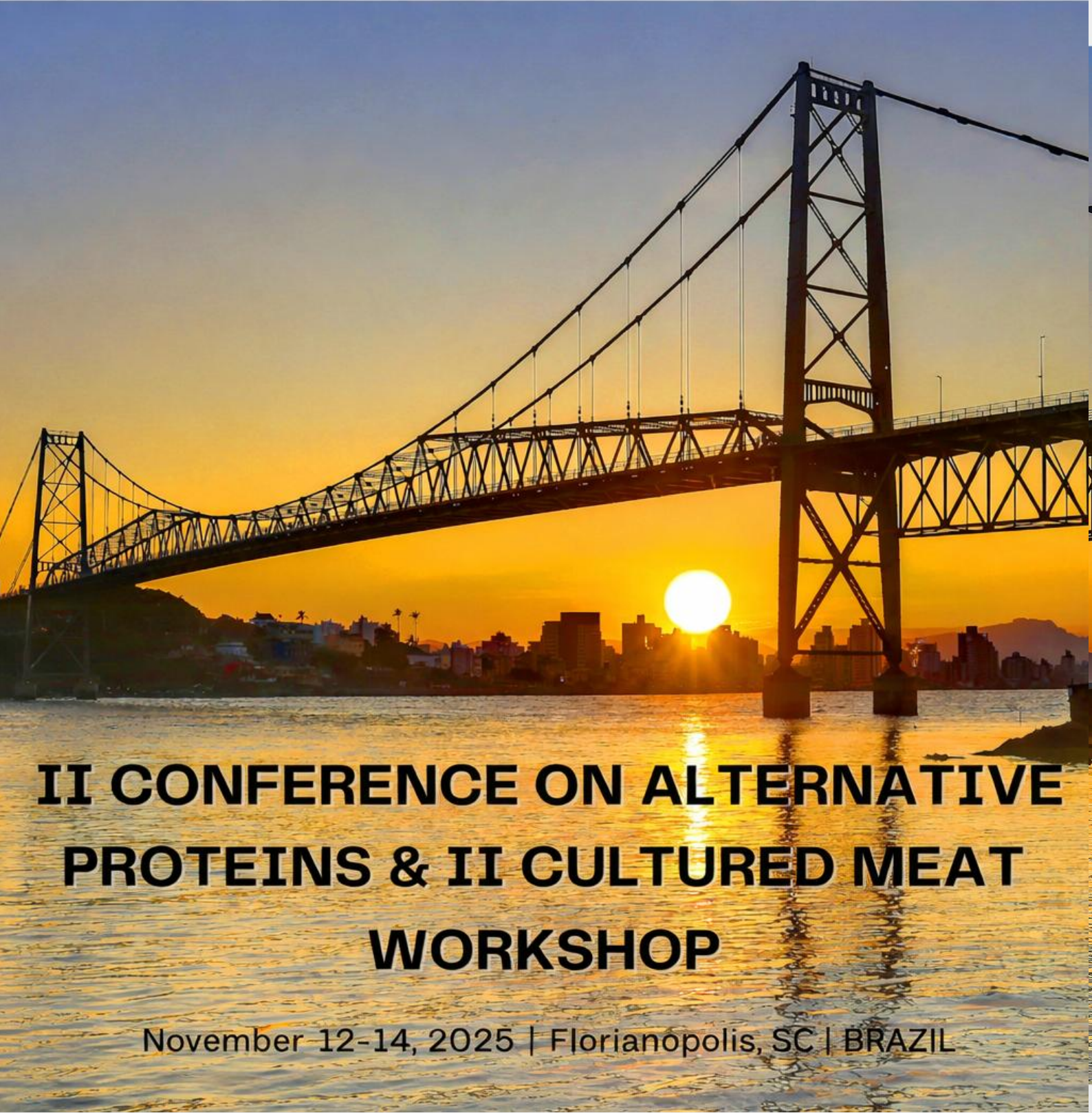




II CONFERENCE ON ALTERNATIVE PROTEINS & II CULTURED MEAT WORKSHOP

November 12-14, 2025 | Florianópolis, SC | BRAZIL

Brazilian Society of Food Science and Technology - sbCTA-SC
Brazilian Agricultural Research Corporation
Embrapa Swine and Poultry
Ministry of Agriculture, Livestock and Food Supply

Proceedings of the II Conference on Alternative Proteins & II Cultured Meat Workshop

November 12-14, 2025

sbCTA-SC
Embrapa Suínos e Aves
Concórdia, SC
2026

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www.embrapa.br/fale-conosco/sac

Responsible for edition

Brazilian Society of Food Science and Technology and
Embrapa Swine & Poultry

Editorial coordination: *Tânia M. B. Celant*

Graphic design: *Vivian Fracasso*

Bibliographical standardization: *Claúdia A. Arrieche*

Cover: *Vivian Fracasso*

**Brazilian Society of Food Science and Technology -
sbCTA-SC**

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Brazilian Society of Food Science and Technology

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1st edition: Electronic version (2026)

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Cataloging-in-Publication (CIP) Data

Embrapa Suínos e Aves

II Conference on Alternative Proteins & II Cultured Meat Workshop (2. : 2025 : *Florianópolis, SC*).

Proceedings of the II Conference on Alternative Proteins & II Cultured Meat Workshop,
November 12-14, 2025. - Florianópolis, SC : sbCTA-SC : Embrapa, 2026.
160 p.

ISBN 978-85-89436-02-1

1. Meat production. 2. Technology. 3. Cultivated meat. 4. Plant-based. 5. Fermentation.
I. Proceedings of the II Conference on Alternative Proteins & II Cultured Meat Workshop

CDD 664.9 (21. ed.)

Claúdia Antunes Arrieche - CRB 14/880

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November 12, 13, and 14th, 2025 | Florianópolis, SC | UFSC Culture and Events Center

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PRESENTATION

The Conference on Alternative Proteins (COAP) is a scientific event organized by the Alt Protein Project (APP) university groups, part of a global student movement created by the Good Food Institute (GFI) to empower students to lead the transformation of the food system at their universities. COAP aims to promote integration between universities and industry, highlighting the sector's main innovations. The event addresses three central pillars: plant-based protein analogues, cultivated meat, and fermentation, featuring discussions on science, technology, and market trends. Since its first edition in 2024, the event has brought together students, researchers, and professionals, strengthening the dialogue between academia and industry in support of more sustainable food solutions.

The Cultured Meat Workshop (JCC) was the first event in Brazil dedicated to the topic, organized by Empresa Brasileira de Pesquisa Agropecuária (Embrapa Swine and Poultry). Held since 2022, the event aims to deepen knowledge about cultivated meat by bringing together perspectives from various sectors, including the food industry, the Sociedade Vegetariana Brasileira, researchers in the field, science and technology institutes, as well as input and equipment industries. It also addresses discussions on future regulation and consumer trends.

Building on the success of previous editions and seeking to intensify connections between strategic sectors by stimulating collaborations and innovations in the field of alternative proteins, the II Conference on Alternative Proteins (COAP) & II Cultured Meat Workshop (JCC) aimed to reach new heights under the theme “Proteins of the Future for a Resilient Food System.” The 2025 event addressed three central pillars: cultured proteins, plant-based proteins, and fermentation applied to the alternative protein market, expanding the scientific and social scope of the alternative proteins field.

The Sociedade Brasileira de Ciência e Tecnologia de Alimentos (sbCTA), the UFSC Alt Protein Project (UFSC APP), the Universidade Federal de Santa Catarina (UFSC), and the Empresa Brasileira de Pesquisa Agropecuária (Embrapa) joined forces to hold the second joint edition of these events. The combined event took place from November 12 to 14, 2025, in Florianópolis, Santa Catarina, at the Centro de Cultura e Eventos Reitor Luiz Carlos Cancellier de Olivo of UFSC, culminating in the participation of approximately 290 attendees, 34 speakers, 17 exhibitors, 7 sponsors, 17 supporters, and 137 scientific submissions.

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Realization

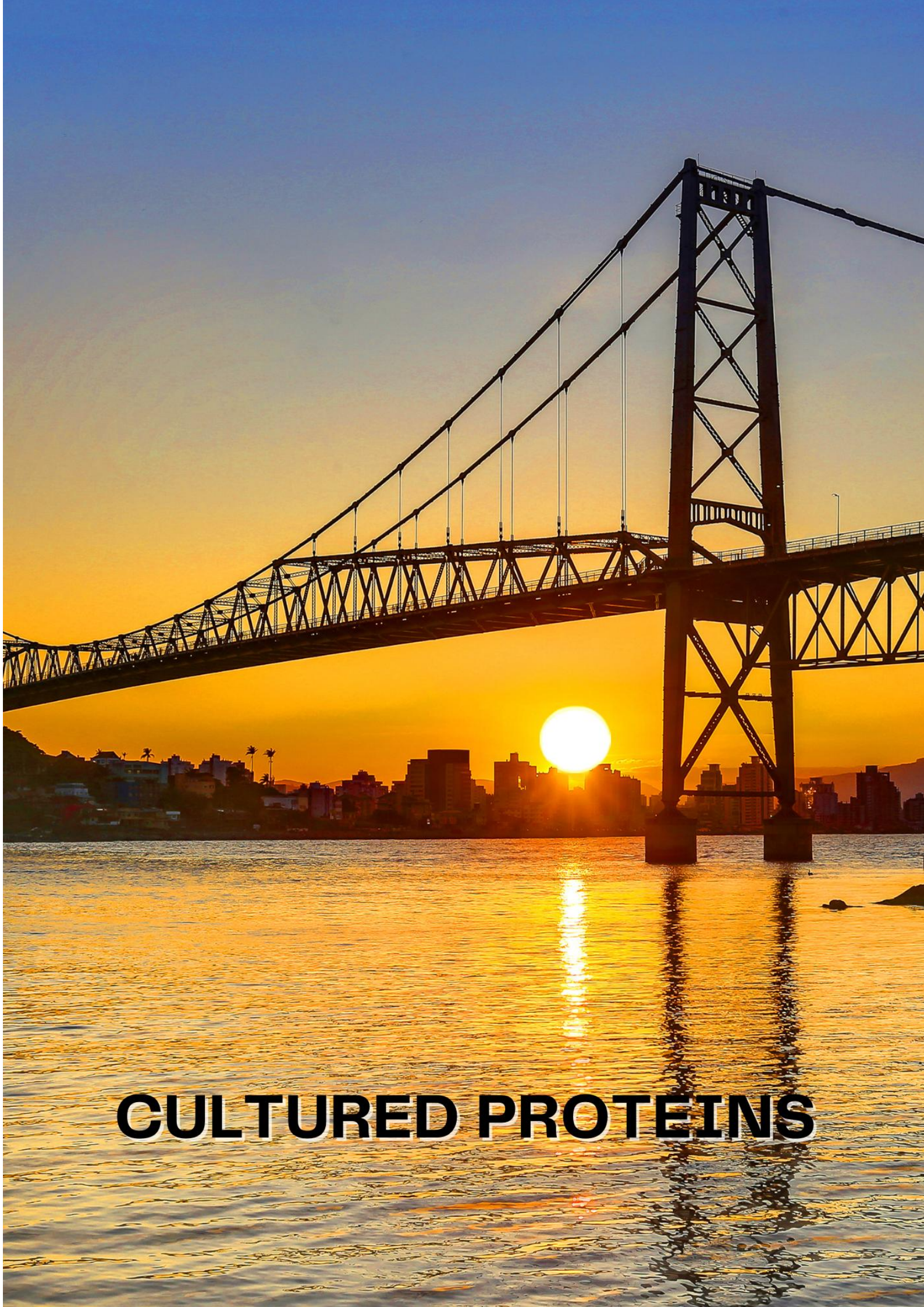


Promotion



Supporter





CULTURED PROTEINS

CULTIVATED FAT PRODUCED BY TISSUE ENGINEERING TECHNIQUE

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ABSTRACT: Cultured fat is fat produced using a tissue bioengineering technique, based on the cultivation of stem cells isolated from animals through biopsies onto structures that mimic the extracellular matrix of the tissue, known as scaffolds. This innovative technology allows the production of a product of animal origin without the negative impacts of intensive domestic animal production on public health, environment and animal welfare. One of the bottlenecks for the production of cultured fat on a large scale is the identification of a scaffold that prevents the buoyancy of mature adipocytes, a condition that occurs due to the decrease in cell density as they accumulate lipid droplets in the cytoplasm during differentiation. This study investigated the ability of a porous plant-based scaffold to support growth and anchorage of adipogenic lineage cells even during their differentiation. Immortalized 3T3-L1 cells were used to test the adhesion capacity, viability, proliferation and adipogenic differentiation on this scaffold. Then, porcine adipose tissue-derived stem cells were isolated from visceral adipose tissue biopsies and evaluated for adhesion, survival and differentiation capacity into mature adipocytes. The results demonstrated that the plant-based porous scaffolds adequately supported the adhesion and viability of both cell lines for up to 3 days of culture. 3T3-L1 and ADSC cells also demonstrated the capacity to differentiate into mature adipocytes, with the activation of key genes in the adipogenic differentiation process and the formation of lipid droplets over the time of culture with specific medium. These findings highlight the potential of plant-based porous scaffolds for the production of cultured fat.

