosity were also analysed up to 28 days after processing.

During the fermentation process, the addition of lentil flour did not affect the decrease in pH, although it did lead to a greater development of acidity, especially in the Pardina and Armuña varieties. The fermented milks elaborated had a total solids content between 82 and 85%. Fat composition showed that the incorporation of lentil flour resulted in products with a lower content of saturated fatty acids and a higher content of polyunsaturated and omega-6 fatty acids. During storage, yoghurts with lentil flour were harder, more cohesive and viscous, although they showed a lower cohesiveness. These differences were more prominent in the case of the Castellana lentil flour. Changes in pH and acidity during storage were not affected by the

presence of lentil flour. The water retention capacity of the yoghurts was better when lentil flour was incorporated, but no differences were observed in the syneresis of the yoghurts.

The sensory analysis of the fermented milks was carried out by means of a consumer preference test. No differences were found in the preference of a fermented milk incorporating lentil flour versus a fermented milk control. These results show that the incorporation of lentil flour from Spanish varieties is viable in the production of fermented milks, although further trials should be carried out to increase the percentage of flour incorporated.

Keywords: lentil, protein-rich, fermented milk

CIPCA25-62441

Influence of Fe^{2+} and ascorbic acid addition on the properties of electrospun gelatin matrices

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Abstract:

Electrospinning is an innovative technique that allows the production of nanofibers from natural and synthetic polymers. Proteins processing through this technology is not straightforward due to the complex structure of these macromolecules, especially with globular ones. Gelatin, derived from collagen, has a more suitable structure for being processed by this technique. This, combined with gelatin biocompatibility and biodegradability, opens the possibility of using this protein in new applications. In a previous work, we managed to obtain electrospun gelatin matrices capable of delivering bioactive compounds. However, its high hydrophilicity limits its structural stability and functionality. In this study, the effect of incorporating ferrous sulfate into electrospun gelatin matrices was evaluated with a dual purpose: to improve the physicochemical properties of the protein matrices and to assess the possibility of encapsulating this mineral into the protein nanofibers.

Six formulations were developed from an aqueous gelatin dispersion (25% w/v) prepared using acetic acid (30% v/v) as solvent. SO4Fe was added to these dispersions at 0, 12, and 25% p/p relative to the weight of gelatin, with and without the addition of ascorbic acid in a 2:1 ratio with respect to SO4Fe. The resulting matrices were characterized in terms of moisture content, solubility, color, contact angle, FTIR, water activity, and Fe^{2+} / Fe^{3+} release in contact with water.

All formulations could be processed, resulting in thin electrospun matrices composed of protein nanofibers. The use of acetic acid as a solvent allowed working with high concentrations of gelatin in dispersion and provided a suitable medium to prevent the oxidation of Fe^{2+}. The incorporation of SO4Fe into the formulation caused a significant decrease in the solubility of the materials and an increase in their surface hydrophobicity (observed as an increase in the contact angle formed when a drop of water was placed on the material), evidencing a crosslinking effect of Fe+2 within the protein matrix. These matrices displayed a color change from white (in the absence of Fe^{2+}) to brown-orange, with increasing values of a* and b* parameters as the SO_4Fe concentration in the formulation rose. Upon analyzing the release of Fe ions from the matrices when in contact with aqueous media, it was found that 60% to 80% of the Fe present in the matrix was released, while only 20% to 30% was oxidized to Fe^{3+}.

The incorporation of ascorbic acid into the formulations facilitated the release of Fe from the matrices, with 80–100% of the ions being released in the form of Fe^{2+}. This highlights the protective role of ascorbic acid against the oxidation of Fe^{2+} ions, which is also evidenced by the color change of the matrix upon its addition. However, the presence of ascorbic acid also diminished the cross-linking effect of iron, making the matrices more susceptible to moisture. Consequently, an increase in the solubility and wettability of the materials was observed, as indicated by a contact angle approaching 0°. Based on these results, further efforts will focus on optimizing the formulation to improve the physicochemical properties of the materials while protecting Fe^{2+} from oxidation.

Keywords: iron(II), ascorbic acid, protein nanofibers

CIPCA25-64956

Does microfluidization induce a protein denaturation state beneficial for gel formation?

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Abstract:

Flaxseed meal is a byproduct primarily used for animal feed or discarded. Extracting proteins from this meal presents an opportunity to increase the global protein supply, add value to the byproduct, and reduce waste disposal. However, some extraction processes may negatively affect the functional properties of the proteins. One technique to reverse this damage is microfluidization. Previous research has shown that microfluidization treatment improves solubility, hydrophobicity, and reduces the particle size of flaxseed protein isolates. In this study, we applied microfluidization to enhance gel properties and investigate whether the treatment could induce a pre-denaturation stage beneficial for both heat-set and cold-set gel formation. To achieve this, we evaluated the rheology of 6% protein (treated or control) solutions, as well as the rheology of heat-set and $CaCl_2$-induced gels (10% protein), comparing gels made with treated (137 MPa/5 passes) and untreated proteins. We also performed electrophoresis and evaluated the self-sustaining gels using uniaxial compression tests, scanning electron microscopy (SEM), and confocal laser scanning microscopy (CLSM). For the treated protein solution, the G' value increased over time at 4 ºC until it crossed over with G", indicating structuring over time. In contrast, the control solution exhibited almost constant elastic behavior across the entire evaluated range. Both heat-set and cold-set gels made from treated proteins showed lower G' values compared to those made from untreated proteins. However, the loss factor (G"/G') was similar between the samples, suggesting that their networks had comparable viscoelastic properties. Both gels exhibited minimal frequency dependence, indicating stable structures. The control sample showed a broader regime of plastic deformation during the strain ramp, allowing for greater deformation before rupture. Mechanical tests also showed that the strain and stress at rupture were significantly higher for the gels formed with untreated proteins. Finally, microstructural analysis revealed that the gels with treated protein were more homogeneous, with smaller, more evenly distributed pores than the gels with untreated proteins. This study demonstrates that microfluidization treatment can induce a state of denaturation that alters the dynamics of gel network formation, resulting in gels that, although more fragile, are more homogeneous. This characteristic could be beneficial for the visual appeal of food products such as puddings or yogurts.

Keywords: heat-set, cold-set, plant protein, flaxseed

CIPCA25-65475

Evaluation of potato protein nanofibrils for the production of oleogels

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Abstract:

The increasing interest in novel, more nutritious and more sustainable food consumption leads to the pursuit of new approaches to incorporate alternative proteins into the human diet more efficiently. Protein amyloid-like fibrils (PAFs) are rod-shape fibrous aggregates that are formed under acidic conditions (pH < 3) and high temperatures, particularly during prolonged heating (1). Among the other applications, PAFs can be incorporated into emulsions to increase the stability due to their long length, flexibility and high hydrophobicity, depending on the protein type (2). In this study, oleogels were produced from potato PAFs. Potato PAFs were obtained by applying heating treatments at 90 ºC with a heating time of 10 hours. Their formation was evaluated through intrinsic fluorescence intensity, ThT fluorescence assay, as well as changes in protein structure were followed through circular dichroism. The microstructure of PAFs was evaluated through transmission electron microscopy (TEM). Fluorescence intensity evaluation and TEM analysis confirmed that the 2 h of heating time increased fibril formation. For the development of oleogels high oleic sunflower oil and PAFs at different concentrations (2-20 wt.%) were used. The oleogel formation was observed with at least 12 wt% PAFs concentration and evaluated through the tube inversion method, rheological analysis and polarized microscopy. This study shows that PAFs can be a promising oleogelator to form plant-based oleogels.

Acknowledgements

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Keywords: oleogel; PAFs

CIPCA25-67148

Lactoferrin for additional coating of β-carotene/xylan complex

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Abstract:

Lactoferrin is a multifunctional protein with beneficial biological properties that make it an attractive nutraceutical for use in foods. It has antibacterial, antifungal, antiparasitic, antiviral, anticarcinogenic and other activities. Due to these properties, it is widely used as a carrier in the synthesis of nanoparticles or as an additional layer in the layer-by-layer method.

β-Carotene is one of the most important carotenoids in the human diet, with high antioxidant activity, and is important not only for its colour but also for the many health benefits associated with it. Despite its benefits, β-carotene is poorly soluble in water, sensitive to light, temperature and oxygen, and chemically unstable. To overcome this problem, various encapsulation methods are used, which are constantly being improved to achieve higher encapsulation efficiency and stability of the encapsulated material. One of the polysaccharides that can be used to encapsulate carotene is xylan. Xylan, an important component of lignocellulosic biomass and agricultural waste, has attracted attention in several industries for its role as an environmentally friendly and renewable raw material. Xylan is non-toxic, biodegradable and has beneficial effects on human and microbiome health.

The aim of this study was to use lactoferrin for the additional coating of water-soluble β-carotene/xylan complexes and to investigate the properties of the resulting derivatives. The synthesis of the β-carotene/xylan complexes (CAR-XYL) was based on the addition of β-carotene in acetone to a heated water dispersion of xylan derived from beech wood, followed by immediate evaporation of the organic solvent. The resulting complex was additionally coated with lactoferrin (LF). The concentration of CAR-XYL was constant (0.8 mg/mL) and the concentration of LF varied between 0-8 mg/mL: 0; 0.1; 0.2; 0.3; 0.6; 3.2; 4.8; 8.0. Dynamic light scattering (DLS) was used to determine particle size and zeta potential and their dependence on lactoferrin concentration. FT-IR and Raman spectroscopies were used to confirm the interaction of the components. The morphology of the particles was determined by SEM. The antioxidant activity of the three-component particles was also determined using DPPH and FRAP methods.

The study showed that the additional coating can successfully improve the properties of the β-carotene complex and extend its range of applications.

Keywords: Lactoferrin, β-carotene, xylan, encapsulation

CIPCA25-69048

Enzymatic modification of commercial pea protein for vegetable dressings design

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Abstract:

This study focuses on the development and characterization of a plant-based dressing based on pea protein, aiming to create a healthier alternative to traditional mayonnaise containing egg yolk. However, the use of commercial protein concentrates presents challenges for use in emulsified products due to their low solubility, grittiness and flavor.

To overcome the technological and functional limitations of commercial pea protein, controlled hydrolysis (bacterial protease) of the protein was used. This increased solubility, eliminated grittiness, and achieved very good emulsification properties as determined by particle size distributions (Malvern Panalytical Ltd, UK) and multiple light scattering using a vertical colloidal dispersion analyzer, model Turbiscan Classic 2 (Formulaction, Toulouse, France).

The initial formulation replaced egg powder with hydrolyzed pea protein, but stability issues such as oiling off were observed. Various modifications were tested and the final successful formulation excluded starch and increased pea protein concentration to 7.5%, achieving stability without phase separation.

The optimized formulation was scaled up to pilot plant production, alongside a control batch of mayonnaise made with egg yolk powder. The plant-based dressing demonstrated smaller and more uniform droplet sizes compared to mayonnaise, indicating better initial stability. Texture analysis (back extrusion test) revealed that the plant-based dressing had lower firmness, consistency, and cohesiveness than mayonnaise, attributed to the absence of starch, which acts as a thickener.

Rheological analysis involved measuring flow curves and tixotropic breakdown. Herschel-Bulkley and Weltman models were applied to characterize flow behavior and structural breakdown. The plant-based dressing showed lower yield stress and consistency index values, indicating easier flow initiation and lower viscosity compared to mayonnaise. These differences were linked to the absence of starch and the use of hydrolyzed pea protein.

The stability of the plant-based dressing was evaluated over three months at 4°C. Visual inspection revealed no changes in appearance, and droplet size distribution analysis showed minimal variations. The plant-based dressing maintained stability better than mayonnaise, which exhibited increased polydispersity and droplet size variation over time.

A sensory analysis was conducted with 95 participants, evaluating general acceptance, aroma, consistency, mouthfeel, flavor, and acidity. The plant-based dressing received high acceptance rates, with 89.4% of participants expressing positive overall acceptance.

The plant-based dressing developed in this study demonstrated good stability, favorable sensory properties, and potential for mass market acceptance. The absence of starch in the formulation contributed to the creation of a product with high nutritional value, low carbohydrate content, and rich in protein, suitable for

people requiring low-glycemic diets. Furthermore, the dressing is allergen-free, as the pea protein is also allergen-free, and no egg is used in the formulation.