Keywords: Cultivated fat; porous scaffolds; adipogenesis; 3T3-L1; ADSC.

Funding: CNPq; FAPEMIG.

CELLULOSE ACETATE NANOFIBER SCAFFOLDS SUPPORT MYOGENIC CELL GROWTH FOR CULTURED MEAT APPLICATIONS

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ABSTRACT: As the need for sustainable and ethical protein alternatives increases, cultured meat presents a promising approach by enabling the *in vitro* growth of muscle cells on biomaterial scaffolds. In partnership with CEFET-MG, our research explores the potential of cellulose acetate nanofiber (CAN) scaffolds for creating three-dimensional structures. This study evaluates how effectively these scaffolds support myoblast proliferation under low-density conditions, a crucial factor for optimizing cell expansion in tissue engineering and regenerative applications. C2C12 immortalized myoblasts and primary chicken muscle satellite cells were seeded at a density of 4×10^3 cells/mL and cultured for 28 days under two maintenance protocols. In the first, cells were maintained in growth medium [DMEM High Glucose (Gibco) supplemented with 20% fetal bovine serum (FBS, Gibco)] for 7 days, followed by a switch to differentiation medium (DMEM High Glucose + 2% FBS) from day 8 onward. In the second, cells were continuously maintained in growth medium for the entire 28-day period. Mitochondrial activity, as evidenced by MTT assays, indicated high viability, with primary cells reaching an absorbance of 1.3 at 595 nm compared to 0.5 in C2C12 cells under the same conditions. Immunofluorescence staining of actin filaments confirmed strong cell adhesion and scaffold colonization under both conditions, with interconnected cellular networks forming over time, particularly under differentiation conditions. Regarding myogenic markers, a significant upregulation was observed when primary cells were cultured in differentiation medium compared to those maintained in growth medium. Specifically, *Pax7* showed a 10-fold increase, *MyoD* a 30-fold increase, and *myosin* a 200-fold increase, indicating a strong commitment towards the myogenic lineage under differentiation conditions. qPCR analysis confirmed consistent expression of myogenic markers, reflecting preserved cellular functionality and early-stage myogenic differentiation, further supporting the scaffold's compatibility with long-term culture. Overall, these results demonstrate that CAN scaffolds effectively support myoblast growth at low cell densities, highlighting their potential for scalable cultured meat production and advanced biomedical applications.

Keywords: cultured meat; cellulose acetate nanofiber; myogenic differentiation; tissue engineering.

Funding: The authors extend their gratitude to the funding institutions such as FAPEMIG and GFI for their financial support.

SCIENTOMETRIC ANALYSIS OF SCAFFOLD DEVELOPMENT FOR CULTIVATED MEAT: TRENDS, GAPS, AND FUTURE PERSPECTIVES

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REVIEW: The increasing global demand for meat in recent decades has raised sustainability and food security concerns, driving research into cultivated meat as a viable alternative. Scaffold development—three-dimensional supports—is essential for facilitating cellular growth and replicating the textures of conventional meat. This study conducted a scientometric analysis using PubMed, Scopus, and Web of Science databases, covering the period from 2014 to 2025, aiming to identify research trends, key authors, and collaborative networks. The analysis revealed a steady annual growth rate of 22.8% in publications on scaffolds for cultivated meat. China, the United States, and South Korea were identified as leading countries, contributing significantly to the field. Research focus areas include the development of innovative biomaterials, advancements in 3D bioprinting techniques, and enhancing scaffold biocompatibility. Despite these advancements, the scientometric analysis highlighted fragmented and limited collaborative networks, suggesting a critical need for increased interdisciplinary and international partnerships. Challenges persist concerning scalability, production cost, standardization of methods, and regulatory approval processes. Selecting appropriate biomaterials requires balancing mechanical properties, biocompatibility, and economic feasibility. Consumer acceptance and regulatory standards also play significant roles in determining the future success of cultivated meat technologies. In conclusion, although considerable progress has been achieved, substantial challenges remain. It is crucial to foster stronger collaborations between academia and industry to address these challenges effectively. Enhanced cooperation can accelerate technological advancements in scaffold development, thereby supporting the commercial viability and sustainability of cultivated meat as a reliable and sustainable protein alternative.

Keywords: Cultivated meat; Scaffold, Biomaterials; Tissue engineering; Scientometric analysis.

APP NA ESCOLA: AN EDUCATIONAL INITIATIVE FOR SCIENTIFIC DISSEMINATION ON ALTERNATIVE PROTEINS

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ABSTRACT: The transition to a fairer, safer, and more sustainable food system requires not only scientific innovation but also public engagement and education. To ensure lasting change, it is essential to disseminate scientific knowledge to younger generations, empowering them as future agents of transformation. In this context, we from the Alt Protein Project UFMG created the initiative “APP na Escola”, which brings discussions on environmental issues, alternative proteins, and the scientific method into public elementary schools in Belo Horizonte, Minas Gerais. Our team conducts playful and interactive activities, designed to communicate complex topics in an accessible and engaging way. The project includes dynamic lectures, historical storytelling about science and Brazilian scientists, and hands-on workshops. One highlight is a simplified cultivated meat simulation using modeling clay and popsicle sticks to represent muscle cells and scaffolds. We also use a custom version of the game “Super Trunfo”, where fictional planets are compared based on environmental indicators, sparking discussion on food systems and sustainability. To evaluate our impact, we apply pre- and post-intervention questionnaires on themes such as climate change, ethics, cultivated meat, and public health. Results show a clear increase in students’ understanding, with a reduction in “I don’t know” responses and greater acceptance of cultivated meat as a viable alternative. Students demonstrated enthusiasm and engagement, with some later choosing cultivated meat as the theme for their school science fair presentations. This initiative not only promotes scientific literacy but also introduces the concept of alternative proteins to a new generation in a context of curiosity, creativity, and critical thinking. As next steps, we aim to expand to more schools and further adapt our materials to enhance learning and impact. We believe in planting seeds of curiosity and hope through science, and with each visit, we see those seeds begin to grow.

Keywords: education; APP na Escola; cultivated meat; science; scientist.

Funding: CAPES, CNPq, FAPEMIG, GFI.

RGMA: FROM NEURONAL MOLECULE TO MUSCLE GROWTH FACTOR FOR CULTIVATED MEAT

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ABSTRACT: Proliferation factors are essential molecules for promoting cell expansion, substantially increasing the number of cells available for various biological processes and industrial applications. In the context of cultivated meat production, identifying and understanding these factors is crucial to improving the efficiency of muscle biomass generation. Despite advances in tissue engineering, there remains a need to discover new molecular modulators that optimize proliferation and the formation of functional muscle fibers. In this context, the Repulsive Guidance Molecule A (RGMa) protein has emerged as an unexpected candidate. Although RGMa is traditionally associated with neuronal biology, it was surprisingly identified in chick somites, the precursor structures of satellite muscle cells, suggesting a previously unknown role in skeletal muscle development. Subsequent studies have shown that RGMa is in the sarcolemma and sarcoplasm of adult muscle fibers. When analyzed in culture of C2C12 cells; an immortalized murine myoblast cell line widely used as a model for studying myogenesis, it enhances both the fusion index and the size of myotubes. Moreover, the administration of recombinant RGMa to healthy and injured muscles considerably increases muscle fiber area, effects consistent with both hyperplasia and hypertrophy. This study shows that although RGMa is a membrane-anchored protein via a GPI anchor, a type of lipid anchor that attaches proteins to the outer surface of the cell membrane, it is also found in the nuclei of mouse satellite cells. This nuclear localization depends on its interaction with its canonical receptor, Neogenin, since Neogenin silencing leads to RGMa exclusion from the nucleus. Furthermore, treatment of satellite cells with recombinant RGMa enhances their proliferation, whereas inhibition of RGMa expression drastically reduces the cell proliferation rate. Finally, the differential proliferative effects of treating primary bovine and chicken cells with recombinant RGMa were analyzed, highlighting its potential as a relevant proliferation factor for cultivated meat production. These findings position RGMa as a key molecule to boost cell expansion strategies in muscle cultivation systems, offering new opportunities for tissue engineering and biotechnology applied to cultivated meat.

Keywords: Proliferation Factors, RGMa, Protein, Skeletal Muscle, Cultivated Meat.

Funding: Fapemig

CULTIVATING A NEW STYLE: MORPHOFUNCTIONAL CHARACTERIZATION OF 3D CULTURES OF IMMORTALIZED FIBROBLASTS ON CARBON BASED SCAFFOLDS FOR CULTURED LEATHER PRODUCTION

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ABSTRACT: Leather production was one of the first manufacturing processes developed by humanity to protect against environmental conditions. It is composed by the preserved dermal layer of vertebrate skin and the final product is comprised mainly of fibrillar collagen (Type I and III). Today, leather has many other applications, including in footwear, bags, and upholstery. However, its production is not cheap and depends on animal slaughter, which has been widely debated in recent years, especially when sourced from wild animals. Therefore, arises the need to develop alternatives to traditional leather. Nevertheless, these alternatives we have today often lack the appearance, texture, or durability of the original product, as they are usually made from non-biodegradable materials and still have a high production cost. Thus, the idea arises to use the principles of tissue engineering to recreate traditional leather in vitro. To achieve this, a scaffold is required to ensure the mechanical properties of the ECM and provide physical support for dermal cells. Carbon based scaffold presents itself as a promising alternative, as it exhibits good mechanical properties, high biocompatibility, and low cytotoxicity. The initial steps of this project involved optimizing the seeding of Mouse Embryonic Fibroblasts onto the scaffold to mimic the dermal layer. For this, cells were seeded onto the scaffolds, either treated or untreated with ECM proteins to optimize cell adhesion. The fibroblasts adhered well to the carbon scaffold, exhibiting an elongated morphology that covered a significant portion of the biomaterial's surface. Additionally, significant cell proliferation was observed between 24 and 72 hours after seeding. Moreover, TEM revealed that the cells were metabolically active, displaying well-developed RER and multiple vesicles fused to the plasma membrane at their periphery. Subsequently, collagen deposition was analyzed by allowing the cells to grow for 10 days onto the scaffold, with and without a collagen secretion induction medium, using both qualitative and quantitative approaches. Additionally, through SEM we observed an extensive collagen network, comprised of thin interconnected fibers intersecting between them and the biomaterial. No differences were observed in collagen deposition after induction medium treatment, possibly inducing

Keywords: Leather; Fibroblasts; Collagen; MEF cells; Scaffold; Carbon-based.

Funding: FAPEMIG; CNPQ.

STABLISHING A PROTOCOL FOR 3D BIOPRINTING OF MICROCARRIER-LADEN BIOINKS FOR CULTURED MEAT

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ABSTRACT: The cultivated meat bioprocess involves two main phases: proliferation and differentiation. Proliferation typically occurs in suspension within large bioreactors, with cells adhering to microcarriers—small spheres that enhance surface area for growth. Before going to the next phase, differentiation and meat structuring, cells can be either separated or proceed together with microcarriers to the final product. At this stage, cells mature and organize into engineered tissues, guided by cues provided by an underlying biomaterial that mimics extracellular matrix, called scaffold. Although various biomaterials, like nanofibrous and porous structures, have been proposed as scaffolds for cultured meat, none allow precise spatial control of cell deposition necessary for marbled meat production. 3D bioprinting offers a promising solution by enabling spatially controlled deposition of muscle and fat cells using bioinks—cell-laden hydrogels. Little is known about whether microcarriers affect cell morphology, elongation, or survival during differentiation. Thus, we here investigated whether C2C12 myoblasts, cultured on commercially available microcarriers, could establish a healthy culture within biomaterials, especially those suitable for 3D bioprinting. For this, cells were first cultured in microcarriers and then transferred into cellulose acetate nanofibers. Cells successfully migrated and adhered into the nanofibers, exhibiting favorable morphology. To evaluate their behavior in printable hydrogels, we tested formulations based on alginate, gelatin, and a fermentation-derived ingredient. We analyzed rheological properties, printability, and cell viability. Hydrogels demonstrated shear-thinning behavior, ideal for printing. Moreover, C2C12 myoblasts printed as single cells remained viable immediately after printing, suggesting that the hydrogels protected them from shear stress. However, prolonged culture reduced viability, likely due to poor adhesion and spreading, as gelatin can leach out and alginate's stable network hinders remodeling. Thereupon, we developed a novel formulation based on a chemical modification on alginate, that increases their remodeling through natural hydrolysis and allows gelatin covalent bonding, which would improve cell attachment. Indeed, C2C12 cells remained viable and spread well over seven days of culture. Our findings confirm that microcarrier-grown C2C12 cells can thrive in biomaterials and that our novel hydrogel formulation supports long-term culture of individualized myoblasts. Next, we will develop microcarrier-laden hydrogel constructs to assess cell behavior during differentiation.

Keywords: Bioink; Bioprinting; Cultivated Meat.

Funding: CNPQ, Moondo Biotech, Rede Mineira de Engenharia de Tecidos e Terapia Celular (Remettec).

DEVELOPMENT OF NEW BIOINKS FORMULATIONS FOR THE PRODUCTION OF SCAFFOLDS FOR CULTIVATED MEAT

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ABSTRACT: The current meat production model is unsustainable for future generations. Global population is expected to reach 10 billion people by 2050, increasing global demand for protein. On the other hand, expansion of livestock areas not only will not meet this demand, but will aggravate deforestation and greenhouse gas emissions, leading to greater environmental impacts. Therefore, alternative protein sources are necessary. Cultured meat emerges as a promising solution, offering lower environmental impact and a more effective way to feed the world's population. Cultured meat production involves three main steps: first, extracting animal cells through biopsies; second, growing these cells in a controlled environment; and third, inducing cell differentiation and structuring the meat. This work focuses on the third stage, aiming to develop new edible bioink formulations for 3D bioprinted scaffolds that support muscle cell adhesion and differentiation. To develop effective bioinks, materials must exhibit viscoelastic behavior and shear-thinning properties — becoming more fluid under pressure. This ensures that cells experience less mechanical stress while passing through the printer nozzle, reducing cell death. Additionally, thermosensitive polymers like gelatin are essential, as they remain liquid at high temperatures but gel at lower ones, facilitating bioink mixing. We developed six bioink formulations (A, B, C, D, E, and F) containing alginate (pristine or oxidized), gelatin, and a fermentation-based product (to improve bioink structure and cell adhesion) at different concentrations in DMEM culture medium. The prepared bioinks were tested using the TissueLabs bioprinter. Printing grid models were used to evaluate each formulation's printability. Flow and print speed parameters were optimized, varying nozzle gauge, flow volume and speed. Using a 25G nozzle, 1000% flow, and 1 mm/s speed yielded the best results. Among the tested bioinks, formulation E demonstrated the best printability, maintaining structural integrity most effectively. These findings contribute to optimizing bioinks for cultured meat, advancing towards scalable and structured meat production with lower environmental impact.

Keywords: Bioink; Bioprinting; Cultivated Meat.

Funding: CNPQ, Moondo Biotech, Rede Mineira de Engenharia de Tecidos e Terapia Celular (Remettec).

PRODUCTION OF ALGINATE-BASED MICROCARRIERS FOR CULTURED MEAT PURPOSES

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ABSTRACT: Cultivated meat is a promising biotechnology to produce a genuine meat product without animal slaughtering. To this end, muscle stem cells (Satellite cell - SC) are cultivated and differentiated in a three-dimensional biomaterial to obtain a structured product similar to animal muscle. However, to scale the production of SC, cells need to be grown in large vessels with culture being performed in suspension, using microcarriers (MC). MC provides an attachment surface for the SC and is usually necessary for cell survival and proliferation. This is an important bottleneck in the bioprocess, since both SC adhesion to and removal from these structures are critical stages in the productivity. Commercially available MCs are usually not edible and very expensive. Therefore, finding efficient MCs that are edible and can compose the final product is ideal. To achieve this goal, we tested different methods to produce a homemade microcarrier based on alginate (used in the food industry). For this, a 3% alginate solution was pushed through a 10ml syringe by extrusion in the absence or presence of an electric field (electrospraying) in a 100mM CaCl₂ bath. Beads were then photographed under a microscope and both shape and particle size evaluated using ImageJ. Both methods were able to generate stable beads with a round shape. However, through extrusion, beads presented a wider range of sizes, when compared to electrospraying. Additionally, most of the electrosprayed beads were smaller (200-350um) when compared to the ones produced by extrusion (>1mm). Since MC should be no larger than 350uM our results indicate that electrospraying was the best method for producing the beads. We also investigated whether we would be able to encapsulate cells using this protocol. For this, we used a fibroblast cell lineage and were able to show that most of the cells survived not only the electrospraying process but also survived in culture for at least 72 hours. This result indicates that this method may be used for production of alginate beads with potential use for cultivated meat purposes. For this, further studies are necessary to evaluate SCs adhesion and proliferation using this MC.

Keywords: microcarrier; alginate; cultivated meat, suspension growth.

Funding: CNPq (403685/2024-9), FAPEMIG (APQ-05625-24).

REDUCING CULTIVATED MEAT PRODUCTION COSTS: EVALUATION OF PLANT-BASED EXTRACTS AS A SUSTAINABLE ALTERNATIVE FOR CULTURE MEDIA

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ABSTRACT: Over the years, the global population has increased geometrically, while meat production has grown arithmetically, as postulated by Thomas Malthus (1798). Furthermore, extensive livestock farming leads to large deforested areas and increase in greenhouse gases, accelerating global warming and increasing the frequency of climate-related emergencies. In this context, cultivated meat has emerged as a sustainable protein alternative to ensure nutritional access, as emphasized by the WHO. The viability of this meat, however, is still impacted by several production bottlenecks, one of which is the high cost of culture media. Therefore, this project aims to develop an alternative, cost-effective, culture medium using plant-based extracts as sugar and amino acid sources, which could also contribute to a circular economy. For this we used LC/MS (Liquid Chromatography/Mass Spectrometry) technique to analyze both hydrolyzed and non-hydrolyzed extracts of oat, black oat, buckwheat, and pea, malted or not, produced at UFPR, to evaluate amino acid content and their potential use, alone or in combination, for media formulation. Grain malting is a controlled germination followed by drying to activate enzymes, especially amylases and proteases, which break down starches into sugars and proteins into peptides or amino acids, making a nutrient-rich and enzymatically active extract. Hydrolyzed extracts from all grains were the poorest samples with low number of available amino acids. Results showed that lysine (essential) and glycine (non-essential), were not detected in any extract, while tyrosine (non-essential) and phenylalanine (essential) were present in all. Methionine detection was likely hindered by ionization issues. Among the samples, non-hydrolyzed pea malt was the most complete. It contained the highest number of amino acids and most of the essential amino acids, lacking only lysine, which may be present in non-hydrolyzed peptides. Also, some detection limitations should be considered, which are attributed to poor ionization (methionine, lysine), overlapping signals (leucine/isoleucine) or low molecular mass (glycine). Therefore, future analyses will focus on non-hydrolyzed pea malt, while other extracts may be combined to complement missing amino acids and/or to optimize the culture medium formulation.

Keywords: plant extracts; amino acid; culture media; LC/MS; plant-based.

Funding: CNPq (403685/2024-9), FAPEMIG (APQ-05625-24).

BIOENGINEERING FOR CULTIVATED MEAT DEVELOPMENT

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ABSTRACT: Various politicians from different countries have gathered at conferences such as the COP (United Nations Climate Change Conference) to discuss ways to mitigate emerging climate change. According to a 2019 UN report, the global population is expected to reach 9.7 billion people by 2050. Therefore, it is crucial to explore ways to feed the population without causing further deforestation, greenhouse gas emissions, and animal slaughter. Muscle tissue engineering (MTE), which initially had a biomedical focus, has come to encompass a wide range of applications. After Mark Post's work on cultivated meat (2013), MTE aimed at the food industry received significant investments. However, creating thick muscle structures with characteristics similar to animal tissue remains a challenge. Previous studies from our group have demonstrated the potential of cellulose acetate nanofibers (CAN) as a scaffold for muscle cell cultivation. With these CAN, a process of stacking cell-loaded mats was developed, generating a three-dimensional meat-like product (16x16 mm). In this study, larger prototypes were evaluated, with four stacked sheets (24x60 mm) loaded with C2C12 myoblasts. Cell viability and organization were analyzed by actin cytoskeleton staining, revealing elongated cell morphology, which indicates cell differentiation. The mechanical characterization of the stacked structures was performed through stress-strain tests using Dynamic Mechanical Analysis (DMA). Stiffness was evaluated by the elastic modulus and compared to mouse muscle tissue. The stacked material showed a modulus of 485 KPa, while the animal tissue had 565 KPa. Tissue morphology before and after cooking was assessed by Scanning Electron Microscopy (SEM) in both stacked structures and animal tissue. Both showed similar morphology and tissue organization before and after cooking. Finally, we quantified the protein content using the Bradford method to compare with animal tissue. The stacked material presented 27.51 mg/g of protein, a value significantly lower than that found in animal tissue, which was 412.54 mg/g. The results indicate that although the stacking technique generates cohesive tissues similar to animal tissue, an equivalent to in vivo protein content has not yet been achieved, likely due to incomplete cell differentiation. Thus, new strategies need to be developed to improve final product quality.

Keywords: cellulose acetate; nanofibers; cultivated meat.

Funding: The Good Food Institute (GFI), INCT-FCx, CNPq, CAPES, FAPEMIG.