Keywords: pea

CIPCA25-72973

Optimizing acorn starch extraction: Valorization of quercus rotundifolia co-products for sustainable food applications

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Abstract:

Quercus trees cover approximately 34% of Portugal's forested land, significantly contributing to acorn production. Despite, acorns are virtually the most abundant fruit in Portugal, and its relevant use in the past as a staple food for famine periods, they remain largely underutilized, with less than 1% being incorporated into human nutrition. Since Portugal is a net food importer, utilizing these nutrient-rich resources with proven nutritional value is extremely important. Furthermore, acorn co-products have significant potential as sources of bioactive compounds, which can help reduce food waste, promote upcycling, and support innovative health-focused applications. Acorn kernels, particularly, contain starch with unique physico-chemical characteristics, which offer promising properties for developing gluten-free, value-added food products. This work aimed to valorize the oxidized kernel (OKL) from *Quercus rotundifolia*, an endemic Portuguese species, as a co-product provided by Landratech, by optimizing the extraction and characterizing its starch.

OKLs were dried, and ground into flour and, before starch extraction, the fat content of the OKL flour was removed. Two different conditions were tested for starch recovery, using alkaline extraction: i) NaOH 0.2% (w/v) extraction, using a 1:2 (w/v) sample to solvent ratio (AL-2) and, ii) NaOH 0.2% (w/v) extraction, using a 1:4 (w/v) sample to solvent ratio (AL-4). An aqueous extraction (1:2 w/v) was used as a control, to compare the starch properties (CE). These processes were all followed by isolation through sieving, and drying (40 °C, 2 days). The starch content within the resulting powder was quantified spectrophotometrically, revealing an overall yield and purity of 78.18±3.32% (w/w) and 67.43±2.29 % (w/w), 79.80±7.54% (w/w) and 74.35±6.39% (w/w), 90.18±2.88% (w/w) and 87.79±3.71% (w/w) for each extraction, respectively. AL-4 revealed that its respective methodology was the most efficient regarding starch yield and extract purity. The FT-IR spectra of the starch samples were also evaluated to assess and compare their chemical structure, which revealed that AL-2 and AL-4 showed similar profiles to the CE, suggesting the preservation of the starch's typical functional chemical groups across the different extraction conditions. Moreover, using a dynamic rheometer, the rheology of these three extracts 'solutions was assessed regarding shear viscosity and dynamic oscillation. Therefore, an aqueous 8% (w/v) solution was prepared and allowed to reach complete gelatinization (90 ºC, 30 min). CE, AL-2, and AL-4 showed a similar shear viscosity profile, revealing a Newtonian shear thinning flow behavior, meaning a decrease in viscosity with an increase in the shear rate4. For dynamic oscillation, AL-4 has shown a similar profile to CE where G ' and G'', as a frequency function, presented higher values than AL-2. This suggests that the former condition better preserves the starch's structural integrity. Furthermore, at lower frequencies (0.2 – 2.2 Hz) both CE and AL-4 presented G''>G', meaning that these solutions presented a liquid-like behavior, different from AL-2 (G'>G''), which showed a more elastic-like behavior.

Overall, these findings highlight that the AL-4 extraction method is the most effective in maximizing the starch yield, purity, and structural integrity, making it a promising approach for future applications in food product development while also supporting a circular economy.

Keywords: co-products; starch; acorn; food applications

CIPCA25-78237

Protein-rich chia by-product from defatted flour as a novel nutritional ingredient in the production of fresh pasta

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Abstract:

Pasta, traditionally made from wheat, is a widely accessible and versatile food primarily composed of carbohydrates. Its nutritional profile can be improved by incorporating functional ingredients such as chia (*Salvia hispanica* L.), which is rich in essential fatty acids, proteins, minerals, and vitamins. The chia oil industry generates by-products with high nutritional value, which can be used to enhance food formulations while reducing waste.

In this study, wheat flour, chia seeds, and chia by-products (whole flour and protein fractions) were used as raw materials. Five pasta formulations were prepared: a control (100% wheat flour) and four with 10% chia substitution. Samples were analyzed for moisture, starch, protein, lipids, ash, texture, cooking properties, color, phytate, and mineral content. The amino acid profile and protein quality (score) were determined, and starch digestion and glycemic index were assessed using enzymatic methods.

Chia protein-rich fractions present a promising alternative to animal proteins, as they contain sufficient lysine—an essential amino acid often limited in cereals. Although pasta enriched with chia by-products showed higher mineral content, its elevated phytic acid levels, typically associated with proteins, may reduce the bioavailability of iron and zinc, as suggested by phytate/mineral molar ratios. Additionally, calcium absorption could be inhibited in formulations containing chia seeds or chia protein, highlighting potential limitations in mineral bioavailability. Despite the reduction on starch content, the incorporation of chia ingredients did not significantly alter the glycemic index of the pasta.

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Keywords: chia proteins; EFSA; pasta

CIPCA25-80417

Tannases, unique biocatalysts for specialized food processing

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Abstract:

Tannases are hydrolytic enzymes that catalyze the breakdown of hydrolyzable tannins, particularly gallotannins and ellagitannins, into their respective monomeric units, such as gallic acid and glucose or ellagic acid and glucose, respectively. These enzymes have broad applications in specialized food processing, including tea, coffee, wine, and fruit juice industries. Tannases are generally glycoproteins that exhibit a dimeric or trimeric structure with multiple catalytic and substrate-binding domains. Exhibit broad substrate specificity, hydrolyzing polyphenolic compounds present in food matrices. Tannases hydrolyze ester and depside bonds in tannins, converting complex polyphenols into bioavailable forms such as gallic acid, ellagic acid and glucose. The reaction products enhance flavor, reduce astringency, and improve the antioxidant properties of food products. It reduces bitterness and astringency by hydrolyzing tannins and enhances the bioavailability of polyphenols, improving antioxidant potential. Tannase prevents haze formation by degrading tannins that interact with proteins and improves the sensory attributes of alcoholic beverages. Tannase reduces turbidity and astringency in pomegranate, apple, and grape juices and enhances stability and nutritional profile by increasing bioavailable polyphenols.This enzyme reduces bitterness in cocoa beans, improving flavor and acceptability. Also, it supports microbial growth in fermentation processes by modifying polyphenolic compounds into fermentable substrates. Their ability to hydrolyze tannins enhances food quality, improves bioavailability, and contributes to the development of functional foods with improved sensory and nutritional attributes. In this work, several research studies of the group on tannase production, recovery, purification, characterization and applications are presented and, we also analyze and discuss these versatile biocatalysts with unique biochemical, catalytic, and physicochemical properties that make them valuable for specialized food processing.

Keywords: Tannases, bioactive compounds, properties

CIPCA25-81509

Effect of formulation on soy-based o/w emulgels: Molding gelation vs. 3d printing processing

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Abstract:

Fats play crucial roles in food by contributing to nutritional value, texture, flavor, and preservation. However, their consumption is often associated with negative health effects. In contrast, polyunsaturated fatty acids are linked to improved cholesterol levels and a reduced risk of heart disease and stroke. Nevertheless, their low oxidative stability and textural limitations present challenges. Structuring oils with biopolymers offers a promising approach to replace saturated fats in food products. Emulgels can be created that mimic the textural properties of saturated fats without altering the degree of unsaturation or producing trans fatty acids. Furthermore, processing these systems through 3D printing enables the creation of foods with personalized shapes, textures, and nutritional profiles. The objective of this study was to analyze the effect of formulation in O/W emulgels prepared with different proportions of soybean oil and mixtures of soy protein isolated (SPI) and k-carrageenan (kC), either gelled by molding or processed through 3D printing. Aqueous dispersions of SPI (5% w/w) and different concentrations of kC (0; 0.5; 1.0% w/w) were prepared at 60°C with magnetic stirring for 1 h. These dispersions were used to prepare O/W emulsions with different soybean oil contents (Φm = 0.1; 0.3; 0.5; 0.7) using an Ultraturrax homogenizer (17,500 rpm for 500 s). The resulting emulsions were either refrigerated in cylindrical molds for 24 h to obtain emulgels by molding or refrigerated for 1 h and processed as bioinks in a 3D printer (FoodIni, Natural Machines) to obtain printed cylindrical emulgels. The emulsions were characterized according to their morphology, global stability, and printability. The emulgels were studied for their visual appearance and textural behavior. All formulations produced fluid emulsions after homogenization, except for those with 1.0% w/w kC, Φm = 0.7, which did not form an emulsion, and those with 0.5% w/w kC, Φm = 0.7, whose emulsions were not fluid. Optical microscopy showed an increase in droplet size and polydispersity with the oil content; although, the kC addition did not significantly affect droplet size. All emulsions showed an increase in the percentages of backscattering (BS%) with the increasing kC concentration and Φm. Emulsions stabilized only with SPI exhibited signs of destabilization after 24 h, while the addition of kC prevented destabilization and enabled gelation. The molded emulgels were self-supporting, smooth, whitish and opaque, retaining all incorporated oil. The textural properties and printability of the emulgels were influenced by kC concentration and Φm. Increasing Φm and kC increased emulgel hardness and adhesiveness without altering cohesiveness. All kC-stabilized emulsions were printable and maintained a stable shape after processing. Visual appearance improved by 0.5% kC and a higher oil phase, making them more homogeneous. These emulgels also exhibited increased hardness with higher oil and kC content, although they showed lower hardness than molded ones with the same formulation. These distinctive characteristics could be advantageous for various products. The emulgels could be used as substitutes for saturated and trans fats in the formulation of healthy foods and to develop foods with defined characteristics and/or for specific nutritional requirements.

Keywords: soy protein, kcarrageenan, oil structuring

CIPCA25-81782

Developing strategies for incorporating insect flour into food products: enhancing nutrition and sustainability

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Abstract:

The growing global population and increasing demand for protein-rich diets present significant challenges to conventional food production systems. Traditional livestock farming is associated with high environmental costs, including extensive land use, water consumption, and greenhouse gas emissions. As a result, there is an urgent need to explore sustainable and efficient alternative protein sources. Edible insects have emerged as a promising solution due to their high protein content, essential amino acid profile, and low ecological footprint. Studies indicate that insect-based proteins can match or even surpass traditional sources like beef, poultry, and fish in terms of nutritional value. Furthermore, insect farming requires significantly fewer resources, producing less waste while supporting circular economy models. The use of insects in food is currently underexplored, as their use in the food industry is being approved.

In this study, our goal is to explore the potential of *Tenebrio molitor*, an insect species approved for human consumption, as an alternative protein source for food spreads. We first examined the behaviour of insect meal in systems containing aqueous and lipid phases. Gelled oil displayed low miscibility and a tendency for phase separation, whereas free oil proved more effective in forming stable emulsions. Similarly, free water systems were more prone to separation compared to gelled water systems. Among the hydrocolloids tested, k-carrageenan exhibited the highest stability when incorporated into insect meal.

To optimize the spread formulation, various pre-treatments were studied, including thermal processing, mechanical shearing, alkaline treatments and combinations of them. These treatments significantly enhanced the solubility of insect meal (54.41% ±3.74) and reduced particle size by 81.96% (from 792.67 µm to 143.00 µm in 90% of the particles of the sample), improving its functional properties and making it more suitable for food applications.

One of the main challenges in incorporating insects into food products is their not-so-pleasant aftertaste. To enhance sensory appeal, we integrated olives into the savory spread and cocoa into the sweet version, improving both flavor and aroma. The resulting spreads were further analyzed for texture and color characteristics.

Using *T. molitor* flour, we successfully developed innovative spreads with strong commercialization potential. Given its high protein content, it was possible to produce savoury and even sweet spreads with the labelling of a protein-rich product (at least 20% of the energetic value provided by the protein component).

In conclusion, insects represent a nutritionally rich alternative protein source that could revolutionise the food industry as indicated by this study. They offer environmental benefits and a viable solution to current global food challenges.

Keywords: Edible insects; Food innovation; Protein

CIPCA25-82630

Determination of functional properties of okara protein concentrate obtained by different methods

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Abstract:

It has little market value, so it is usually used as animal feed or discarded. The growing consumption of plant-based products has led to the search for more sustainable vegetable products processing with greater profits. Okara is a protein-rich byproduct from soymilk production. The process includes a step of grinding in cold or hot water in to broke cell walls, but, some cells remained undamaged. In this work enzymatic-assisted pretreatment (EAP) was used to improve protein extraction by hydrolyzing these cells walls. The effect of the milling type and the EAP on the extraction yield, thermal behavior and protein solubility were evaluated on okara protein concentrates.

The grinding of okara was made okara with hammer (water at 90ºC) (HO) and disc (water at 20°C) (DO) mills. The extraction of protein concentrates were made from DO and HO by solubilization at pH 8.0 and subsequent precipitation at pH 4.5 (traditional method) and named PCD-T and PCH-T . In addition, an EAP with Viscozyme was performed before the extraction at pH 8.0 to, obtain PCD-E and PCH-E. On the protein concentrates were determined the protein content (Kjeldahl method, Nx6.25) was determined by the and protein solubility in a pH range between 2.5 and11.5(BCA method) (). Thermal behavior was analyzed by DSC for HO and DO and their protein concentrates.