STUDY OF THE RHEOLOGICAL PROPERTIES OF HYDROGELS FOR 3D BIOPRINTING OF CULTIVATED MEAT

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ABSTRACT: Currently, protein consumed globally comes mainly from animal slaughtering. However, producing just one kilogram of beef requires around 15,000 liters of water and releases about half a kilogram of methane into the atmosphere, significantly impacting the environment and contributing to climate change. Similar concerns exist for most animal-derived food products. In light of this, cultivated meat has emerged as a promising research area aimed at reducing, and eventually overcoming, the environmental and ethical issues linked to traditional meat production. The process of producing cultivated meat involves three main steps: collecting progenitor muscle and fat cells from a selected animal, expanding these cells in a bioreactor, and inducing their differentiation and assembly into structured muscle tissue with similar morphology and nutritional value as conventional meat. A key technology for the final stage is 3D bioprinting, which enables precise placement of cells to replicate the architecture of natural tissue. In this technique, a printer extrudes a bioink—consisting of a hydrogel mixed with cells—through a nozzle, forming layers according to a digital model. The formulation of the bioink is a critical factor in ensuring successful bioprinting. It must be viscous enough to retain its shape after deposition but fluid enough to pass through the printer nozzle without damaging the embedded cells. Evaluating these properties falls under the field of rheology, which studies the flow and deformation of materials. In this study, three bioink formulations containing varying concentrations of alginate, gelatin and a fermentation based ingredient were developed and tested for mechanical performance. An ARES-G2 parallel plate rheometer was used to analyze their viscosity over a shear rate range of 1 to 100 s⁻¹, following a capillary rheometry model that assumed a syringe flow rate of 12 mm³/s and a 1.2 mm nozzle diameter. The results confirmed that all bioinks exhibited viscoelastic and shear thinning behavior—ideal characteristics for bioprinting. It was concluded that the specific concentrations of the components influenced the maximum shear stress experienced by the cells, which is critical to maintaining cell viability during printing.

Keywords: Bioink; Rheology; 3D Printing; Cultured Meat.

Funding: CNPq (310381/2022-3).

ESTABLISHMENT OF A PROTOCOL FOR ISOLATION OF BOVINE SATELLITE CELLS FROM ADULT MUSCLE

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ABSTRACT: The traditional meat production model based on cattle farming is responsible for major environmental issues, including biodiversity loss, greenhouse gas emissions, and water pollution and depletion. Cultivated meat has emerged as an alternative to this model, promising to reduce its environmental impact and address ethical concerns. Cultivated meat is produced using tissue engineering techniques, by culturing myogenic lineage cells on scaffolds designed to mimic the extracellular matrix of muscle tissue. Satellite cells (SCs) are the most commonly used due to their natural ability to generate new muscle fibers. However, the most widely adopted protocols for SC isolation were developed for murine models, which has hindered their application in livestock species relevant to meat production, such as cattle. In this study, we investigated the potential for isolating SCs from adult bovine muscle tissue (freshly slaughtered) using two protocols: (i) enzymatic digestion with collagenase II and trypsin, and (ii) the explant method. We assessed cell yield and proliferation capacity in culture, in addition to the expression of myogenic markers through immunofluorescence. Results indicated that both protocols are effective for isolating SCs from adult bovine muscle. The population doubling time for cells obtained via enzymatic digestion was 25.7 ± 1.0 hours, while the doubling time for the explant method was 34.9 ± 1.5 hours. This increase in doubling time must be a reflection of the delay in satellite cell activation due to less damage induced to the native tissue during cell isolation. The cells are being maintained in growth medium containing DMEM supplemented with 20% FBS and 1% antibiotic-antimycotic, and are currently at passages 4 (enzymatic digestion) and 3 (explant). Immunofluorescence analysis revealed the expression of MyoD and myosin, confirming their myogenic potential. By standardizing these protocols, we aim to contribute to the development of bovine cultivated meat.

Keywords: cultivated meat; satellite cells; bovine; explant.

Funding: CNPq, CAPES e FAPEMIG.

ADVANTAGES AND CHALLENGES OF ELECTROSPINNING IN THE PRODUCTION OF FIBROUS SCAFFOLDS FOR THE CULTURED MEAT MARKET

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REVIEW: Electrospinning is a well-established technique for synthesizing nanoscale fibrous architectures, which offer exceptional advantages when applied as scaffolds for cellular applications [1]. Despite its promise, the integration of electrospun matrices into the cultured meat industry – a sustainable solution to address escalating global demand for animal protein while mitigating environmental burdens – remains underexplored [2]. This study conducts a literature review to critically evaluate the benefits, limitations, and translational challenges of electrospun scaffolds in cultured meat production. Electrospun nanofibers have shown potential in promoting cell adhesion, proliferation, and alignment in *in vitro* models due to their tunable porosity and biomimetic topography [3]. However, their application in cultured meat systems is initial compared to their use in regenerative medicine, where studies predominantly employ non-edible animal models (e.g., murine or human cells) [4]. While the electrospinning apparatus (comprising a high-voltage generator, precision syringe pump, and collector) is technically accessible [1], limited commercial availability elevates capital expenditures (USD 10,000 – 50,000), posing a barrier to scalability [5]. For application in cultured meat, nanofiber production must involve food-grade, safe, non-toxic, and nutritionally suitable materials while ensuring optimal structural characteristics [6]. This requires strict control of process parameters, electrospinning solution properties, and environmental conditions, as well as specialized labor [7]. Although electrospinning demonstrates scalability potential for scaffold production through biomaterial innovation and eco-friendly solvents, industrial adoption remains constrained by knowledge gaps, including insufficient validation with agriculturally relevant species (bovine, porcine, poultry, and piscine cells) and cost-prohibitive infrastructure. Future studies should focus on optimizing biomaterial applications, establishing regulatory frameworks, standardizing production processes, and reducing costs to enable the industrial implementation of this technology in the cultured meat sector.

Keywords: Cultivated meat; Scaffold; Nanofibers.

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Funding: The authors would like to thank FINEP for project number 2892/22, FAPESC (Grant number 48/2021), and CAPES (Grant number 001) for providing scholarships to students.

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INDUCTIVE EFFECT OF MANGO PULP ON LIPID AND BONE TISSUE FORMATION

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ABSTRACT: The growing demand for alternative protein sources has driven the development of more sustainable technologies for cultured meat production. However, challenges such as prolonged cellular differentiation and the low productivity of complex tissues — such as fat and bone, which are essential to replicate the sensory and nutritional characteristics of conventional meat — still limit progress in the field. In this context, fruits, known for their richness in bioactive compounds and low cytotoxic potential, are proving to be promising natural sources for modulating cellular processes and stimulating tissue differentiation. In particular, polyphenols and carotenoids are often associated with the induction of osteogenic and adipogenic processes that promote extracellular matrix formation and lipid accumulation. The aim of this study was to investigate the potential of dehydrated mango pulp (DMP) to promote the differentiation and production of bone and adipose matrices in human adipose- derived mesenchymal stem cells (ADSCs). DMP was obtained from the Tommy Atkins cultivar using the foam mat drying method and characterized by liquid chromatography coupled with mass spectrometry (LC-MS). ADSCs were cultured for 21 days under four experimental conditions: Control (standard culture medium), MG (standard medium + 5 mg/mL DMP), Ind A/Ind O (adipogenic and osteogenic induction media) and Ind A/Ind O + MG (induction media supplemented with 5 mg/mL DMP). At the end of the culture period, the cells were stained with alizarin red (to assess the mineralized matrix) and oil red (to assess lipid accumulation) and analyzed by phase- contrast optical microscopy. The DMP-treated induced groups showed a significant increase in both bone matrix deposition and lipid accumulation. In terms of chemical characterization, DMP was identified as a matrix rich in carotenoids and polyphenols. These results suggest that the bioactive compounds present in mango may contribute to the differentiation of ADSCs into structural tissues relevant to cultured meat production, such as bone and adipose tissue, which are essential for reproducing the sensory and nutritional properties of conventional meat — especially in structured cuts that require higher tissue complexity.

Keywords: osteogenesis; adipogenesis; mangifera; bone matrix; adipose tissue.

Funding: This work was supported by Coordination for the Improvement of Higher Education Personnel – Brasil (CAPES) - (CAPES: 88887.919621/2023-00) for scholarship.

STRUCTURAL AND FUNCTIONAL PROPERTIES OF ZEIN FIBERS OBTAINED BY ELECTROSPINNING FOR CULTIVATED MEAT SCAFFOLDS

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ABSTRACT: The growing demand for sustainable alternatives to conventional meat production has accelerated research into cultivated meat. Scaffolds are essential components in cultivated meat production, as they provide a three-dimensional support structure that allows cells to adhere, proliferate, and differentiate. Among the technologies under exploration, electrospun fibrous scaffolds stand out for their ability to mimic the architecture of muscle tissue. This study aimed to develop and characterize zein-based fibers with potential application as scaffolds for cell culture. Zein (20 kDa) was dissolved in an ethanol:water solution (80:20 v/v) at a concentration of 24% (w/v). Fibers were produced using an electrospinning setup with the following parameters: a 5 mL syringe fitted with a 0.75 mm steel needle, an applied voltage of 12 kV, a 10 cm working distance, and a feed rate of 1.55 mL h⁻¹ at room temperature. Scanning electron microscopy (SEM) revealed the formation of continuous and bead-free fibers, with a mean diameter of 999 ± 5.16 nm. Contact angle analysis revealed a highly hydrophilic surface, likely due to the exposure of polar groups during fiber formation and drying. Fourier Transform Infrared Spectroscopy (FTIR) analysis confirmed the presence of zein's characteristic functional groups but also indicated conformational changes in polypeptide chains due to electrospinning. Differential scanning calorimetry (DSC) further supported these structural modifications by showing a decrease in enthalpy values, suggesting a less ordered molecular arrangement. Tensile testing demonstrated that the fibers exhibit predominantly elastic behavior, with high deformation capacity before rupture. UV light exposure for 30 min per side of the fiber surface effectively inhibited the growth of bacteria, fungi, and yeasts. The results suggest that zein-based electrospun fibers hold potential for use as scaffolds in cultivated meat applications, due to their favorable morphological and structural characteristics. Further investigations may help enhance their performance under cell culture conditions.

Keywords: Nanofibers; Zein-based scaffold.

Funding: The authors would like to thank FINEP for project number 2892/22, FAPESC (Grant numbers 48/2021, 2024TR002561, and 2024TR002301), CNPq, and CAPES (Grant number 001) for providing financial support and scholarships to students.

FROM LIVESTOCK TO FISH: ADVANCES AND CHALLENGES TOWARD MUSCLE AND FAT CO-CULTURING FOR MEAT PRODUCTION

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REVIEW: Co-culturing is a promising strategy to enhance the structural and sensory complexity of cultivated meat, but practical applications still face technical challenges, particularly during the differentiation stage. Thus, this review describes the main challenges currently faced during co-culture for meat production, focusing on cell interactions during the differentiation stage, and the innovative approaches for muscle and fat integration using cattle, swine, poultry, and fish cells. Simultaneous differentiation of muscle and fat cells promotes inhibitory interactions and does not seem to be efficient, although specific supplementation in the culture medium (with arginine or conjugated linoleic acids) demonstrated great potential to circumvent this behavior. Sequential differentiation using the same cell population (e.g., fibroblasts) in scaffolds seemed to be the most promising strategy for producing muscle and fat cells in a single system. Recommendations for future studies involve applying non-GMO cell transformation techniques and developing edible scaffolds using this innovative approach. Scaling up co-culture systems also remains a major technological challenge. While perfusion reactors offer a promising method for creating structured cultivated meat, optimizing the co-culture approach and bioreactor designs will be crucial for commercial production.

Keywords: cell-based meat; chicken; beef; fish; pork.

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Funding: This work was financed in part by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001; and the Financier of Studies and Projects (FINEP) 2892/22.

NEXT-GENERATION RISK ASSESSMENT FOR NOVEL FOOD PROTEIN SAFETY: TOOLS AND CURRENT CHALLENGES

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REVIEW: Next-Generation Risk Assessment (NGRA) has emerged as a hypothesis-driven and exposure-led approach that integrates *in silico*, *in vitro*, and modeling tools to support animal-free safety evaluations. Therefore, this work aims to explore NGRA-integrated tools to assess the toxicity of novel cultivated proteins. *In silico* approaches currently play a central role in early safety screening. After obtaining protein sequence through genomic or proteomic analysis, tools such as *ToxinPred2* and *Toxify* may be used to predict toxicity based on the protein primary structure, employing machine learning and deep learning algorithms, respectively. These are often complemented by resources like *InterPro*, which support the identification of conserved functional domains and associations with known toxic mechanisms. Structural prediction tools (e.g., *AlphaFold*, *I-TASSER*) further aid in understanding protein conformation, with *AlphaFold* relying on deep learning to offer additional insights into potential biological activity and toxicity. Since defining similarity thresholds or mechanistic alerts that would be required for further *in vitro* testing is still debated, regulators favor hypothesis-driven and case-by-case evaluation. Sequentially, *in vitro* assays support key cellular endpoints and protein digestibility evaluation. Advanced tools like *ToxProfiler* provide mechanistic insights via stress pathway activation in human cells, while digestion models simulate the stability and potential hazards of resulting protein fragments under gastrointestinal conditions. Concurrently, physiologically-based toxicokinetic (PBTK) and *in vitro* to *in vivo* extrapolation (IVIVE) models estimate a human toxic dose from *in vitro* data, enhancing exposure relevance. Integrating these tools provides a comprehensive toxicity assessment for cultivated proteins. However, future perspectives include the need for standardized digestion models, validation of computational methods for regulatory acceptance, and real-world case studies. Collaborative efforts to develop harmonized bioinformatic frameworks and shared databases among regulators, academia, and industry will also ensure more robust and reliable safety evaluations.

Keywords: safety assessment; alternative proteins; bioinformatic; artificial intelligence; toxin database.

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Funding: This work was financed in part by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001; and the Financier of Studies and Projects (FINEP) 2892/22.

POROUS STARCH-BASED SCAFFOLDS FOR CELLULAR AGRICULTURE: DEVELOPMENT AND STRUCTURAL EVALUATION

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ABSTRACT: In the context of cellular agriculture, edible scaffolds are essential to support the three-dimensional (3D) cultivation of animal cells by mimicking the extracellular matrix and enabling nutrient diffusion. Plant-based biomaterials, such as starch and plant proteins, offer cost-effective and food-grade alternatives for scaffold fabrication. This study aimed to develop and characterize starch-based porous scaffolds with potential applications in the 3D cultivation of muscle, adipose, and skin cells for food biofabrication. Six formulations (F1 – F6) were developed containing cassava starch in native (2, 6, 10, and 15%) or pregelatinized (2 and 6%) forms, associated with κ -carrageenan (1.6%) and soy protein concentrate (3%). To obtain the porous matrices, different drying methods were employed: freeze-drying, vacuum oven drying, and air fryer drying. All samples were sterilized by autoclaving. Structural characterization was performed by scanning electron microscopy (SEM) to evaluate pore formation, wall morphology, and general architecture. Among the formulations, the most promising was F6 (6% native starch), particularly when freeze-dried. This process yielded scaffolds with interconnected macropores and thin, rough walls, features that favor nutrient transport, fluid retention, and potential cell adhesion. In contrast, scaffolds made with modified starch or low starch concentration (<6%) collapsed under moisture, forming fragile and dense structures without effective porosity. Rapid thermal drying (e.g., air fryer) produced compact, laminar architectures unsuitable for 3D culture. The results demonstrate that both the concentration of native starch and the freeze-drying process are critical to achieving mechanically stable and functionally porous structures. The study provides a structural and technological basis for the use of starch-based scaffolds in cultured meat systems. The F6 scaffold, combining intermediate starch content and freeze-drying, proved to be the most suitable for further biological validation. Future experiments will involve swelling, compression, and cell viability tests to confirm its applicability in food-grade tissue engineering.

Keywords: edible scaffold; cassava starch; freeze-drying; cultured meat; cellular agriculture.

Funding: C.S.W. to acknowledge the Coordination for the Improvement of Higher Education Personnel (CAPES) for financial support through scholarship (Code 001). S.V. to acknowledge Financiadora de Estudos e Projetos (FINEP 2892/22), Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC 2024TR002561), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq200015/2025-7) for the financial support.

CULTIVATED PORCINE SKIN AS A FOOD PRODUCT: BRIDGING TISSUE ENGINEERING AND FOOD SCIENCE

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REVIEW: The biofabrication of edible tissues is an emerging field that integrates concepts from tissue engineering and food science, with the potential to diversify and sustain novel food systems. In this context, in vitro–cultivated porcine skin stands out as both an innovative final food product and a versatile platform for the development of structured cultured meat. Its applications extend beyond muscle-based cultivated meat, including structured cuts, edible coatings, and functional snacks, as well as layered assemblies integrating skin and fat tissues. This review explores the scientific advances that enable the production of edible cultivated porcine skin, highlighting its main cellular components, scaffold materials, cultivation strategies, and technological challenges. The bilayer architecture of the skin, stratified epithelium supported by a fibroblast-rich dermis, serves as a biologically relevant model for scaffold-guided tissue construction. The cultivation of porcine keratinocytes and dermal fibroblasts in monolayer, co-culture, or stratified systems is feasible through the use of Transwell® inserts and air–liquid interface (ALI) conditions, promoting the formation of a functional epidermal barrier. Plant-derived biopolymers have been explored as edible scaffolds due to their compatibility with food-grade standards and their ability to mimic the extracellular matrix of skin. Advanced techniques such as 3D bioprinting of epithelial layers and bioreactors tailored for thin tissue cultivation are also discussed as process intensification strategies. Beyond production aspects, this review addresses regulatory, safety, and consumer acceptance issues, considering the novelty of applying dermal constructs to food innovation. The anatomical and biochemical similarities between porcine and human skin reinforce the relevance of porcine models for developing edible tissues for human consumption. Cultivated porcine skin, whether as a final product or a structural platform, represents a novel category within cellular agriculture, combining technological value, functional versatility, and sustainability, and contributing to the diversification of future food systems.

Keywords: cultivated porcine skin; cultured meat; food-grade scaffold; tissue-engineered foods; cellular agriculture.

Funding: C.S.W to acknowledge the Coordination for the Improvement of Higher Education Personnel (CAPES) for financial support through scholarship (Code 001). S.V. to acknowledge Financiadora de Estudos e Projetos (FINEP 2892/22), Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC 2024TR002561), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq200015/2025-7) for the financial support.

ENGINEERING ECM-MIMETIC SCAFFOLDS WITH COLLAGEN AND BACTERIAL NANOCELLULOSE FOR CULTURED MEAT APPLICATIONS

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ABSTRACT: The production of cultured meat (CM) requires a thorough understanding of the interactions between cells and the extracellular matrix (ECM). ECM components play essential roles in the migration, proliferation, and differentiation of myoblasts, directly influencing their physiological behavior. Muscle tissue relies heavily on the ECM for organization, structural support, and mechanical function, with collagen being its main constituent. Although not derived from food species, cell lines such as C2C12 are still used as standard models for validation and performance of scaffolds in CM applications. In this context, the present study aimed to evaluate the influence of collagen I on the migration, proliferation, and differentiation of murine myoblasts (C2C12) cultured on bacterial nanocellulose (BNC) scaffolds. BNC membranes were prepared in the format of 24-well plates, into three groups: pure BNC, BNC incorporated with collagen I, and BNC cross-linked with glutaraldehyde and collagen I. Approximately 20 C2C12 spheroids were seeded together with 150,000 single C2C12 cells per scaffold type. The experiment was performed in triplicate and maintained in an incubator at 37 °C with 5% CO₂ for 14 days. After seven days, the medium was replaced with differentiation medium containing 2% Fetal Bovine Serum (FBS). Cell behavior was monitored by light microscopy on days 1, 7, and 14. Finally, the scaffolds were fixed with 4% paraformaldehyde for immunofluorescence analysis using anti-MYF5 and anti-MYHC antibodies and counterstained with DAPI and rhodamine-phalloidin. Scaffolds containing collagen exhibited enhanced cell adhesion and proliferation, as evidenced by the migration of cells from the spheroids to the material and the strong F-actin staining. Although the same initial cell density was used in all models, rhodamine-phalloidin staining revealed a greater abundance of F-actin filaments in the collagen-containing scaffolds, correlating with a higher number of adhered C2C12 cells and indicating a more favorable microenvironment. Immunostaining revealed MYF5 expression, characteristic of proliferating myoblasts, and stronger MYHC staining, indicative of myotube differentiation. It is concluded that the presence of collagen promotes the formation of an ECM more conducive to myogenesis, enhancing cell adhesion, proliferation, and differentiation, critical steps for the advancement of CM development.

Keywords: Cultured Meat; Extracellular Matrix; Collagen; Bacterial Nanocellulose; Spheroids.

Funding: FINEP.

BIBLIOMETRIC AND TECHNOLOGICAL ANALYSIS OF STARCH- BASED SCAFFOLDS FOR CULTURED MEAT PRODUCTION

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REVIEW: This study presents a comprehensive bibliometric analysis of scientific publications on starch-based scaffolds, with emphasis on their applications in tissue engineering and cultured meat production. A total of 54 experimental articles published between 2000 and 2024 were selected from the Scopus database, excluding systematic and narrative reviews to focus strictly on applied and experimental studies. The temporal evolution of publications shows a stable period between 2000 and 2015, with 0 to 2 articles per year, totaling only 13 in 16 years. From 2018 onwards, research output increased sharply, with 5 articles in 2018, 6 in 2019, and a peak of 9 in 2020. Slight declines were observed in 2021 (4 articles) and 2022 (3 articles), possibly due to COVID-19 disruptions. In 2024, output rose again to 9 articles, and in 2025 (partial), 5 have been identified. This trend reflects advancements in bioengineering and growing interest in sustainable, biocompatible materials for medical and food uses. Geographically, Portugal leads with 11 publications, followed by Iran (9), China (8), Brazil (4), and India (4), with additional contributions from Mexico, the U.S., and Canada. Key international collaborations include Iran–Canada, Canada–Turkey, Portugal–Italy, and Portugal–U.S., highlighting global cooperation. Keyword analysis reveals country-specific research focuses: Iran on porous and nanostructured starch; Brazil on 3D printing and surface modifications; Portugal and the U.S. on micro- and nanofibrillar scaffolds; and India on electrospinning and starch hydrogels. Most studies (70.4%) did not specify the starch source. Among those that did, corn starch was most used (11.1%), followed by wheat, potato, and cassava starches (3.7% each). The most explored scaffold types were porous structures and hydrogels, followed by 3D-printed forms, fibrous meshes, membranes, and aerogels. In terms of research fields, medical sciences accounted for 68.5% of studies, followed by food science (25.9%) and materials science (5.6%). Overall, starch emerges as a promising, versatile biomaterial in an expanding multidisciplinary landscape.

Keywords: Cultured meat; starch-based scaffolds; tissue engineering; bibliometric analysis; patent prospection; food biofabrication; cellular agriculture.

Funding: C.S.W to acknowledge the Coordination for the Improvement of Higher Education Personnel (CAPES) for financial support through scholarship (Code 001). S.V. to acknowledge Financiadora de Estudos e Projetos (FINEP 2892/22), Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC 2024TR002561), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq200015/2025-7) for the financial support.