The heat-treated samples (hammer mill) showed lower protein extraction yield being the . extraction yield in the EAP samples of 17 times for HO and 3 times for DO higher than the traditional method. The thermal profiles of HO, PCH-E and PCH-T did not showed the typical endothermic peaks of soy proteins, which means that thermal treatment caused the denaturation of proteins probably denatured them. For all the concentrates the protein solubility vs. pH curve showed the typical U-shape with a minimum at pH 4.5.

In conclusion, disc milling leads to higher protein yields and lesser protein denaturation. The EAP treatment increased the protein extractability. These findings encourage the potential utilization of okara protein concentrates to obtain functional food ingredients.

Keywords: okara, grinding method, protein concentrates

CIPCA25-82683

Functional and sustainable food innovation: incorporation of insect hydrolysates in tuna pâté

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Abstract:

The growing demand for alternative protein sources has led to an increased interest in insect-derived proteins due to their sustainability, high nutritional value, and functional properties. Many insect species contain approximately 60% protein and are recognized as a good source of other important nutrients, such as iron, zinc and calcium. Insect protein hydrolysates have demonstrated promising bioactive properties, including antioxidant, anti-hypertensive and anti-inflammatory effects, making them suitable for incorporation into food products. This study explores the development of a novel tuna pâté enriched with insect protein hydrolysate, aiming to enhance its protein content and assess its potential health benefits, as well as its behavior throughout gastrointestinal digestion.

The pâté was formulated using fresh tuna, dried tomatoes and the hydrolysate, in proportions that ensured an appealing texture and flavor for consumer acceptance. The insect hydrolysate used in this study contained 57.1% protein, with a significant proportion of low molecular-weight peptides: 47.35% within the 1–3 kDa range and 45.85% below 1 kDa. The incorporation of this hydrolysate into the pâté formulation is expected to improve not only its protein profile but also its bioactive properties, given that the hydrolysate exhibited antioxidant, anti-inflammatory, anti-obesity and anti-hypertensive activities. To evaluate the true potential of the pâté, protein stability and bioavailability after ingestion must be assessed. For this purpose, an *in vitro* gastrointestinal (GIT) tract digestion simulation was performed, following the standardized static digestion model of the INFOGEST 2.0 protocol. Post-digestion characterization included protein quantification to determine the impact of digestion on protein content and structure. Additionally, the bioactive properties of the digested product were evaluated, including antioxidant, antihypertensive, and antidiabetic activities, to assess its potential functional benefits.

This study contributes to the advancement of novel protein-rich foods, demonstrating that insect hydrolysates can be used to enrich food formulations, not only enhancing their protein content but also providing new properties that may align with EFSA health claims, contributing to the development of new functional foods. Additionally, insects serve as a sustainable protein source, addressing the global demand for alternative proteins and supporting sustainable development goals by potentially reducing the reliance on traditional protein sources. However, despite being consumed in several countries, insect-based products face acceptance challenges in Western markets due to consumer stigma. Therefore, it is necessary to overcome these barriers by increasing awareness and highlighting the nutritional and environmental benefits of insect proteins. Future work will focus on sensory analysis with a consumer panel to validate the acceptability of this innovative formulation, as well as on developing effective communication strategies to improve consumer acceptance of the product.

Keywords: Sustainability; INFOGEST; Bioactivities; Peptides; New-Food

CIPCA25-85495

Lignin nanoparticles and chitosan-coated lignin nanoparticles for pickering

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Abstract:

The shift toward eco-friendly materials has increased interest in lignocellulosic biomass as a renewable resource. Lignin nanoparticles (LNP) have emerged as promising candidates for various applications due to their nanoscale properties, including potential use in Pickering emulsions, where solid particles stabilize oil-in-water (O/W) interfaces. This study explores lignin extraction from corncob using the organosolv method, followed by the synthesis of LNP and chitosan-coated lignin nanoparticles (chi-LNPs) as stabilizers for O/W emulsions. LNP from 60 nm and 120 nm (by number), PDI of 0.160 and 0.260, and ζ-potential of approximately – 30 mV were produced. Chitosan modification imparted a positive charge to the chi-LNPs, affecting their interfacial behavior. Chi-LNPs presented a higher contact angle than LNPs, but no changes in the surface tension of LNPs was verified. However, higher particle concentrations resulted in lower surface tension. These particles were used in the development of Pickering emulsions at different O/W ratios. Chi-LNPs resulted in higher droplets than LNPs. Stability analysis revealed that the LNP-stabilized emulsion with a 1:99 O/W ratio exhibited no creaming, whereas other formulations, particularly those with chi-LNPs, demonstrated limited stability with creaming indices exceeding 65%. However, the increase in LNP and chi-LNP concentrations led Pickering emulsions with lower creaming index, reinforcing the potential of LNP in creating sustainable emulsions, offering an environmentally friendly alternative to conventional stabilizers.

Keywords: lignin, nanoparticles, Pickering emulsions, chitosan

CIPCA25-88964

Texturized ingredient rich in protein from babassu biomass

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Abstract:

The babassu is a native palm from the Brazilian Amazon, Cerrado, and Caatinga transition zone. The fruit consists of four parts: epicarp (the thin peel), mesocarp (starchy layer), wooden endocarp, and kernel. Babassu kernel is a key product with great socioeconomic importance for agro-extractivist families working in the babassu forest. The mesocarp flour of babassu is particularly notable for its nutritional composition, which includes proteins (1.5 %), lipids (0.18 %), ash (0.81 %), dietary fiber (14.3 %), and carbohydrates (83.21 %). Within this carbohydrate content, 72.9 % consists of starch, making the mesocarp flour an intriguing matrix for bioprocesses. Enzymatic hydrolysis presents an efficient method for converting starch into fermentable sugars, providing a sustainable alternative to carbohydrates derived from other crops. This work aimed to apply enzymatic-hydrolysis on babassu mesocarp, increase protein content with fermentation, and finally produce texturized protein from babassu. The mesocarp babassu flour was enzymatic-hidrolyzed using α-amylase and glucoamylase. The hydrolyzed was centrifuged (5000 rpm, at 4°C, 20 min), the supernatant was autoclaved, and the precipitate was dried in an oven at 50°C. Using a stirred-tank biorreator, the babassu mesocarp hydrolyzed was used as substrate for yeast biomass production. Saccharomyces cerevisiae fed-batch cultivation was performed at pH 5.2, 400 rpm, 32°C for 42 h. The biomass was centrifuged (5000 rpm, at 4°C, 20 min), and dried in an oven at 50°C for 48h. The texturized ingredient rich in protein was obtained by a mix of soy concentrate (68.8%), babassu flour (14.2%), yeast (10.0%), and precipitate of the hydrolyzate (10.0%). The condition of extrusion: temperature zones from 2 to 8, and die, 40°C, 60°C, 80°C, 110°C, 120°C, 145°C, 140°C and 140°C; gravimetric feeder, 1120 Kg/h, liquid feeder, 30% (v/h); speed, 500 rpm (Process 16, Thermo Fisher Scientific, Germany). The extruded was evaluated regarding expansion ratio, water absorption index (WAI), and protein. After the hydrolysis process, total reducing sugar levels increased from 7.8 g/L at the beginning of the process to 147.7 g/L, representing a 19-fold increase. The fed-batch cultivation resulted in a biomass production of 29.77 g (dry matter), yielding a conversion factor of substrate to yeast biomass (YX/S) of 0.40. The extruded showed an expansion ratio of 1.15 mm/mm; WAI 4.27 g/100g, and protein 46.33 g/100g. In conclusion, the utilization of babassu mesocarp as a substrate for bioprocesses presents significant potential for both the agro-extractivist families in Brazil and the broader food industry. Through enzymatic hydrolysis, followed by fermentation and extrusion processes, it is possible to increase the protein content of babassu mesocarp, producing a sustainable source of texturized protein.

Keywords: fermentation; biomass; protein

CIPCA25-89251

Nanoemulsions as delivery systems for algal lipids: Stability, antioxidant enhancement, and food application potential

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Abstract:

Algae-derived lipids, particularly those rich in omega-3 polyunsaturated fatty acids, have garnered significant attention as functional ingredients due to their health benefits and potential as a sustainable alternative to fish oil. However, their application into food systems is hindered by poor stability, susceptibility to oxidation, and low bioavailability. To overcome these limitations, lipid-based nanosystems, such as nanoemulsions (NEs), have been explored. Thus, in this study, we developed and characterized lipid-based NEs for the encapsulation of a food-grade algal lipid extract (LE).

First, LE was obtained via ultrasound-assisted extraction with ethyl acetate from an algae blend (Algaessence®). Then, NEs were formulated by incorporating LE into medium-chain triglyceride oil and emulsifying using rhamnolipids (0.05 wt%) as a biosurfactant. Two lipid-phase concentrations (1 and 5 wt%) were tested. NEs were produced through a two-step process combining pre-homogenization followed by high-energy ultrasonication. Control NEs, without LE, were prepared for comparative analysis. Physicochemical properties, including particle size (PS), polydispersity index (PDI), zeta potential, and stability under different pH conditions, were assessed. The antioxidant activity, including the LE, was evaluated using the ABTS assay. Additionally, NEs stability was monitored over 1 month at room temperature (RT) and refrigerated (4ºC).

At day 0, LE–NEs exhibited favorable physicochemical characteristics, including PS <200 nm (1wt%: 137.4±2.6 nm; 5wt%: 187.2±0.6 nm), low PDI (1wt%: 0.205±0.026; 5 wt%: 0.201 ±0.015), and zeta potential of 1wt%: –70.9±1.9 mV and 5wt%: –66.9±2.1 mV, suggesting good colloidal stability. After 1 month of storage, the 1wt% formulation exhibited a small increase in PS, while PDI remained stable, and zeta potential decreased at both RT and 4°C conditions. In contrast, the 5wt% formulation maintained all parameters stable throughout the storage period, but a slight creaming layer was visually observed at the surface. In both formulations, these changes were more pronounced at RT, suggesting a higher susceptibility to destabilization under non-refrigerated conditions. Notably, LE–NEs exhibited a smaller PS compared to control NEs, indicating an effect of the LE within the system. Additionally, LE–NEs demonstrated superior resistance to pH-induced destabilization (pH 2.5–9) compared to unloaded NEs, reinforcing their

potential for incorporation into diverse food matrices. Encapsulation also significantly enhanced the antioxidant activity of LE, as evidenced by an increase in $ABTS^{\bullet+}$ scavenging capacity confirming the protective role of the emulsion system. However, a decrease in antioxidant capacity was observed after 1 month of storage, indicating a gradual loss of activity over time.

In conclusion, the successful encapsulation of algal lipids in NEs highlights their potential as effective delivery systems for functional food ingredients. As a novel colloidal system, developed NEs exhibited good stability and enhanced the bioactive properties of the LE. Future research will focus on assessing the digestibility and gastrointestinal fate of these NEs, comparing the bioavailability of the encapsulated versus non-encapsulated extract, and evaluating their functional performance in real food systems.

Keywords: nanoemulsion, algal lipids, antioxidant activity

CIPCA25-89270

Enhancing protein content and reducing antinutritional factors in beluga lentils through combined germination and fermentation

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Abstract:

As leguminosas ganharam atenção significativa devido ao seu rico perfil nutricional e potenciais benefícios à saúde, levando a uma maior demanda de mercado por esses produtos. As lentilhas (*Lens culinaris*spp.) ganharam destaque como uma cultura valiosa devido ao seu rico perfil nutricional, incluindo altos níveis de proteína (20-30%), fibra e micronutrientes essenciais, como ferro, zinco e folato. No entanto, a aplicação da maioria dos grãos de leguminosas, incluindo lentilhas, permanece limitada devido a compostos antinutricionais que interferem na absorção de nutrientes, especificamente proteínas, reduzindo sua digestibilidade e biodisponibilidade ao limitar a ação das enzimas de digestão. Várias técnicas de processamento foram exploradas para melhorar o perfil nutricional das leguminosas e melhorar a acessibilidade e biodisponibilidade de seus nutrientes, inativando ou eliminando antinutrientes. Assim, este estudo teve como objetivo investigar o efeito da germinação e fermentação no conteúdo de proteína de lentilhas Beluga, acessando sua influência no conteúdo de proteína e antinutrientes específicos que reduzem sua assimilação e aceitabilidade geral. Este estudo investigou os efeitos sinérgicos da germinação (3 dias em condições escuras) e fermentação (24 horas a 50 °C) usando bactérias do ácido láctico (*Lactobacillus rossiae* , *L. brevis*) e levedura probiótica (*Saccharomyces boulardii*) em lentilhas Beluga. Os resultados mostraram que o conteúdo de proteína aumentou significativamente em todos os métodos de processamento, aumentando de 26,8 g/100g dw em lentilhas cruas para 38,4 g/100g dw após a combinação de germinação e fermentação com *L. rossiae* e *S. boulardii* . Esses resultados se alinham com aqueles obtidos para antinutrientes específicos, ou seja, taninos condensados, que formam complexos estáveis com proteínas dietéticas, reduzindo sua bioacessibilidade, e cujas concentrações diminuíram significativamente durante o processamento combinado.