STABILITY IN CULTURE MEDIUM: EVALUATION OF THE INTEGRITY OF STARCH-BASED POLYMERIC SCAFFOLDS FOR APPLICATIONS IN CELLULAR AGRICULTURE

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ABSTRACT: This study aimed to evaluate the structural stability of polymeric scaffolds composed of cassava starch (native or modified), κ -carrageenan, and concentrated soy protein when immersed in culture medium for up to 72 hours. The investigation focused on the physical integrity of the scaffolds under wet conditions, targeting their application as three-dimensional supports for cell cultivation in the context of cellular agriculture. Four formulations were tested, all containing 1.6% κ -carrageenan and 3% soy protein concentrate, with variations in starch type and concentration: 2% and 6%, both native and modified. The scaffolds were subjected to different drying methods, including lyophilization, vacuum oven, and air fryer, and were subsequently sterilized by autoclaving prior to immersion. Samples were evaluated at 0, 24, 48, and 72 hours for structural integrity, collapse, and pH variation in the medium. Among the tested conditions, the formulation containing 6% native starch showed the most promising results, maintaining a cohesive and stable structure throughout the 72-hour period, with minimal pH fluctuation. In contrast, scaffolds containing modified starch demonstrated early disintegration and partial collapse after 24 hours, likely due to their reduced ability to form stable polymeric networks under aqueous conditions. Additionally, formulations with lower starch concentrations (2%) were also less stable, showing greater susceptibility to degradation and solubilization in the culture medium. Several samples presented significant pH variation over time, suggesting either leaching of solubilized components or partial polymer degradation, which may compromise scaffold functionality in long-term applications. These findings highlight the importance of starch type and concentration, as well as the drying method, in determining scaffold performance under wet conditions. Overall, lyophilization followed by autoclaving was identified as the most effective combination for preserving scaffold structure and medium compatibility. The formulation containing 6% native starch will be used as the basis for future experiments involving crosslinking agents and advanced morphological and biological analyses.

Keywords: Starch-based scaffold; culture medium stability; lyophilization; cellular agriculture; polymeric matrix.

Funding: C.S.W to acknowledge the Coordination for the Improvement of Higher Education Personnel (CAPES) for financial support through scholarship (Code 001). S.V. to acknowledge Financiadora de Estudos e Projetos (FINEP 2892/22), Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC 2024TR002561), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq200015/2025-7) for the financial support.

ASSESSING ULTRAFILTRATION IMPACTS ON SOYBEAN MEAL HYDROLYSATE FOR USE IN CULTURED MEAT MEDIA

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ABSTRACT: Hydrolysates derived from agro-industrial residues, free of animal-derived components, offer sustainable and cost-effective alternatives for cell culture media in cultivated meat production. However, their use is limited by significant batch-to-batch variability. This study investigates ultrafiltration as a strategy to standardize the biochemical profile of hydrolysates, using soybean meal hydrolysate as a model. Hydrolysis was performed at 65 °C, pH 8.0, with 5.0% enzyme, using an optimized mixture of 87.5% Alcalase® and 12.5% Flavourzyme®, previously established to maximize the degree of hydrolysis. A portion of the hydrolysate was ultrafiltered using a pressurized nitrogen cell (10 kgf/cm²) with a polyethersulfone membrane (NADIR® UP010) of 10 kDa at 0.15 mL/min and 100 rpm. The crude and ultrafiltered hydrolysates were analyzed for protein content, molecular weight distribution (SEC), FTIR spectral profile, and cytotoxicity in C2C12 cells using the MTT assay. Characterization by SEC and FTIR analysis revealed subtle differences between raw and ultrafiltered samples, despite the 4.21% decrease in protein content. These results indicate that ultrafiltration induced only minor physicochemical changes in the hydrolysates. In the cytotoxicity assay, cells were exposed to hydrolysate concentrations of 100, 500, and 2000 µg/mL, supplemented with 10% FBS. No cytotoxic effects were observed for any of the hydrolysates, and no statistically significant differences were found between treatments ($p > 0.05$). Despite its potential, ultrafiltration of soybean meal hydrolysate under the tested conditions did not yield important advantages. Process limitations, including low flux rate and high cost, combined with the lack of meaningful differences in outcomes, indicate limited effectiveness. Therefore, ultrafiltration may not be a viable strategy for standardizing hydrolysates intended for cultivated meat production.

Keywords: cultivated meat 1; standardization 2; cell culture 3; agro-industrial wastes 4

Funding: This work was supported by the Brazilian National Council for Scientific and Technological Development [CNPq, research project no 403675/2021-9 and productivity grant no 311333/2023-0], the Universidade Estadual de Campinas (UNICAMP) Teaching, Research, and Extension Support Fund [FAEPEX, 2451/23], the Brazilian Federal Agency for Support and Evaluation of Graduate Education [CAPES, Finance code 001], and the São Paulo Research Foundation [FAPESP, research project numbers 2022/15221; 2023/17148-2; 2024/02154-0; 2024/12776-8; and thematic project 2022/05731-2].

DEFINITION OF PLURIPOTENCY MARKERS FOR THE ANALYSIS OF REPROGRAMMING AND CELL DIFFERENTIATION IN FISH

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ABSTRACT: The implementation of innovative technologies, such as *in vitro* meat production, is necessary to mitigate the environmental, social, and economic impacts resulting from overfishing. *Astyanax lacustris* (lambari) has become a model organism for applying conservation strategies. This study aimed to characterize embryonic stem cells (ESCs) to define pluripotency and differentiation markers for the subsequent generation and characterization of induced pluripotent stem cells (iPSCs), which will be used to differentiate into muscle lineages of lambari. The research involved *in vitro* fertilization, followed by the isolation, cryopreservation, and culture of the ESCs. Pools of 50 embryos at the blastula stage (1,000 cells) were used for cryopreservation. The isolated blastospheres were placed in DMEM medium supplemented with 1% antibiotic-antimycotic and 0.05% BSA. Two freezing methodologies (cryotubes and straws) were tested using 10% DMSO. After thawing, viability (assessed with 0.4% trypan blue), pluripotency (assessed with alkaline phosphatase-AP), and culture in 24-well plates coated with 0.1% gelatin were evaluated. Samples from the early stages of embryonic development such as blastula stage (256 cells, 1,000 cells, elongated, spherical and dome morphological phases) and gastrulation with 30% and 70% epiboly were collected for evaluation of pluripotency and differentiation genes by RT-qPCR. The results showed a significant difference in cell viability post-thawing between the two methodologies ($P = 0.0049$). The viability of cells cryopreserved in cryotubes was $65 \pm 3.26\%$, while in straws it was $23 \pm 14\%$. Additionally, the cells were reactive to AP. However, it was not possible to maintain cell viability after long-term culture. Three pluripotency markers were defined: *OCT4*, *KLF17*, and *TDGF1*, whose expression began at the 1,000-cell stage and subsequently decreased during gastrulation. The differentiation markers *GATA4* (endoderm), *TBXTA* (mesoderm), and *SOX2* (ectoderm) was verified, showing expression during the final blastulation stage, with a progressive increase during gastrulation. These pluripotency markers in stem cells constitute an essential step for the reprogramming and characterization of iPSCs which have the potential to differentiate into muscle lineages. This project aims to contribute to a more conscious management of neotropical fish species, promoting the field of fish meat production from cell cultures.

Keywords: embryonic stem cells, neotropical fish, cryopreservation, reprogramming.

Funding: FAPESP

DIFFERENTIATION OF BOVINE INDUCED PLURIPOTENT STEM CELLS (biPSCs) INTO SKELETAL MUSCLE CELLS

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ABSTRACT: Traditional livestock farming, despite its economic importance, ends up having a major impact on the environment, such as illegal deforestation and greenhouse gas emissions. Population growth brings with it an increase in demand for protein sources, and so new technologies are emerging to meet this demand. Cultured meat is emerging with the goal of producing high-quality protein in a sustainable manner. In this context, the use of induced pluripotent stem cells from cattle (biPSCs) enables the differentiation and large-scale production of muscle cells. The objective of the study was to differentiate biPSCs into functional muscle cells in 2D culture over a period of 45 days. During differentiation, biPSCs received DI-CL, DI-CLF, DK-HIFL, DK-I, and DK-HI media on days D0, D3, D6, D8, and D12, respectively. From D27 to D45, the cells were maintained in SKGM medium supplemented with 2% Horse Serum or 1% N2, resulting in 10 groups to be evaluated. After analysis via RT-qPCR of the myogenic markers BMP4, MYOG, MYOD, and PAX7, an increase in the expression of the MYOG gene was noted, as well as an increase in the expression of the BMP4 gene, a mesoderm marker. Adjustments will be necessary in the initial stages of differentiation, with the use of small molecules such as CHIR, responsible for activating the WNT pathway, in order to improve the efficiency of differentiation in the mesodermal destination, favoring the obtaining of a culture of homo muscle cells.

Keywords: Stem cells; iPSCs; cultured meat; muscles cells.

Funding: Fapesp

DEVELOPMENT OF AN ANIMAL-FREE SCAFFOLD FOR CULTIVATED MEAT BASED ON AGAR-AGAR AND AQUEOUS EXTRACT OF ORA- PRO-NOBIS

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ABSTRACT: The development of scaffolds for cultivated meat still requires significant improvements. One critical aspect is the replacement of animal-derived components in the process. The creation of fully animal-free scaffolds remains a key challenge to be addressed in the field. In this context, the present work aimed to produce agar-agar hydrogel with different concentrations of ora-pro-nobis aqueous extract (OAE) for the development of cultivated meat scaffold. The hydrogel was produced using 1.5 g of agar-agar and different concentrations of OAE obtained by ultrasonic bath from 0.5 g and 1.0 g of dehydrated leaves, the extract was incorporated into the formulations at a proportion of 20% (v/v). The hydrogel were characterized in relation to visual analysis, gel formation, color parameters and total phenolic compounds. The agar-agar scaffolds with OEA exhibited gel formation, also were homogeneous and transparent, an important characteristic for applications in the food sector. In relation to the color parameters there was an increase in chroma a^* , being 0.57 ± 0.01^b to 0.75 ± 0.01^a and total compound phenolic, being 9.79 ± 1.35^b to 12.23 ± 1.28^a mg GAE/ 100 g for hydrogel with addition 0.5 g and 1.0 g OEA, respectively. The reduction of chroma a^* denote a tendency to green color in hydrogel, possibly due to the presence of chlorophyll in ora-pro-nobis leaf. Because of this, the scaffolds can be considered a potential source of phenolic compounds. The formulation demonstrates potential for the development of a functional and structurally stable scaffold for cultivated meat production. The resulting hydrogel may represent a promising alternative for cellular cultivation, as it offers a biocompatible, homogeneous, and transparent matrix that could support cell adhesion and proliferation. Additionally, this scaffold is animal-free, low-cost, and enriched with bioactive compounds, being derived from a sustainable, plant-based source. Its composition reinforces its potential as a functional biomaterial, aligning with current demands in the food industry for innovative, ethical, and environmentally sustainable solutions within the field of cellular agriculture.

Keywords: Agar-Agar; Active Compound; Ora-pro-nobis; Cultivated Meat.

Funding: This study was partly funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

ASSESSMENT OF *HAEMATOCOCCUS PLUVIALIS* GROWTH UNDER DIFFERENT NUTRITIONAL CONDITIONS FOR APPLICATION IN CULTIVATED PROTEINS

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ABSTRACT: The biomass of the microalga *Haematococcus pluvialis* stands out as a promising ingredient for application in cultivated protein formulations, due to its high protein content and production of astaxanthin, a carotenoid with well-recognized antioxidant potential and added value for the functional food and nutraceutical industries. This study aimed to evaluate the growth performance of two *H. pluvialis* strains (0028 and 0055), cultivated under three different concentrations of culture medium, to identify the most productive condition for biomass generation with potential use as a bioactive ingredient in culture media or scaffolds for cultivated protein production. Cultivation was carried out for 22 days in 3X, 4X, and 5X concentrations of the base medium described by Hata et al., with cell density monitored using flow cytometry (Cytek Northern Lights). The results showed that strain 0055 achieved the highest flow growth in Hata 3X medium, reaching a concentration of 9.82×10^6 cells/mL on the final day of cultivation. Strain 0028, in turn, showed the highest growth in Hata 4X medium, with 1.66×10^7 cells/mL. Under less favorable conditions, strain 0055 cultivated in Hata 5X medium reached a cell density of 8.05×10^5 cells/mL. The observed growth response of the strains under different medium concentrations demonstrates that variations in essential nutrient levels, such as nitrogen and phosphorus, directly influence the metabolic modulation and cell division rate of microalgae, leading to biomass accumulation. The findings reinforce the potential of *H. pluvialis* biomass as a source of ingredients for use in cellular agriculture technologies. However, to enable its large-scale application, optimized cultivation is essential. In this context, the results represent a strategic advancement by contributing to increased productivity and reduced production costs, which are key factors to making the solution more accessible and sustainable for the cultivated protein sector.