Este estudo é o primeiro relatório sobre a aplicação combinada de fermentação de germinação em lentilhas, fornecendo insights pioneiros sobre seu potencial para elevar simultaneamente o conteúdo de proteína e reduzir componentes antinutricionais de leguminosas. Essas descobertas destacam o papel significativo da integração de diferentes técnicas de processamento, especificamente fermentação, para alcançar melhorias notáveis na disponibilidade de nutrientes e redução de fatores antinutricionais, contribuindo para o conhecimento do processamento de leguminosas e abrindo caminho para o desenvolvimento de produtos alimentícios inovadores, ricos em nutrientes e funcionais à base de plantas.

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Keywords: Protein; Antinutritional Factors; Lentils; Fermentation

CIPCA25-89533

Improving the physical properties of carioca bean protein flour through high-shear wet granulation

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Abstract:

Alternative plant protein sources are key in diversifying raw materials for protein-based products, a growing trend in the food industry. The large-scale production of carioca bean (*Phaseolus vulgaris* L.) in Brazil makes this legume a promising raw material for plant-based protein extraction. Plant-based proteins are usually commercialized in powder and used either as a food supplement or as an ingredient in food formulations. However, most of these powders contain fine particles with low wettability and poor flowability, which can limit their application. The agglomeration process is performed to improve the physical properties of powders such as wettability, flowability, mechanical resistance, and appearance. In this study, the high-shear wet granulation of carioca bean protein flour was investigated as a potential method to produce a protein powder with enhanced physical properties. Polydextrose and gum Arabic were used as binder agents since they are dietary fibers, providing a healthier alternative to maltodextrin, which is commonly used. Granulation was performed in a high-shear mixer granulator using 0.30 kg of carioca bean protein flour (CBPF, protein content 40.16% dry basis). Binders were applied in powder form and as solutions, with polydextrose at 5% and 50% (w/w), gum Arabic at 5%, 10%, and 20% (w/w), and polydextrose and gum Arabic in powder form at 5% and 10%, respectively. The powders were characterized by moisture content, water activity, color, particle size, flowability, and wettability. The granulation of CBPF was successfully performed in a high-shear mixer, producing large granules with a short wetting time, low moisture content (≤15%), water activity (<0.60), and improvement of the flowability. These results demonstrate the effectiveness of polydextrose and gum Arabic as alternative binder agents. The median particle size of the granulated powders ranged from 19.00 μm to 339.01 μm, which was at least 1.2-times larger than the CBPF powder (D50 = 15.76 μm). Most of the granulated powders exhibit flowability levels changing from poor (CBPF powder) to good or even excellent. Furthermore, the powders showed better wettability compared to the CBPF, as indicated by a reduction in wetting time, which ranged from 79% to 99%. The shortest wetting times (10 s and 8 s) were achieved under conditions that also produced particles with the largest particle sizes (198.30 μm and 339.01 μm), resulting from the use of polydextrose and gum Arabic as powdered binders. Additionally, a noticeable color difference ($\Delta E > 5.0$) was observed when comparing CBPF powder to the granulated powders. In general, the best conditions for granulating CBPF were achieved using 50% polydextrose and 5% gum Arabic solutions, as well as polydextrose and gum Arabic in powder form. These conditions resulted in powders with the shortest wetting times (< 40 s) and flowability levels ranging from good to excellent. Finally, once the technical feasibility of granulating CBPF was demonstrated, the next steps should focus on evaluating a broader range of process conditions using an experimental design approach to identify the optimal operational parameters for producing a protein powder with the desired characteristics.

Keywords: plant protein; bean; high-shear; granulation

Oral Communications

Colloids for edible films

CIPCA25-15679

Development and characterization of chitosan-alginate films incorporated with cinnamon essential oil nanoemulsion or zinc oxide nanoparticles

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Abstract:

Agricultural products are highly perishable and susceptible to physical damage, moisture loss, and microbial contamination after harvest. Biopolymer-based packaging, incorporated with antimicrobial agents, represents a sustainable alternative for preserving product quality. This study focused on developing chitosan-alginate composite films, incorporating cinnamon essential oil (CEO) nanoemulsion and zinc oxide (ZnO) nanoparticles, to enhance the mechanical, physicochemical, moisture barrier, and antifungal properties for food packaging applications. Polymeric solutions (1.5% w/v) were prepared using chitosan:alginate ratios of 100:0 and 75:25, with active agents, including 0.25% w/v CEO nanoemulsion (Cc) and 0.05% w/v ZnO nanoparticles (Cz), incorporated separately into the films. Chitosan was dissolved in 1% v/v acetic acid under magnetic stirring, while alginate was solubilized in distilled water at 60°C (Ultra-Turrax, 6,500 rpm). CEO nanoemulsions were prepared by homogenizing CEO:polysorbate 80 in the proportion 1:1 w/w and water (Ultra-Turrax, 10,000 rpm, 5 min), followed by sonication (150 W, 3 min). The nanoemulsions and ZnO nanoparticles were separately dispersed into the polymer solutions (Ultra-Turrax, 6,500 rpm), poured onto acrylic plates, and dried at 35°C for 48 h. Polymeric films without the addition of active agents were considered as control. The films were characterized in terms of moisture content, swelling, solubility, mechanical and barrier properties. Additionally, their antifungal activity against Colletotrichum gloeosporioides was assessed. The mechanical properties of the films were significantly influenced by the incorporation of ZnO compared to the control. ZnO-containing films (100Cz and 75Cz) exhibited the highest tensile strength (41.10–43.66 MPa) and Young's modulus (23.42–24.52 MPa), indicating that ZnO nanoparticles contributed to increased rigidity and reduced flexibility, as reflected by lower elongation at break (4.42–5.73%). Physicochemical analysis showed that ZnO-containing films were thinner (53.3–58.9 μm) and exhibited lower moisture content (21.76–25.28%) and solubility (14.32–22.86%) compared to CEO films, suggesting that ZnO reduced the hydrophilicity of the films, improving their moisture resistance. ZnO films also showed increased opacity, particularly in the 75Cz formulation (6.06 A600/mm), indicating light protection that may extend the shelf life of sensitive food products. CEO-containing films, on the other hand, showed a higher swelling capacity (89.92–605.03%), particularly in the 75Cc formulation, indicating that CEO increased the water absorption capacity of the films, which could benefit moisture retention in fresh fruits and vegetables. Water vapor permeability (WVP) results indicated that films containing 100% chitosan (100Cc and 100Cz) exhibited lower WVP, suggesting improved moisture barrier properties. In contrast, formulations containing alginate (75Cc and 75Cz) showed increased WVP, indicating higher wa-

ter vapor transmission. Films containing CEO exhibited lower WVP compared to those with ZnO, particularly in the 100Cc formulation. Possibly the hydrophobic character of the CEO components influenced the film's permeability. All films containing nanoemulsions and nanoparticles inhibited fungal growth by contact, whereas films without nanometric components allowed fungal proliferation. These results emphasize the importance of incorporating active agents into polymeric films to prevent post-harvest pathogen growth. These data reveal the potential of these films for sustainable food packaging and post-harvest quality maintenance of agricultural products.

Keywords: Antimicrobial agents; Fungal inhibition; Packaging

CIPCA25-27609

Chitosan active packaging incorporated with bioactive extract from jaboticaba (*Myrciaria jaboticaba*): Development, characterization and application

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Abstract:

Prevent the oxidative process is crucial to extend shelf-life and maintain the food quality. Synthetic antioxidants are chemical additives widely used in the food industry to prevent food oxidation, however, due to potential human risk exposure if consumed in high levels, novels alternatives are sought to replace these chemicals. Among these novel technologies, active packaging incorporated with natural antioxidants stands out. This work aimed to develop and characterize biodegradable chitosan-based film incorporated with anthocyanins extracts from jaboticaba's peel (AEJP) and study its effect on oxidative stability of cooked pork meat. The extracts showed an average content of anthocyanins and phenolics compounds of 1.77 mg of cyanidin-3-o-glucoside and 30.91 mg of gallic acid equivalents (GAE) per ml of extract, respectively, and antioxidant activity of 317.13 mM Trolox·mL-1 and 602.27 mM Trolox·mL-1 by the methods of ABTS*+ and DPPH* radicals scavenging, respectively. Films were produced by casting with the incorporation of 0%, 0.3%, 0.5%, 0.8% and 1.0% (w/w) of anthocyanins. The incorporation of EAJP in the polymer matrix resulted in increase on thickness, solubility in water and oxygen permeability (OP), and decrease on moisture content, swelling index in water and water vapor permeability rate. The mechanical properties were influenced by the incorporation of the extracts, the films showed an increase in strength and rigidity and decrease elongation at brake with the addition of small percentages of extracts, however in superior concentrations the biopolymers turned less rigid and resistant, with greatest elongation at brake, due to the plasticizing characteristic of anthocyanins extract. Infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) corroborated the changes in the functional properties of the biopolymers developed, as chemical interactions between polymer chain and phenolic compounds as well as the structural of the films could be observed. Pork cooked meat packed in the active films incorporated with AEJP showed higher oxidative stability. The levels of thiobarbituric acid reactive substances (TBARS) from treatment starting with 0.5% anthocyanins, were lower than the values reported in the literature as a rejection threshold, even after 15 days refrigerated storage. Meanwhile, for the control treatment and 0.3% anthocyanins, TBARS values for rejection were reached at the fourth and sixth day of storage, respectively. Demonstrating the effectiveness of the active film developed in inhibit the oxidative process of cooked pork meat, and hence its potential use in the food industry.

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Keywords: natural antioxidant; anthocyanins; chitosan; meat

Emerging technologies in food protein research

CIPCA25-18122

Evaluation of p24's impact on a corn- and soy-based chicken feed formulation

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Abstract:

Optimizing protein digestibility in poultry feed is critical for maximizing nutrient absorption, improving gut health, and enhancing overall feed efficiency. Protein hydrolysis primarily begins in the stomach, where proteins are broken down into peptides and amino acids, which are then absorbed in the small intestine. This study evaluated the use of a novel protease, P24, in the pre-processing hydrolysis of chicken feed containing Smart Distillers Dried Grains (DDG) and soy as protein sources. The goal was to improve protein digestibility and understand its impact on the gut microbiome, assessing P24's suitability for poultry feed formulations.

P24 is a protease produced via precision fermentation by Digestiva (US). It belongs to the S53 family of enzymes, known as sedolisins or serine-carboxyl peptidases, which are particularly effective under acidic conditions.

Chicken feed was hydrolyzed using P24 under acidic conditions, and the degree of hydrolysis (DH%) was measured. The resulting hydrolysates were then subjected to a simulated chicken digestion protocol to assess protein digestibility during both the gastric and intestinal phases. Results showed that P24 treatment significantly enhanced protein hydrolysis—up to three times higher than in untreated samples.

Digestive simulations revealed improved protein breakdown during both the gastric and intestinal phases in feeds treated with P24, underscoring its key role in facilitating digestion before the feed reaches the intestine.

To further explore the effects on gut health, in vitro cecal fermentation was conducted to assess microbiome responses. The P24-treated hydrolysates did not significantly alter the overall microbiome composition within each feed type. However, the feed supported the growth of beneficial bacteria, including lactic acid bacteria (LAB), Bifidobacterium, and total bacterial populations, all associated with improved gut health.

Microbial metabolic activity analysis revealed that fermentation of the P24-treated feed increased the production of beneficial short-chain fatty acids (SCFAs), such as acetate and propionate, as well as lactate cross-feeding, a microbial interaction vital for maintaining gut function.

These findings demonstrate that P24 enhances protein digestibility in chicken feed formulated with DDG and soy, creating a more favorable environment in the cecum. This promotes beneficial microbial populations and supports optimal fermentation dynamics. The study highlights the importance of evaluating both digestibility and microbiome interactions when developing poultry diets, to ensure efficient nutrient use and improved gut health.