Keywords: *Haematococcus pluvialis*; cultivated proteins; microalgae; biomass.

Funding: Financial support for the author Joseane C. Bassani was provided by the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES), Finance Code 001.

CULTIVATED MEAT AND COMPUTATIONAL MODELING: CHALLENGES AND PERSPECTIVES FOR INDUSTRIAL SCALE VIABILITY

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ABSTRACT: As the global population grows, demand for meat is expected to increase significantly in the coming decades, intensifying the environmental, ethical, and economic challenges associated with traditional livestock farming. In this context, cultivated meat emerges as a sustainable alternative, although it still faces technical, economic, and cultural obstacles. This study aims to map the use of computational modeling in the scaling of cultivated meat, identifying its limitations and its potential as a tool to support decision-making in large-scale production. To this end, a systematic review was carried out in the Web of Science, Scopus and The Lens databases, using the terms: “cult* meat AND simulat* (Title, Abstract OR Keywords)”. Furthermore, a filter was applied to exclude review articles and a 5-year time limit was not established, in order to broaden the scope of the search. 38 non- duplicate articles were found, of which 7 were considered relevant to the research objective. Process simulators that support this scenario include Ansys Fluent, COMSOL, and SuperPro Designer, which are used to simulate physical-chemical conditions and sizing equipment. Using these tools, studies indicate that the viability of scaling is associated with the development of customized bioreactors capable of maintaining ideal conditions for cell growth and avoiding mechanical damage to cells during cultivation. Another critical point is the culture medium used in the bench tests that precede the simulation, corresponding to the main cost of the process and requiring studies aimed at replacing it with sustainable inputs free of components of animal origin. In this sense, computational modeling supports decision-making by simulating different experimental scenarios, anticipating the behavior of the system under different operational conditions. This makes it possible to identify critical variables, estimate expenses, size equipment, and predict risks, such as failures in cell cultivation or the impact of contamination. Therefore, although cultivated meat represents a long-term alternative, its consolidation will depend on integrated efforts between researchers, industry, and regulatory bodies, which, with the support of simulations, can contribute to optimization, cost reduction, and a safer transition to industrial scale.

Keywords: Cultivated meat; simulation; computational modeling; scaling; viability.

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Funding: CELLMEAT3D / Finep.

ALTERNATIVE PROTEINS IN FOOD DEVELOPMENT: EMERGING SOURCES FOR A SUSTAINABLE FOOD FUTURE

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REVIEW: The urgent global demand for sustainable food systems is catalyzing innovation in alternative protein development, particularly in meat and fish analogs [1]. Traditionally, these products have relied on plant-based ingredients like soy and wheat gluten to replicate the texture and nutritional value of conventional foods. However, such formulations often fall short in replicating the complex sensory and structural characteristics of animal-based products [2]. This review explores the potential of emerging protein sources, with a focus on the synergistic integration of legumes, fungi, and microalgae to expand the global nutritional matrix and overcome current formulation limitations. Legumes contribute essential amino acids, fiber, and gelling capacities, serving as a structural base for analog development. Fungi and mycoproteins offer substantial functional advantages, enhancing viscoelasticity, juiciness, and internal microstructure—especially when applied in innovative technologies such as 3D food printing, which allows for greater precision in texture and appearance [3,4]. Mycoproteins also contribute to a favorable nutritional profile, with high digestibility and bioavailability. Among these novel sources, microalgae stand out as a highly promising and multifunctional ingredient, delivering not only high-quality protein but also essential fatty acids, pigments, and distinctive volatile organic compounds [5]. These compounds are particularly effective in reproducing authentic marine flavors, making microalgae ideal for plant-based fish analogs. Their established status as a superfood further enhances their appeal to health-conscious consumers and may help mitigate neophobia toward novel ingredients [6]. Importantly, the future of sustainable food innovation lies not in isolated ingredients but in the strategic combination of diverse sources. The synergy between the structural role of legumes, the textural benefits of fungi/mycoproteins, and the sensory-nutritional attributes of microalgae offers a robust framework for developing next-generation analogs. These products have the potential to satisfy consumer expectations for taste, nutrition, and sustainability, while contributing to the diversification of the global food supply.

Keywords: Alternative proteins; Sustainable food systems; Fungi and mycoproteins; Microalgae; Plant-based innovation

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Funding: The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES), The National Council for Scientific and Technological Development of Brazil (CNPq), Research Support of the State of Rio Grande do Sul (FAPERGS) and Ministry of Science, Technology, and Innovation (MCTI) for the support.

POTENTIAL OF *Spirulina* BIOMASS AS A FUNCTIONAL INGREDIENT FOR PROTEIN-ENRICHED FOODS

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REVIEW: The growing demand for alternative protein sources has been driven by several factors, including population growth and the environmental impacts associated with conventional protein production ^[1-2]. In this context, microalgae have emerged as a promising alternative to address global challenges related to food security and environmental sustainability ^[2]. Recognized as sustainable protein sources, microalgae stand out for their high biomass productivity and their ability to be cultivated on non-arable land, using brackish water, seawater, or wastewater, without competing with conventional agricultural crops ^[2-3]. Microalgal biomass has been used in food products, particularly species of the genus *Spirulina*, due to its high protein content (50-70% w/w), the presence of essential amino acids, minerals, vitamins, and bioactive compounds in its composition ^[4,5,6]. In this context, this review presents recent advances in the use of *Spirulina* biomass for protein enrichment in food products and highlights the functional properties of microalgal proteins. The addition of *Spirulina* biomass to foods such as snacks (2.6%) ^[7], yogurt (1.6%) ^[8], gluten-free bread (5%) ^[9], vegan cookies (20%) ^[5], and gummy candies (5%) ^[6] resulted in increases in protein content of 22.6%, 125%, 39%, 40%, and 37%, respectively, compared to control formulations. Moreover, snacks enriched with 2.6% *Spirulina* biomass not only showed protein enrichment but also exhibited a protein digestibility of approximately 90% ^[7]. Regarding the amino acid profile, replacing part of the wheat flour with 20% *Spirulina* biomass in vegan cookie formulations not only increased the protein content but also maintained protein quality, while maintaining a chemical score of approximately 100% ^[5]. In addition to the nutritional value of the biomass, *Spirulina* protein isolates stand out for their functional properties, including high solubility and performance under alkaline pH conditions, as well as good emulsifying and foaming capacities, water retention ability, oil retention ability, and the potential to form films and gels ^[10]. Therefore, *Spirulina* biomass represents a promising source of proteins and bioactive compounds for the development of functional foods, while also offering an alternative and environmentally sustainable platform for protein production.

Keywords: Alternative protein; Microalgae; Nutritional value; Supplementation.

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Funding: The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) (Finance Code 001).

OPTIMIZATION OF NON-ANIMAL-DERIVED HYDROLYSATES AS A SUSTAINABLE REPLACEMENT FOR FETAL BOVINE SERUM IN CULTURED MEAT PRODUCTION

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ABSTRACT: Large-scale cultured meat production faces a central challenge: the replacement of fetal bovine serum (FBS), a high-cost component associated with ethical issues and contamination risks. To enable sustainable and scalable production, it is crucial to develop effective alternatives that do not depend on animal-derived inputs. This review focuses on strategies to develop effective alternatives by optimizing hydrolysates from various sustainable sources. These include plant-based materials (soy), microbial sources (algae, yeast), and other agro-industrial byproducts (okara), which are explored as raw materials rich in essential nutrients, bioactive peptides, and high protein content necessary for cell proliferation. The main optimization strategies include the use of specific enzymes (such as Alcalase) to control the degree of hydrolysis, fractionation to obtain low molecular weight peptides, and microbial fermentation to enrich the nutritional profile. Studies demonstrate that supplementing these hydrolysates with recombinant growth factors and other additives enhances cell proliferation and differentiation, allowing for the partial or total replacement of FBS. The optimization of hydrolysates from alternative sources is a promising strategy to reduce costs and increase the sustainability of cultured meat, despite challenges with standardization and compositional variability.

Keywords: fetal bovine serum; hydrolysates; serum-free media; sustainability.

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Funding: The authors acknowledge FINEP (2892/22), FAPESC (2024TR002561), CNPq (303672/2024-2, 406222/2025-8), and CAPES (Code 001) for their financial support and student scholarships.

BIOACTIVE PEPTIDES FROM DIGESTED PORCINE GROWTH FACTORS: AN IN-SILICO ANALYSIS

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ABSTRACT: The increasing demand for alternative protein sources, such as cultured meat, necessitates a thorough functional assessment of its components, particularly growth factors (GFs) like FGF-2, HGF, IGF-1, IGF-2, IL-6, NRG-1, PDGF-B, TGFB-1, TGFB-3, VEGF-A and EGF (fibroblast growth factor 2, hepatocyte growth factor, insulin-like growth factor, insulin-like growth factor 2, interleukin-6, pro-neuregulin-1, platelet-derived growth factor subunit B, transforming growth factor beta-1 proprotein, transforming growth factor beta-3 proprotein, vascular endothelial growth factor A and pro-epidermal growth factor, respectively). This study performed an *in silico* gastrointestinal digestion of eleven key porcine GFs to characterize the bioactive potential (BP) of the resulting peptides. Using the BIOPEP database, the GFs' amino acid sequences were subjected to simulated complete sequentially digestion with pepsin, trypsin, chymosin, and pancreatic elastase. The analysis revealed that digestion releases numerous peptides with potential bioactive properties. The specific percentage of peptides with predicted BP for each GF was as follows: FGF-2 (16.51% of 129 peptides); HGF (11.67% of 437); IGF-1 (15.74% of 108); IGF-2 (14.73% of 129); IL6 (18.57% of 140); NRG-1 (9.89% of 435); PDGFB (8.33% of 12); TGF-B1 (17.65% of 255); TGF-B3 (11.37% of 255); VEGF-A (22.22% of 99); and EGF (8.97% of 747). Analysis also showed that despite presenting several different activities, the most prominent and consistent across the GFs were Dipeptidyl Peptidase IV (DPP-IV) inhibitor and Angiotensin-Converting Enzyme (ACE) inhibitor. Of all the 44 different bioactivities identified, 7 were present for the trypsin, chymosin and trypsin digestion in the following GFs: HGF, NRG-1, TGBF-1 and EGF. The overlap was even more evident between IGF-1 and IGF-2, that shared 8 bioactivities when digested with same enzymes. These overlaps provide critical information on the most commonly presented bioactivities across all GFs analyzed, making it easier to continue further studies. Overall, 400 out of 3270 of the peptides were identified as potentially bioactive, linked to promising functions like antihypertensive and antioxidant activities. The *in silico* digestion of these growth factors can generate peptides with valuable biotechnological and health-promoting properties. These findings are significant for highlighting the critical need for further *in vitro* and *in vivo* validation to confirm these effects.

Keywords: Bioactive peptides; In silico digestion; Growth Factors; Pork.

Funding: The authors acknowledge FINEP (2892/22), FAPESC (2024TR002561), GFI Brazil, and CNPq (303672/2024-2, 406222/2025-8) for their financial support and student scholarships.