Keywords: Chicken feed; Protease; DDG; Digestibility

CIPCA25-50964

Alternative protein research in widening countries: The APRISE project

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Abstract:

The global food system faces critical challenges in meeting increasing nutritional demands while reducing environmental impacts. Alternative proteins (APros)—including plant-based proteins, insect-derived ingredients, and cultivated meat—offer sustainable solutions by significantly lowering resource use and greenhouse gas emissions compared to conventional animal farming. Despite the recognized potential, Widening Countries (WCs) such as Türkiye, Poland, Greece, Malta, and North Macedonia face significant obstacles in advancing their APros sectors. These challenges include limited research infrastructure, high production costs, evolving regulatory frameworks, and insufficient governmental and private sector support. Türkiye, for example, despite hosting innovative startups producing plant-based alternatives, currently lacks strategic governmental initiatives and adequate investments, limiting its competitive potential.

To address these challenges and unlock the untapped potential of WCs, the APRISE project, funded under the Horizon Europe ERA Talents call, has been initiated. The project aims to strengthen research and innovation capabilities specifically within these countries by enhancing research skills, professional development, intersectoral and geographical mobility, policy advocacy, stakeholder engagement, and sustainable practices. APRISE employs a comprehensive approach involving targeted training programs, workshops, extensive cross-sectoral secondments, and structured dissemination activities.

Expected outcomes include increased scientific and innovation capacities among researchers and industry professionals, strengthened professional networks, and enhanced competitiveness within the global APros market. Additionally, APRISE will facilitate the integration of sustainability and policy advocacy into the broader APros ecosystem, helping these Widening Countries capitalize on their unique biodiversity, favorable agricultural conditions, and existing strengths to emerge as influential contributors to the global alternative protein sector.

Keywords: alternative proteins , widening countries

CIPCA25-53550

Applications of proteomics in determining the geographical origin of tiger nut (cyperus esculentus)

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Abstract:

Tiger nut is a grass-like plant belonging to *Cyperaceae*family with edible tubers popularly known in Spain as "Chufa" that can be consumed raw, roasted or grounded to manufacture a milky beverage called "horchata". Nutritional studies demonstrated benefits of chufa tubers consumption as a natural source of lipids, amino acids, starch, fibre, vitamins C and E and minerals, pointing out its positive effects against cardiovascular diseases, muscle cramps and erectile dysfunctions (Edo *et al.* 2024).

Cultivation of chufa pant mainly occurs in Western Africa and the Valencian community (Spain) that protects its cultivation since 1995 through the protected denomination of origin (PDO) "Chufa de Valencia". Despite the annual Valencian production of chufa is about 8000 tons this seems not to be enough to cover all market demands of horchata. Consequently, it is estimated that around 3000 tons/year of tiger nuts are imported from Western-African countries to cope with these needs. The African tiger nut is larger and cheaper, but compared to its Valencian counterpart it is poorer in fat, organoleptic properties and horchata yield. In this sense, characterization of indigenous Valencian tubers and their differentiation from foreign varieties is essential for quality assessment of chufa-derived products such as horchata mainly considering the aforementioned PDO. Thus, this research addressed the characterization and discrimination of chufa tubers from Africa (Burkina Faso and Mali) and Valencia through a proteomic approach as a way to authenticate raw materials of commercial foodstuffs.

Tubers (300 g) were grounded and mixed (1:5) with water, allowed to stand for 10 minutes, sieved (0.3 μm) and the filtrate was centrifuged, obtaining an intermediate aqueous layer that was collected, centrifuged and filtered. Protein profiles of eluates were assessed by SDS-PAGE followed by Coomassie staining and protein band quantification by densitometry analysis. Gel bands exhibiting different intensities between samples assayed were tryptically digested and peptides characterized by LC-ESI-MS/MS using Mascot 3.0 as search engine loading Unipot KB and NCBInr protein databases. As a result, seven protein bands in the 40-120 kDa MW range showed different abundances between sample groups assayed, and among them, six bands had a higher intensity in African tubers. Among them, it is worth highlighting the overexpression in African varieties of chloroplast envelope membrane 70 kDa heat shock-related protein. In line with this finding, we hypothesized that, because of harvesting, clime and soil peculiarities of the region, a higher expression of proteins related to drought stress was observed in African varieties. Results demonstrated how the proposed proteomic approach enabled differentiation of geographically different tiger nut tubers through the elucidation of reliable protein biomarkers of authenticity.

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Keywords: proteomics; tiger nut; certification; origin

CIPCA25-66299

Structuring plant-based pickering emulsions: Tunning lupin protein functionality

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Abstract:

Pickering emulsions (PEs) are stabilized by colloidal particles adsorbed at the oil-water interface, offering high stability 1. Recent advances in biomaterials have led to the development of plant protein-based particles, offering adjustable surface properties and improved biocompatibility 2. Despite their advantages, plant proteins face challenges in dispersion systems, including self-aggregation and low solubility 3. Thus, developing surface modification is essential to enhance their functionality in food applications4.

This study aimed to assess the effects of pH-shifting and heat treatments (HT) on plant-based protein particles prepared from lupin flour, focusing on their Pickering capacity. The impacts of pH-shifting (2, 8, and 12), evaluated both individually and in combination with HT (70 and 90ºC), were measured by assessing protein solubility, zeta potential, particle size, and emulsifying capacity through the preparation of a 50/50 oil/water PE at a 1% particle concentration (w/w, aqueous base). The PEs were analysed using optical microscopy.

The untreated protein dispersion could not form an emulsion, while the treated particles showed promising results. The pH-shifting process disrupted the native protein structure, and the heat treatment induced further unfolding and amino acid exposure, both contributing to the particles 'stabilizing capacity. At pH 2, reduced protein charge promoted aggregation and increased particle size, intensifying this effect at 70°C HT. At 90°C, structural reorganization reduced particle size, producing smaller and more stable PE droplets. At pH 8, pH-shifting alone resulted in larger PE droplets, which combined with HT improved the zeta potential and solubility, enhancing PE formation. At pH 12, pH-shifting significantly increased solubility, while HT at 90°C further improved the zeta potential, leading to smaller and more stable droplets.

The synergistic effect of pH-shifting and HT effectively modified the lupin protein structure, offering a promising strategy to overcome its limitations in food functionalization applications. Future work will systematically optimize the protein particle formulations and PEs production to enhance their properties and applications.

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Keywords: Plant protein; Pickering emulsion

CIPCA25-70925

Optimizing protein extraction from microalgae: A multi-step approach for improved yield and bioactive properties

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Abstract:

Microalgae are a promising source of protein-based functional ingredients; however, their complex polysaccharide matrix can hinder protein extraction. This study evaluated the impact of four extraction techniques on protein yield from six microalgae species: *Spirulina platensis*, *Chlorococcum*sp., *Tisochrysis lutea*, *Chlorella Yellow*, *Chlorella White*, and *Tetraselmis striata*. Conventional and non-conventional methods were investigated, including acidic extraction with citric acid, thermal treatment, ultrasound-assisted extraction, and enzymatic extraction using cellulase (6 U/mL). These methods were tested individually and in combination to enhance protein recovery by disrupting the cellulose-sensitive carbohydrate matrix. Protein content was measured using the Bradford method. In*S. platensis*, acidic extraction followed by ultrasound-assisted extraction yielded 18% protein (dry weight, DW). However, adding enzymatic treatment significantly improved extraction efficiency, resulting in the highest protein yield of 38% DW. Species such as *Chlorella Yellow, Chlorococcum*sp., *Chlorella White, Tisochrysis lutea*, and *Tetraselmis striata*, exhibited protein recoveries ranging from 13% to 23% DW, respectively. Antioxidant assays demonstrated notable activity in the ABTS assay, with *Tisochrysis lutea* exhibiting the highest value (99 µmol TE/g DW), followed by *Chlorococcum* sp. (86 µmol TE/g DW) and *Tetraselmis striata* (80 µmol TE/g DW). Similarly, strong reducing antioxidant capacity was observed in the FRAP assay, with *Tisochrysis lutea* achieving the highest value (30 µmol TE/g DW). The FPLC analysis revealed that the recovered proteins predominantly ranged between 1 and 5 kDa. This study demonstrates that combining extraction techniques enhances protein recovery from microalgae, with*Spirulina platensis* yielding the highest. The proteins exhibited potential antioxidant activity, underscoring their potential for food applications. However, further analysis and studies on the optimization parameters are needed to boost microalgal protein bioactivity.

Keywords: Alternative proteins Microalgae biomass

Food colloids and health (bioactive peptides, allergenicity)

CIPCA25-12582

Neuroprotective effect of hemp protein hydrolysates as anti-aggregant of β-amyloid peptide

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Abstract:

Non-communicable diseases (NCDs), including cardiovascular diseases, neurological diseases, diabetes, respiratory diseases, certain cancers and mental disorders, kill 41 million people every year, accounting for 74% of all deaths worldwide, with 90% of deaths from environmental stress in Europe attributable to NCDs. In Spain, people aged 65 and older will represent more than 30% of the total population by 2050, jeopardising progress towards achieving the Sustainable Development Goals (SDGs), including reducing deaths from NCDs (>33%) by 2030. In particular, the total number of people with Alzheimer's disease (AD) is expected to reach 82 million by 2030 and 152 million by 2050, with more women than men affected. AD is the most common neurodegenerative disease, responsible for 60-90% of cases of dementia. Misfolding and extracellular aggregation of Amyloid-β (Aβ) peptides are recognized as the main cause of AD progression, leading to their oligomerization or fibrillization and to the deposition of β-amyloid plaques in the brain, representing the hallmarks of AD. The World Health Organization (WHO) recognises the social impact of AD as a public health priority and the transformation of the agricultural and food system as a strategy for sustainable and inclusive growth. Green industries, such as industrial agricultural hemp (*Cannabis sativa* L.), represents a new source of sustainable plant protein and bioactive peptides that can exert biological functions and promote health. In previous reports, we undertook the characterisation of two hemp protein hydrolysates, HPH20A and HPH60A15F, obtained at laboratory scale with two food grade enzymes, Alcalase and Flavourzyme, respectively, including physicochemical composition, ultrastructural characterisation and anti-inflammatory properties (against murine BV-2 microglial cells, primary human monocytes and Caco-2 cells). However, until now, few studies have investigated the ability of these biomolecules to enter the bloodstream and reach the central nervous system (CNS). Recently, our research team also identified the peptidomes of HPH20A and HPH60A15F by LC-TIMS-MS/MS, in collaboration with the Proteomics Unit of the Central Research Support Service of the University of Cordoba. The bioavailable peptides contained in HPH20A and HPH60A15F (bioHPH20A and bioHPH60A15F, respectively) were synthesised by the ICTS NANBIOSIS-CSIC from the fraction collected from the transwell system using Caco-2 cell culture as an absorption model according to the procedure described in our patent P202230873. The results obtained by chromatography and multi-omics analysis allow us to confirm that almost 50% of the peptides present in both protein hydrolysates were able to cross Caco-2 cells. The aim of this study is to evaluate the β-Amyloid anti-aggregation capacity of the bioavailable peptides present in both HPHs. The neuroprotective capacity of HPHs was evaluated by the thioflavin T (Th-T) fluorimetric assay owing to their measure of capacity to inhibit the formation of oligomers and/or β-Amyloid fibres. *In*

vitro cell-free experiments showed that both hydrolysates inhibit the formation of oligomers, but that only the hydrolysate obtained with Alcalase (HPH20A) was able to disintegrate fibre formation after 13 days. These findings open new opportunities for developing nutritional strategies with hemp as a dietary source of biopeptides to prevent the development and progression of neurodegenerative-related diseases.

Keywords: Hemp Protein hydrolysates, biopeptides, neuroprotective

CIPCA25-68784

Inclusion of *Limosilactobacillus fermentum* CECT5716, a probiotic strain from human milk, in a novel fermented caprine milk: Technological and nutritional characterization

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Abstract:

The strain *Limosilactobacillus fermentum* CECT5716 (LC40®), a lactic acid bacteria originally isolated from human milk, has been proven to exert beneficial long- and short-term effects, in particular on gut microbiota composition, prevention of infections and barrier dysfunction. The effect of this promising probiotic in the fermentation of milk is unknown and could pose a challenge to the physical chemical properties of the final product. The aim of this work was to evaluate the inclusion of this strain in a novel fermented goat's milk product. The source milk was partially skimmed and subjected to ultrafiltration to improve the nutritional profile and mineral bioavailability. Fermentation with the thermophilic milk culture composed of *L. delbrueckii* spp. *bulgaricus* and *S. salivarius*ssp. *thermophilus* was conducted in presence or absence of *L. fermentum* CECT5716 at different temperatures. The rheological behaviour in terms of apparent viscometry and viscoelastic behaviour, and the casein micelle size and zeta potential were determined and compared to various commercial products including traditionally crafted products. The addition of the probiotic strain had a marked impact in the apparent viscosity and viscoelasticity, but the use of 42ºC in the fermentation led a gelled product whose rheological parameters were compatible with a creamy texture easy to use with the spoon and to dose. Moreover, these parameters remained stable along the shelf life. The microbiological analysis indicated the dominant role of *S. salivarius* spp. *thermophilus* and *L. fermentum* CECT5716 with average viable cell populations ranging from 8 to 9 logCFU/g. The final product is a high protein product, with a complete amino acid profile, and a moderate content in carbohydrates and fat, that could fit the infant population.

Keywords: Fermented goat milk; probiotic; characterization

CIPCA25-80411

Enhancing the techno-functional properties of quinoa proteins: influence of extrusion, germination, and controlled enzymatic hydrolysis

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Abstract:

The study of emulsifiers based on protein hydrolysates or peptides has grown rapidly in the food sector due to its multifunctional properties including interfacial activity and improved nutritional attributes like reduced allergenicity, high digestibility, antioxidant and immunomodulating activities compared to native proteins. In this study, quinoa protein at different hydrolysis degrees (HD%), prepared from germinated and extruded quinoa seeds, was evaluated for their solubility (PS, % sol.) and emulsifying activity index (EAI, m^2/g). Proteins were extracted and hydrolysed by pH-stat technique (alcalase, pH 8, 50°C), generating the following samples at 0, 2, 5, 10 and 20HD%: Unprocessed Quinoa Protein (UQP0, UQP2, UQP5, UQP10, and UQP20), Extruded Quinoa Protein (EQP0, EQP2, EQP5, EQP10, and EQP20) and Germinated Quinoa Protein (GQP0, GQP2, GQP5, GQP10, and GQP20). Results showed that PS in samples from unprocessed, extruded, and germinated seeds increased progressively with the increase in pH (4→7). Maximal solubilities were 34.28±1.30 (EQP20, pH 7), 42.18±2.52 (UQP20, pH 7) and 44.01±4.91(GQP20, pH 7). At pH 4, 5, 6, and 7, a similar trend was observed, extrusion of quinoa seeds diminished significantly ($p<0.05$) the PS and germination of seeds improved slightly the PS compared to unprocessed seeds. Furthermore, the PS was positively correlated with the HD extent up to 10HD% in all samples, and no significant ($p>0.05$) increase was observed with respect 20 HD% samples. Regarding emulsifying capacity, maximal EAI values were 22.07 (EQP20, pH 7), 32.37 (UQP20, pH 7) and 38.83 (GQP20, pH 7), which shows extrusion reduced the EAI and germination augmented it, compared unprocessed seeds protein. This tendency was observed for all HD% and pH values. In extruded samples, the EAI varied irregularly (EAI 2.9–26 m^2/g) regarding the pH. A significantly upward trend was noticed for all different hydrolysates from germinated seeds than in unprocessed hydrolysates, while no tendency was observed for samples from extruded seeds. In this sense, extrusion and germination pre-treatments improved differently the EAI in the order: germinated>unprocessed>extruded hydrolysed samples. In conclusion, the pre-treatment of quinoa seeds through extrusion and germination, prior to protein extraction and hydrolysis, resulted in protein hydrolysates with varying emulsifying activities derived from the same native protein. This modulation of the emulsifying activity resulted in emulsifiers agents not only with bioactive properties due to peptides but also with different potential techno-functional applications: from low-moderate EAI in extruded samples (suitable for air-water emulsions in highly digestible foods such beverages, infant formulas) to emulsifiers agents with strong interfacial activity for oil-water emulsions or advanced formulations (mayonnaise, plant-based creams, encapsulation). Results showed that pre-treatment of seeds may affect solubility and emulsifying activities, and may change behaviour of the protein in emulsions prepared with protein hydrolysates at various hydrolysis degrees.

Keywords: peptides, interfacial activity, hydrolysis, emulsifier

Food colloids as vehicles for bioactives: digestibility and bioavailability

CIPCA25-48747

Age-related anorexigenic responses in enteroendocrine cells to plant-protein digestion products

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Abstract:

Protein digestion and absorption are dynamic processes influenced by multiple physiological factors, including age and the composition of the protein source. Digestive efficiency dictates the bioavailability of protein digestion products, such as peptides and amino acids, which are essential for various metabolic functions. Moreover, digestion products, particularly resistant peptides, can stimulate the secretion of intestinal anorexigenic hormones such as glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK). However, aging can decrease digestive and absorptive capacity, altering the release of protein digestion products. In this context, understanding how different proteins are degraded along the gastrointestinal tract across different life stages is crucial for optimizing nutritional strategies to enhance their utilization and functionality. This study investigated the influence of age digestive conditions on protein digestion and secretion of anorexigenic hormones. To this end, simulated gastrointestinal digestions were performed following the INFOGEST protocol under both adult and elderly conditions using as substrates three plant-proteins: Rapeseed Protein Isolate (RPI), Pea Protein Isolate (PPI), and Corn Protein Meal (CPM).

The results revealed important variations in protein digestion depending on both age and protein source. In adults, RPI and PPI underwent extensive protein degradation, reaching near-complete digestion by the end of the gastric phase. In contrast, CPM showed substantial resistance to gastric digestion, and after the intestinal phase, approximately 10% of the protein remained undigested. In the elderly gastrointestinal simulation, RPI maintained high protein degradation during the digestion process, while PPI showed approximately 60% of undigested protein at the end of the gastric phase, although it was almost completely degraded at the end of the intestinal phase. Additionally, under adult conditions, CPM digestion resulted in approximately 80% of the protein remaining intact after the gastric phase, a percentage that decreased to 10% at the end of the gastrointestinal digestion. Peptide profile analysis revealed that, in adult simulation, the digestion-resistant peptides were predominantly short (5–9 amino acids), whereas a higher proportion of longer peptides was observed under elderly conditions.

Hormonal secretion in the enteroendocrine cell line STC-1 also exhibited age- and protein source-dependent variations. The substrate effect was notable, with a higher CCK and GLP-1 response for RPI and CPM than for PPI. The impaired digestion in elderly conditions produced lower hormonal secretion for CPM and

higher for RPI and PPI than in adult. These findings remark the impact of aging on protein digestion and the anorexigenic hormonal response, highlighting its relevance in the development of nutritional strategies in different age groups.

Keywords: Plant proteins, Anorexigenic-hormones, INFOGEST

CIPCA25-51885

The impact of gastrointestinal digestion on a protein-rich soup adapted to a 65+ population: Chemical characterization and the influence of different protein sources

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Abstract:

According to the United Nations data, the proportion of older people - 65 years and over (65+) - is growing faster than any other age group. Indeed, the number of people aged 65+ worldwide is projected to more than double by 2050. These demographic changes require societal and governmental responses to adequately address ageing challenges. Some metabolic processes are compromised in older people, and sensory modalities undergo significant changes. Thus, changes in gastrointestinal function are also associated with ageing. Sarcopenia is a relevant condition in senior adults, and it is characterized by a progressive loss of muscle mass and function, reducing mobility and diminishing quality of life. Although it is a complex and multifactorial process, a deficient diet is a well-recognized factor. It is known that moderately increasing daily protein intake may enhance muscle protein anabolism and be important for reducing the progressive loss of muscle. The Diet65+ - High nutritional and functional value food products integrated with tradition and sustainability adapted to elderly 65+ consumer - is a project that intends to develop food products tailored and adapted to 65+ years individual specific nutritional requirements, respecting their food traditions.

Given the importance of high protein intake for elderly individuals, we evaluated different legume pulps as a sustainable and nutritionally rich source of protein. We have also assessed the use of legume flours (chickpea, lupin and pea flours) and protein isolates (chickpea and pea concentrates, and whey protein isolates) for developing rich protein products. The potential use of the mentioned flours and protein powder concentrates was determined by evaluating parameters such as protein digestibility, water solubility, optimal pH, water and oil holding capacity. Subsequently, six different instant soup/purée formulations (6.76±0.93 g of protein/ 100 g of edible product), comprising versions with and without a vegetable protein concentrate (chickpea protein concentrate), were developed to fulfil the distinct protein needs of the elderly population. After, we assessed the impact of the gastrointestinal tract on these formulations using a static *in vitro* digestion model adapted for the older adult population (INFOGEST). We analyzed the impact of the gastrointestinal tract on protein, fat, carbohydrates and total dietary fibre content, as well as fatty acids and amino acid composition. Notably, while the gastrointestinal tract affected the nutritional composition of the tested formulations, a considerable amount of the tested molecules remained detectable after digestion. Interestingly, when comparing the versions with and without the protein concentrations, differences have arisen regarding the impact of the gastrointestinal tract on other bioactive compounds, such as fatty acids. When comparing the intestinal digestive fraction of mungo bean soup with and without a protein concentrate, we observed that the retention indexes for the major fatty acids—stearic, palmitic, and oleic acid—ranged from an average of 31.57 ±6.13% in the standard version to 74.25 ±12.19% in the protein concentrate version. This initial screening allowed us to select the optimal ingredients and develop products fully adapted for this population.

Keywords: Gastrointestinal digestion; High-protein foods; Elderly

CIPCA25-55153

Impact of rice processing on patients with phenylketonuria: Bioaccessibility and bioavailability of phenylalanine.

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Abstract:

Phenylketonuria is an autosomal recessive genetic disorder that affects phenylalanine metabolism, leading to its accumulation in the blood at toxic levels. Untreated PKU can result in severe intellectual disability, behavioral issues, and cognitive deficits**.** The primary treatment involves a protein-restricted diet and phenylalanine-free amino acid supplementation.While rice is permitted in moderation, it is crucial for dietary preference, energy, and nutrient intake. Despite nutritional tables listing phenylalanine content, bioaccessibility and bioavailability data are lacking.This study investigated phenylalanine bioaccessibility and bioavailability in white and brown rice, examining the effects of cooking, polishing, and the food matrix on absorption post-in vitro digestion. Rice samples were cooked using standard methods: 1:2 water ratio (w/v) for 15 minutes (white rice) and 1:2.5 for 20 minutes (brown rice). After cooling, samples were ground, fractionated, and stored at -12±1 ºC. Chemical characterization was followed by simulated gastrointestinal digestion (SGD) to assess bioaccessibility. Soluble digestates were applied to Caco-2 cell inserts (1:100 dilution) to determine bioavailability.Amino acid profiles were obtained by reverse-phase HPLC with pre-column derivatization. Results showed carbohydrates as the main component (88.77% white rice, 86.07% brown rice) and proteins as secondary (9.76%, 9.02%, respectively). Brown rice contained more fiber (7.77%) than white rice (5.48%), likely due to polishing process. Pre-digestion, white rice had higher phenylalanine (168 mg/100g) than brown rice (120 mg/100g). Post-digestion, levels were similar: 20 mg/100g (white) and 23 mg/100g (brown). SGD revealed higher phenylalanine bioaccessibility in brown rice (37.09%) than white rice (26.80%). However, white rice exhibited greater bioavailability (6.83%) compared to brown rice (1.43%), indicating fiber interference. This study demonstrates that phenylalanine absorption depends not only on its content but also on the food matrix and processing, impacting dietary guidelines for PKU patients.

Keywords: Bioaccessibility; Bioavailability; Phenylalanine; Rice

Interactions between biopolymers and their impact on structure

CIPCA25-12108

Influence of transglutaminase on the rheology and gelation kinetics of pea protein

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Abstract:

Plant proteins are widely used in the food industry to create substitutes for conventional meat products. While these proteins can be textured by extrusion to improve their techno-functional properties (such as gelation), these processes are not sustainable. Simple and environmentally friendly structuring is crucial, and in this regard, microbial transglutaminase (an enzyme that catalyzes protein cross-linking) constitutes a viable option. The objective was to study how different concentrations of native pea protein isolate (PPI) and commercial microbial transglutaminase (TG) influence gelation time and the rheological and textural properties of the resulting gels.

The tilting test method was used to determine the gelation time, varying the concentration of PPI (8-18%) and TG (0.2-1%) at 37°C. An experimental design was applied, analyzing the hardness response (texture analysis, TA-XT2i Stable Micro Systems, UK), and the crossover time and loss factor determined in an Anton Paar Rheometer (RheoCompassTM, Germany) by means of an analysis of the elastic (G´) and viscous (G”) modulus for 1.5 hours at 37°C.

It was found that increasing PPI concentration significantly reduced gelation time, while TG showed a smaller but statistically significant reduction (up to 50%). Hardness was greatest at a concentration of 14% PPI and 0.8% TG. Particularly, it was evident that the higher the %PPI, the shorter the crossover time, regardless of the %TG. Furthermore, the loss factor decreased (viscoelasticity increased) with higher %TG. These findings suggest that by adjusting TG and PPI concentrations both gelation kinetics and textural properties can be controlled, adapting them to various food applications.

Keywords: Pea protein, plant-based, transglutaminase, gelification

CIPCA25-20306

β-Cyclodextrin/chitosan particle dispersions as natural stabilisers for high internal phase pickering emulsions

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Abstract:

Food-grade natural solid particles, such as polysaccharides, have gained attention as effective emulsifiers in Pickering emulsions. Among others, β-cyclodextrin (β-CD) stands out due to its amphiphilic structure, enhancing interfacial stabilisation and complexation capabilities. High internal phase Pickering emulsions (HIPPEs) are recognized for stabilising high oil fractions (≥74%) using solid particles, eliminating the need for synthetic stabilisers. Various strategies for modulating the continuous phase have been explored to develop HIPPEs with high viscosity and shear-thinning behaviour. In this context, combining chitosan (CS) with β-CD can enhance the viscosity of the continuous phase and improve HIPPEs' stability. The interactions between β-CD and CS in the dispersion structure the continuous phase, affecting rheology and emulsification properties. Understanding these interactions is crucial for optimising their functionality. Therefore, this study aims to develop and characterise β-CD/CS particle dispersions and evaluate their performance as HIPPEs stabilisers, assessing their physicochemical attributes, emulsification properties, and emulsion stability over time. Particle dispersions were produced using a green chemistry approach involving the dropwise addition of biopolymer solutions followed by pH adjustment to 5. Different β-CD/CS weight ratios (1:1, 2:1, and 3:1) were used at total biopolymer concentrations of 3-4% (w/v). Characterisation included zeta potential, atomic force microscopy (AFM), and wettability (contact angle, CA) measurements. HIPPEs were formulated with a fixed sunflower seed oil fraction of 75% homogenised by Ultra-Turrax. Zeta potential analysis at pH 5 showed similar values across the dispersions, ranging from +10.01 to +11.62 mV, comparable to β-CD solutions. The β-CD/CS dispersions exhibited CA ranging from 50.2° to 81.73°, indicating a predominantly hydrophilic nature suitable for oil-in-water (O/W) emulsion stabilisation. AFM analysis confirmed the complex formation, with structural differences across formulations. The 4% (w/v) 2:1 β-CD/CS dispersion exhibited the smallest particle diameters and a uniform morphology, suggesting improved interaction between β-CD and CS. The HIPPEs demonstrated promising storage stability, and confocal laser scanning microscopy (CLSM) confirmed their O/W structure, and packed arrangement characteristic of HIPPEs. Most formulations displayed bimodal droplet size distributions on day 1, while the 4% (w/v) 2:1 HIPPE showed an initial unimodal distribution (D4,3=8.02 μm). Significant droplet size changes occurred between days 1 and 15, stabilising thereafter. Accelerated stability tests confirmed the physical stability of the HIPPEs, as all samples exhibited phase separation to a similar extent after

centrifugation, with no noticeable differences between them. These findings demonstrate that β-CD/CS particle dispersions effectively stabilise HIPPEs, with the 4% (w/v) 2:1 formulation offering superior performance. The emulsions exhibited long-term stability, enhanced viscoelastic properties, and bimodal droplet size distributions, making them promising for food structuring applications.

Acknowledgements: This work was financed by the Portuguese Foundation for Science and Technology (FCT), the national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the framework of the project 2022.01308.PTDC (NutriPick3D) (DOI 10.54499/2022.01308.PTDC); FCT/MCTES (PIDDAC) support to CIMO (UIDB/00690/2020 (DOI:10.54499/UIDB/00690/2020), UIDP/00690/2020 (DOI:10.54499/UIDP/00690/2020)); SusTEC LA/P/0007/2020 (DOI:10.54499/LA/P/0007/2020); LSRE-LCM (UIDB/50020/2020 (DOI:10.54499/UIDB/50020/2020), UIDP/50020/2020 (DOI:10.54499/UIDP/50020/2020)); ALiCE LA/P/0045/2020 (DOI:10.54499/LA/P/0045/2020); IT1690-22 (GV/EJ, Spain). FCT for the PhD research grant of A. C. Lima (2021.05378.BD) and the scientific employment contract (A. Santamaria-Echart).

Keywords: biopolymers; particles; stabilisers; emulsions; HIPPES.

Interfacial Engineering

CIPCA25-12323

Colloidal lignin particles as pickering stabilizers: study of stability and interfacial mechanisms

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Abstract:

Synthetic emulsifiers such as Tweens and Spans, widely used in the food and healthcare sectors, have been associated with environmental and health concerns, driving research into bio-based alternatives. Colloidal lignin particles (CLPs) recently emerged as a natural-based solution to stabilize Pickering emulsions, where the solid particles replace conventional emulsifiers, providing stability and additional functional attributes (e.g., antioxidant and UV protection). However, to have a competitive advantage, CLPs must demonstrate superior stabilizing capacity over conventional emulsifiers. Therefore, this work aimed to obtain a completely emulsified system solely stabilized by CLPs and to gain insight into their stabilization mechanisms. For this, CLPs from softwood kraft lignin were applied in the production of oil-in-water Pickering emulsions, whose composition, namely the CLPs concentration (from 10 to 50 g/L) and the oil volume fraction (from 0.3 to 0.7) were optimized using a two-factor Central Composite Rotatable Design (CCRD). The formed emulsified layer (EL) and the volume-mean droplet diameter (D4,3) were selected as objective responses. Then, the optimized formulation was evaluated regarding storage stability, where the EL, droplet size, optical microscopy, color, and rheology were analyzed after 1 and 30 days of storage. The emulsion's interfacial microstructure was also investigated using confocal microscopy and cryo-SEM. The CCRD revealed that increasing the CLPs concentration and the oil volume fraction favored forming a high EL and small oil droplets, consequently leading to a wholly emulsified and stable system. The optimized formulation, having a CLPs concentration of 50 g/L and oil volume fraction of 0.7, was characterized by oil-in-water emulsion type with D4,3 of 3.9 ±0.2 μm, a light brown color, and excellent long-term stability, with no significant changes in droplet size, morphology, and homogeneity up to at least 30 days of storage. Nevertheless, a color difference (ΔE = 1.6), only perceptible through close observation, was observed over the period. Additionally, the emulsion displayed a shear-thinning non-Newtonian fluid behavior and gel-

like structure, with a slight increase in viscosity after 30 days of storage. Interestingly, cryo-SEM and confocal microscopies revealed that particle bridging was the primary stabilization mechanism of CLPs in emulsion structure, which resulted in a highly efficient packing of the oil droplets. Overall, this work highlights the capability of CLPs to act as effective natural-based emulsifiers, with promising applications in strategic industrial sectors such as food, cosmetics, and pharmaceuticals.

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Keywords: Lignin, Colloidal Particles, Pickering Emulsions

CIPCA25-58979

Improvement of colloidal and emulsifying properties of commercial pea protein through high-pressure homogenization

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Abstract:

The growing interest in plant-based protein beverages, driven by ethical, health, and sustainability concerns, has highlighted the need to optimize functional ingredients. Although pea protein isolate is recognized for its high nutritional value, low allergenicity, and non-GMO status, its limited functional properties, primarily low solubility and emulsifying capacity, restrict its application in food formulations.The objective of this study was to evaluate the impact of high-pressure homogenization (HPH) on the solubility, colloidal stability, and emulsifying properties of a commercial pea protein isolate.

Solutions of commercial pea protein isolate (3% w/w) were prepared and subjected to six HPH treatments within a range of 0 to 2000 Bar (PandaPLUS, GEA, Germany). The solutions were characterized in terms of soluble fraction, colloidal stability using the Turbiscan Stability Index (TSI) (Turbiscan, Formulaction, France), and particle size distribution (Mastersizer, Panalytical Ltd, UK). Additionally, oil-in-water emulsions were formulated by mixing 10% olive oil with 90% of a 3% (w/w) pea protein solution at 25 °C. The emulsions were obtained using an Ultra-Turrax T25 (IKA-Werke, Staufen, Germany) and high-intensity ultrasonication (Sonics, VCX 750, United States) and the droplet size distribution and emulsion stability were evaluated.

The results showed that the application of HPH significantly increased protein solubility, rising from 17.6% (0 Bar) to 94% (2000 Bar), attributable to the disruption of insoluble aggregates. Similarly, a notable reduction in particle size was observed, decreasing from 48.1 μm to 0.5 μm as pressure increased. Treatments at 1000, 1500, and 2000 Bar exhibited the lowest TSI values after 89 days of evaluation, reflecting higher colloidal stability.

Regarding emulsifying properties, the increase in pressure reduced droplet size, reaching 0.14 μm at 1500 Bar compared to 0.59 μm at 0 Bar. Emulsions prepared with HPH-treated protein (1000 Bar) exhibited the lowest TSI, identifying this condition as optimal. Under these conditions, although a decrease in pH (3 and 5) increased droplet size; a substantial improvement in emulsifying stability was observed, with reductions in TSI of up to 85% at pH 3, possibly linked to an increase in solution viscoelasticity.

These findings suggest that the structural modification of pea protein, induced by HPH and pH adjustments, optimizes its functional properties and expands its potential application in foods.

Keywords: pea protein, HPH, colloidal stability

New processes and ingredients

CIPCA25-20476

Bulk behaviour of pea protein microgels on rheology aqueous dispersions

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Abstract:

High-protein beverages are among the most practical and feasible options for consumers to meet the recommended daily protein intake. However, most commercially available products are derived from animal proteins, which not only have a high environmental impact of production, but also limit consumption by individuals with dietary restrictions, such as vegans and those allergic to such proteins. In this context, the development of products using plant-based proteins (e.g., pea protein) represents a crucial step for the food industry. However, the astringency associated with the incorporation of plant proteins into formulations, especially at high concentrations, remains a major challenge that must be addressed to enhance consumer acceptance. To mitigate these sensory drawbacks, the transformation of plant proteins into microgels emerges as a promising strategy to modulate the perceived sensation of astringency and viscosity. In this study, four gelation methods were investigated for the formation of pea protein microgels, and their subsequent impact on the bulk rheological behavior of aqueous dispersions was evaluated. Initially, 15% (w/w) pea protein isolate (PPI) dispersions were hydrated (24 h at 25°C), thermally denatured (90°C for 30 min), and cooled in an ice bath (30 min). To induce macrogel formation, a control dispersion was prepared without the addition of a crosslinking agent. In the other formulations, crosslinking was induced using transglutaminase (20 U/g protein), Glucono-δ-lactone (GDL) (0.5% w/w), and calcium chloride dripping (100 mM). The resulting gels were then diluted (10% w/w) and disrupted using a Silverson homogenizer at 10,000 rpm for 5 min to obtain microgels. These microgels were characterized in terms of particle size distribution and zeta potential. The microgel concentration was adjusted to obtain dispersions at 1, 2.5, 5, 7.5, and 10% (w/w), which were further analysed from flow curves (0 to 300 s^{-1}). Due to strong aggregation, PPI and heat-treated microgels (HTM) exhibited a bimodal particle size distribution. Despite using the same disruption method, the type of crosslinking agent influenced the size of the resulting microgels. However, all microgels showed similar surface charge profiles at pH 2.0–10.0, with the highest value at pH 2.5 (30 mV) and isoelectric point at pH 4.5. HTM, GDL-induced (GLM) and calcium chloride-crosslinked (CCM) microgels exhibited Newtonian behavior at concentrations ranging from 1% to 7.5% (w/w), while a notable viscosity change was observed at 10% (w/w) microgel concentration, suggesting a change in the oral perception of these dispersions. In contrast, both PPI and transglutaminase-crosslinked microgel (TGM) dispersions exhibited non-Newtonian behavior starting at 2.5% (w/w). Among all samples, TGM dispersions exhibited the highest viscosity, regardless of microgel concentration. The visual aspect of all dispersions indicated a strong tendency for microgel interactions, leading to aggregation and phase separation during storage (7 days). Understanding bulk behavior is essential to tailor rheological properties and predict oral perception of these dispersions, ultimately contributing to sensory acceptance of pea protein-based food products.

Keywords: pea, microgel, bulk, rheology, beverages

CIPCA25-20844

Surface functionalized membrane bioreactors for sustainable protein production

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Abstract:

The development of innovative membrane bioreactors that convert methane and other waste materials into valuable agricultural proteins is essential for meeting global commitments to greenhouse gas reduction by 2050. Methane, a potent greenhouse gas, poses significant environmental challenges, making effective removal strategies essential. This project focuses on utilizing protein-producing microorganisms in a continuous process to generate high-value proteins with minimal energy input. By leveraging advanced surface technologies in energy-efficient membrane bioreactors, we aim to optimize gas exchange and maximize biomass yield, establishing scalable pathways for transforming waste into alternative protein sources.

Biofilm reactors offer a promising strategy for this approach. Biofilm-forming bacteria provide key advantages, including increased microbial stability, shielding against harmful environmental conditions, and enhanced substrate utilization. Their direct attachment to surfaces allows for more efficient resource use, leading to higher productivity. The resulting higher biomass concentrations are crucial for maximizing protein yields and meeting global demand.

Another benefit of biofilm-based systems is their increased resistance to inhibitors. Microorganisms within biofilms exhibit greater resilience to toxic substances, often a challenge in traditional cultivation systems, contributing to more robust production processes. Additionally, biofilms significantly improve gas exchange, as microbes growing near the gas-liquid interface can more effectively absorb gaseous substrates like methane. This leads to higher metabolic activity and improved protein synthesis.

The project also explores plasma and laser surface engineering to create structured substrates that ensure stable gas and nutrient transport over extended periods. These modifications further enhance biofilm reactor performance by fostering optimal conditions for microbial growth.

Harvesting is simplified, as biofilms grow attached to surfaces rather than being suspended in liquid, reducing processing costs. They also withstand various sterilization methods, further lowering operational expenses.

Overall, integrating biofilm-forming bacteria in membrane bioreactors enhances efficiency and productivity in protein production while supporting environmental sustainability and food security.

Keywords: Surface, Material, Functionalization, Bioreactor, Protein

CIPCA25-30565

Impact of water and lecithin-phytosterol ratio on the structural and tribological properties of lecithin-based oleogels

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Abstract:

Oleogels are edible metamaterials with specific structural properties, offering a carefully engineered microstructure that can tailor key attributes like oil binding, mechanical strength, and stability. Their designed functional behaviour allows them to be customized for various applications, including modifying textures and improving the stability in food formulations. This study investigated the multi-component system of lecithin, plant sterols, and water to analyse the effect of small water addition and ratio modifications on the bulk arrangements, microstructure, and rheological and tribological properties of lecithin-based oleogels. Results showed that introducing a polar component (1 wt% water) into this multi-component gelation system reinforced the structural network in formulations with a fixed lecithin-phytosterol ratio, yielding the strongest gels. Beyond this threshold, further water addition led to destabilization, characterized by reduced crystal size, and decreasing oil binding, which resulted in phase separation for some formulations. Microscopy confirmed water's influence on the crystalline network, reinforcing it up to a point before structural disorganization occurred. Rheological tests supported these findings, revealing that gels with 1 wt% water exhibited the highest gel strength, demonstrated by an approximately 150-fold increase in elastic modulus (G'), averaging around 4000 Pa—compared to water-free samples. Additionally, the tribological analysis provided insights into frictional behaviour and lubrication regimes. Studies under small-angle X-rays highlighted the bulk system evolution with the increase in lecithin. The existence of a highly ordered, compact hexagonal phase was observed for small amounts of lecithin, suggesting cylindrical micelles. The rise of lecithin concentration led to lower *d*-spacings that resulted in the increase of the packing density. Then, as lecithin content reached similar values to the phytosterol amount, its amphiphilic nature seemed to facilitate a bulk structural transition into a less ordered structure with inversed micelles. These findings contribute to understanding how lecithin-phytosterol interactions influence edible systems' textural and tribological properties, potentially expanding their applications in food matrices.

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Keywords: lecithin; oleogels; multi-component; structure; rheology

CIPCA25-57308

Innovation in natural ingredients: effect of ultrasound on tarwi okara (*Lupinus mutabilis*) and its use in gluten-free and vegan products

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Abstract:

Tarwi okara (*Lupinusmutabilis*), a byproduct of the alkaline protein and lipid extraction of this Andean legume, is mainly composed of dietary fiber and insoluble proteins. The objective of this research was to evaluate the effect of high-intensity ultrasound (HIU) on the technofunctional properties of tarwi okara and its potential application as an ingredient in gluten-free baking and emulsified systems. Treatment with high-intensity ultrasound (HIU) significantly modified its properties, reducing protein content by 11% and increasing crude fiber by 16%, due to protein solubilization and fiber defibrillation. These structural alterations, confirmed by ATR-FTIR spectroscopy, translate into technofunctional improvements, such as a 30% increase in water absorption capacity (WAC), 14% in oil absorption (OAC) and 26% in emulsifying activity (EAI), which expands its applications. In gluten-free baking, tarwi okara, both native and sonified, acts as a technological enhancer by improving the consistency of doughs based on rice flour and potato starch, forming dense networks that retain gases during baking. Breads with 5% native okara or 3% sonified okara achieve specific volumes (1.82-1.83 mL/g) and crumb textures comparable to those made with xanthan gum (0.5%), although higher concentrations of sonified okara can negatively affect the structure. In addition, its high protein and fiber content promotes the darkening of the bark through Maillard reactions, a favorable sensory attribute. In emulsions, the sonified okara demonstrated good stability, with a 577% increase in the emulsifying stability index (ESI) and 31% in the swelling capacity (SC), allowing the preparation of O/W (30:70) stable vegan emulsions for 24 hours, with pseudoplastic behavior and adaptation to the Herschel-Bulkley rheological model. Emulsions with 4%-6% post-extraction treated okara (OKS2) exhibit greater consistency and initial tension, ideal for dressings or spreads. These results show tarwi okara, specially modified by HIU, as a sustainable and versatile ingredient, capable of replacing synthetic additives in gluten-free baking and stabilizing emulsions, aligning with clean food and circular economy trends.

Keywords: lupinus, mutabilis, okara, tarwi

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Anthocyanin-based double emulsion gels with 3D-printable ability as potential food solutions for dysphagia patients

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Abstract:

This work investigates using double emulsion systems to create anthocyanin-rich structured foods using commercial black carrot (Daucus carota L.), a natural anthocyanin source targeting 3D printing for customized nutrition. The main objectives included the printability evaluation of the produced emulsion gels, focusing on properties such as viscosity, yield stress, and the structural integrity necessary to create 3D-printed structures proposed for dysphagia diets. Additionally, the bioaccessibility and stability of the encapsulated anthocyanins were assessed by simulated in vitro digestion tests. Double emulsion gels of type water-in-oil-in-water (W1/O/W2) were developed with a constant composition of the external aqueous phase (W2), namely gum Arabic (6.5% wt%) and Tween 80 (3% wt%) while varying the gelatin concentration (4%, 6.5%, and 10% wt%; samples named DE_4%, DE_6.5%, and DE_10%, respectively) to evaluate its effect on gel properties.

The droplet size results revealed a rising pattern with gelatin concentration increase, showing volume-mean droplet sizes (D4,3) values of 22.6±0.34 μm, 31.5±0.73 μm, and 51.4±1.62 μm for DE_4%, DE_6.5%, and DE_10%, respectively. The effective entrapment of the anthocyanins in the inner aqueous phase (W1) was evidenced in all formulations through an encapsulation efficiency of 99.9%. The bioaccessibility tests, performed in the intestinal phase of the simulated in vitro digestion, demonstrated a considerable increase in the DE_6.5% and DE_10% formulations, corresponding to a 3.71- and 2.70-times higher release, respectively, compared to the free forms. This enhanced bioaccessibility can be attributed to the protective effect of the double emulsion structure, which prevents anthocyanin degradation during gastric digestion.

With all formulations demonstrating shear-thinning behavior, typical of non-Newtonian fluids, the rheological study revealed that an increase in gelatin concentration improved the printability of the emulsions. The formulations showed promising shape retention and structural integrity during the 3D printing, except for the DE_4% sample, whose structural stability was limited. The textural profile analysis (TPA) demonstrated an increased hardness in the printed gels with the gelatin content increase (the higher value of 135±5 g for DE_10%), with all formulations remaining below the maximum recommended hardness of 203 g for dysphagia foods as measured by TPA. Additionally, when evaluated according to the International Dys-

phagia Diet Standardizing Initiative tests, DE_6.5% and DE_10% formulations complied with the guidelines for minced and moist foods (Level 5), confirming their suitability for individuals with swallowing difficulties. This study demonstrates that combining double emulsion gels and 3D printing offers a promising approach to developing functional foods capable of protecting bioactive compounds while addressing dietary needs, particularly for individuals with swallowing difficulties. The produced systems can serve as carriers of additional nutrients, broadening their potential range of applications in customized nutrition.

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Keywords: Anthocyanins, 3D-printing, W/O/W gels, dysphagia

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Chia mucilage as a hydrocolloid to improve cowpea protein concentrate ink for 3d printing

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Abstract:

Extrusion-based food printing is an emerging technology that enables the creation of customized food products, allowing precise control over shape, design, and nutritional composition. As one of the world's largest bean producers, Brazil has commercially available cowpea protein concentrate (CPC), obtained through dry fractionation, which presents great potential for structuring printed food. Chia mucilage (CM), extracted from the byproduct of chia seeds oil extraction, is rich in fiber and exhibits excellent stabilizing, water and oil holding properties. Its biocompatibility and non-toxicity make it a promising hydrocolloid for food applications. This study focuses on the optimization of CPC-based ink formulations by incorporating CM to enhance water retention, rheological properties, and printing performance. The proximate composition of CPC was 37.03 ±0.25% protein, 10.08 ±0.07% moisture, 4.08 ±0.14% lipids, 4.77 ±0.02% ash, and 44.04% carbohydrates and for CM was 19.96 ±0.04% protein, 5.87 ±0.41% moisture, 14.9 ±0.41% lipids, 5.80 ±0.45% ash, and 53,47% carbohydrates. The gel-forming capacity indicated that the lowest gelling concentration was 0.10 g/mL of CPC. Formulations containing 10, 20, and 30% (w/w) CPC were dispersed overnight in distilled water, heating at 90°C for 30 min, and stirred for 2 min before being loaded in a syringe and extruded onto paper (line test). The 10% CPC formulation lost its structure after extrusion, behaving like a liquid, while the 30% CPC formulation exhibited a brittle appearance. Therefore, the 20% CPC formulation was selected for syneresis, rheological and printing tests.To test the hydrocolloid, 1% of the CPC solid content was replaced with 1% CM of solid content and the same tests were performed. Rheological tests showed that the ink exhibited elastic behavior (G ’> G”) within the linear viscoelastic region (LVER).The intersection point of G ’and G” in a shear stress sweep test for CPC ink was approximately 350 Pa, while for CPC/CM ink, it was around 280 Pa. The loss tangent (tan δ, G”/G’) was close to 0.18, with low frequency dependency, indicating good structural stability for both samples. The G ’and G” values from the Three-interval thixotropy (3ITT) test confirm that the inks behave as a solid under low shear stress (stabilization), as a liquid under high shear stress (extrusion), recovering their solid structure during the third step (regeneration). The CPC/CM ink exhibited better flowability than the CPC ink, and the lower shear stress required for CPC/CM ink is generally preferred for smooth extrusion, benefiting the transitions that occur during the 3D printing process. Printing tests using a 1.2 mm nozzle demonstrated good printability and stability for both inks; with CPC/CM ink showing superior water-holding capacity and overall stability. The results contribute to the development of more stable and high-quality edible inks, paving the way for advancements in 3D food printing and sustainable plant-based food solutions.

Keywords: Rheology; pulses; emerging-technology; alternative-protein; printability

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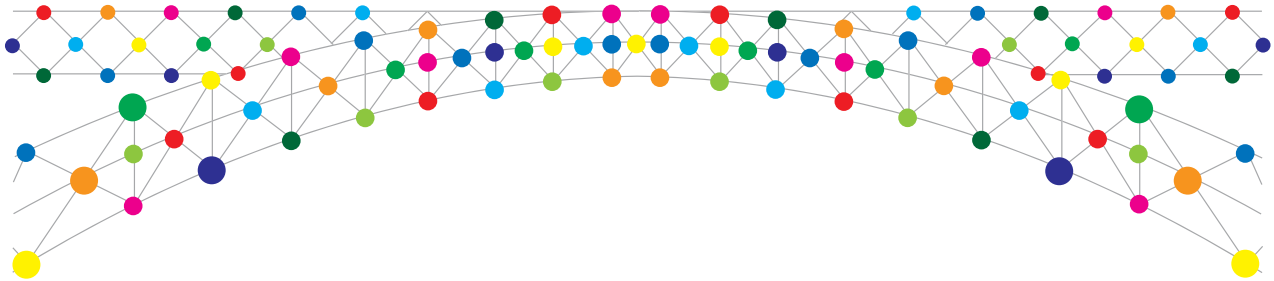

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