PREDICTED TOXICITY AND ALLERGENICITY OF PEPTIDES FROM DIGESTED PORCINE GROWTH FACTORS: AN IN- SILICO STUDY

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ABSTRACT: With the advent of cultured meat as a sustainable protein alternative, a comprehensive safety assessment of its components is necessary, and for the components such as the growth factors, mainly FGF-2, HGF, IGF-1, IGF-2, IL-6, NRG-1, PDGF-B, TGFB-1, TGFB-3, VEGF-A and EGF

(fibroblast growth factor 2, hepatocyte growth factor, insulin-like growth factor, insulin-like growth factor 2, interleukin-6, pro-neuregulin-1, platelet-derived growth factor subunit B, transforming growth factor beta-1 proprotein, transforming growth factor beta-3 proprotein, vascular endothelial growth factor A and pro-epidermal growth factor, respectively). It is imperative to have a better understanding to ensure consumer health and confidence. This research provides an *in silico* evaluation of the toxicogenic and allergenic potential of peptides released from the simulated digestion of the aforementioned porcine growth factors (GFs). The potential toxicity and allergenicity of peptides from the simulated gastrointestinal digestion of GF proteins were evaluated using the ToxinPred 3.0 and AlgPred 2.0 tools, using pepsin, trypsin, chymosin and pancreatic elastase as enzymes for sequential hydrolysis. The analysis indicated that 34.1% of peptide fragments showed potential toxicity, where each distinct sequence was counted only once. Conversely, the vast majority of peptides were predicted to be non-toxic. It's important noting that all of these peptides are already present in the digestion of conventional meat, without specific toxic response reported. For allergenicity, only 12.1% of the peptides were predicted to be potential allergens, while the literature for milk shows that all of the proteins present – caseins (80% of the protein fraction) and serum proteins (20% of the protein fraction) - have potential to cause allergic reactions, highlighting the difference between the food matrix. In conclusion, while *in silico* analysis identified a minority of peptides with predicted allergenic potential, and around one-third of peptides with potential toxicity, the overwhelming majority appear safe for ingestion. These computational findings provide a valuable preliminary safety profile, underscoring the importance of subsequent *in vitro* and *in vivo* experiments to confirm the safety of these peptides for food applications.

Keywords: Food Safety; In silico analysis; Toxicity; Allergenicity.

Funding: The authors acknowledge FINEP (2892/22), FAPESC (2024TR002561), GFI Brazil, and CNPq (303672/2024-2, 406222/2025-8) for their financial support and student scholarships.

ISOLATION AND IMMUNOPROFILING OF PORCINE MYOBLASTS FOR CULTURED MEAT APPLICATIONS

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ABSTRACT: For the development of cultured meat, robust protocols to isolate and expand myogenic precursors are required. However, preserving lineage identity while obtaining workable yields could be challenging. We aimed to isolate and immunophenotypically characterize porcine myoblasts from skeletal muscle using a combined mechanical–enzymatic workflow and to verify if the recovered cells predominantly represent an early myogenic stage suitable for differentiation. Fresh muscle was cooled at 4 °C for 24 h, separated from subcutaneous tissue under aseptic conditions, washed in DPBS with antibiotics, and mechanically minced in 2 mL DPBS. Enzymatic dissociation used type I collagenase (1 mg/mL in DPBS per 2 g tissue) at 37 °C for 1 h with gentle trituration (20 mL syringe, ≥5 times every 15 min), followed by digestion with 0.25% trypsin (no EDTA, 37 °C, 20 min. Viable cells were counted and seeded. Cells were cultured with high-glucose DMEM + 10% FBS, 2 mM glutamine, and 1% antibiotic–antimycotic with daily medium changes for 7 days. Immunofluorescence demonstrated nuclear MYF5 positivity and nuclear MYOG positivity, membrane ITGA7, weak cytoplasmic MYHC, and absent signals for MYOD and DESMIN. These results support an early myoblast profile not yet engaged in active differentiation, consistent with MYOD negativity and modest MYHC, while DESMIN absence likely reflects low expression at this stage and/or limited cross-reactivity in swine. Methodologically, the sequential collagenase–trypsin dissociation steps yielded cells that retained myogenic markers, indicating suitability as starting material for myotube induction. This workflow isolates and characterizes porcine myoblasts with an immunophenotype compatible with pre-differentiation states, providing a practical foundation for controlled myogenic differentiation and scalable applications in cultured- meat research.

Keywords: Cultured Meat; Porcine Myoblasts; Cells.

Funding: FINEP

PRODUCTION OF SCAFFOLDS BY VEGETABLE DECELLULARIZATION FOR STRUCTURING CULTIVATED MEAT

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ABSTRACT: Cultivated meat represents a promising innovation in the food industry, offering a more sustainable alternative to conventional meat production. However, several significant challenges remain, particularly in tissue structuring, since three-dimensional tissue cell growth only occurs when supported by a scaffold. Among emerging approaches, the use of decellularized plants as three-dimensional scaffolds for animal cell growth stands out. Decellularization involves removing cellular components from plant tissues while preserving their cellulose-rich structural matrix, which can serve as a scaffold for cultivated meat. Fruits and other decellularized vegetables have been explored due to their natural vascular structures, biocompatibility, porosity, and abundance, which mimic muscular tissue architecture and facilitate nutrient and oxygen supply to cultured cells. This study aims to develop and test decellularized plant scaffolds derived from apples, eggplants, radishes, potatoes, sweet potatoes, purple potatoes, beets, and chayote as potential alternatives for scaffolds in cultivated meat production. The proposed technique consists of plant decellularization through membrane disruption using sodium dodecyl sulfate (SDS) as the analytical standard, compared with sodium linear alkylbenzene sulfonate (LAS), a widely used commercial surfactant. There were no significant differences in decellularization among the different surfactants, suggesting that LAS may have greater commercial applicability due to its substantially lower cost. Preliminary results using LAS indicated that structures obtained from apples, eggplants, and radishes performed better in decellularization, with mass increases of up to 49%, 71.4%, and 68.7%, respectively, after the process, and average mass losses of over 95% after lyophilization compared to their natural state. These results demonstrate a substantial increase in porosity required for cell growth, as well as sufficient vascularization to enable nutrient flow within the scaffold. Sweet potatoes, however, showed only a 1.1% average mass gain during decellularization and an average 80% mass loss after lyophilization, values considerably lower than those of the other vegetables. Further studies, including imaging, DNA detection, and physicochemical analyses such as magnetic resonance, are required to confirm the feasibility of both the process and the selected plant materials as scaffolds for cultivated meat production.

Keywords: edible scaffolds¹; culture meat ²; decellularized plants³; sustainability⁴

Funding: Santa Catarina State Research and Innovation Support Foundation (FAPESC) for financial support for projects (No. 2024TR001596 and No. 2024TR002658) and for the FAPESC scholarship (No. 00001321/2025 and code 18V6MYL8).



CULTURED MEAT PRODUCTION AND ITS ROLE IN MITIGATING HUMAN EXPOSURE TO MYCOTOXINS

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REVIEW: The rising demand for animal-derived proteins has raised concerns about environmental impact, animal welfare, and food safety, especially regarding mycotoxin contamination. These secondary metabolites, produced by toxigenic fungi, are widespread in plant products and derivatives like animal feed, with some known for their toxicity and bioaccumulation potential in the food chain [1]. Evidence confirms their presence in animal-based foods and even in human fluids and tissues, indicating a public health concern [2,3,4]. Their thermal stability prevents elimination through conventional processing methods [1], highlighting the need for alternative technologies to ensure food safety. This issue becomes more critical amid extreme weather events, such as prolonged droughts in northern Brazil and heavy rains in the south in 2024, which favor fungal growth and mycotoxin synthesis [5]. In this context, cultivated meat (CM) offers a promising alternative to reduce mycotoxin risks in the food chain. This study aims to assess the potential of CM in decreasing the bioavailability of mycotoxins and human exposure to these contaminants. The highly controlled nature of cellular cultivation tends to drastically minimize or eliminate traditional contamination sources. Furthermore, it allows modulation of cell types and lipid profiles, including the incorporation of unsaturated fatty acids, to improve nutritional quality [6]. Dietary fatty acids have been shown to influence intestinal absorption of lipophilic compounds such as flavonoids and other bioactives [7]. Given that certain mycotoxins (e.g., aflatoxin B1, ochratoxin A, and zearalenone) are lipophilic [8], adjusting the fatty acid profile in CM may reduce their absorption by limiting solubilization or incorporation into intestinal transport structures. However, this hypothesis requires direct investigation. Another critical factor is the use of scaffolds in cell cultivation [9]. Many are plant-derived and may remain in the final product, posing a contamination risk if residual impurities are present. Their safety must be carefully evaluated. Despite technical and ethical challenges, CM presents a viable strategy to mitigate toxicological risks from mycotoxins, contributing to the development of safer and more sustainable food systems.

Keywords: toxic metabolites; contaminant control; risk reduction.

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NUTRITIONAL ASPECTS AND CONSUMER ACCEPTABILITY OF CULTIVATED MEAT: A LITERATURE REVIEW

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REVIEW: Beef production is highly resource-intensive and environmentally harmful, while cultivated meat has emerged as a more sustainable and ethical alternative. Nonetheless, challenges persist, particularly in consumer acceptance and nutritional optimization. This study aimed to present a review of the current literature on the nutritional aspects and acceptability of cultivated meat. Scientific articles published between 2020 and 2025 were collected from academic databases. The results show that one of the challenges involves achieving flavor and texture comparable to traditional meat, as sensory quality strongly influences consumer acceptance.¹ While consumer curiosity and willingness to try cultivated meat are increasing, willingness to pay premium prices remains limited.² Reducing production costs, especially biomaterial scaffolds, will be essential for commercial scalability. Another aspect is nomenclature and labeling, since cultivated meat does not clearly fit into existing categories, directly affecting consumer perception.³ Acceptance is also shaped by psychological and cultural barriers, such as perceptions of unnaturalness and technophobia.² In contrast, younger, health-conscious, environmentally aware, higher-income, and more educated groups are more open to adoption. In the nutritional composition aspects, studies indicate that cultivated meat may present lower protein and amino acid levels compared to conventional meat. Such as, cultivated pork reported 11.24% amino acids versus 21.89% in traditional pork, chicken 4.8% versus 22%, and beef 6.3% protein compared to 11.3%.^{1,3} Lipid content is equally important, since fat influences flavor, juiciness, and texture.³ Strategies such as co-culturing muscle and fat cells have been developed to improve these attributes. Cultivated fat can reproduce conventional fatty acid profiles, including oleic, linoleic, and docosahexaenoic acids.⁴ Moreover, opportunities exist to optimize nutrition, such as tailoring fatty acid composition or fortifying with iron and omega-3.^{1,3} In conclusion, cultivated meat shows a sustainable protein source, but its adoption depends on advances in nutrition, cost reduction, regulation, and consumer acceptance.

Keywords: cultured meat; food technology; food safety.

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STRATEGIES TO IMPROVE CELL ADHESION ON ALGINATE SCAFFOLDS FOR CULTURED MEAT APPLICATIONS: A REVIEW

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ABSTRACT: Efficient cell adhesion to alginate scaffolds is a central challenge in tissue engineering, particularly for cultivated meat production, which aims to avoid animal-derived components. Alginate's inherent bio-inert nature necessitates modifications to enhance cell-matrix interactions. This review aims to summarize the main strategies developed to improve cell adhesion on alginate scaffolds using exclusively animal-free inputs. A comprehensive literature review shows that the primary strategies involve chemical, physical, and biological modifications. Chemical functionalization of the alginate backbone with cell adhesion peptides, such as the RGD sequence, is a well-established and widely validated approach to create specific binding sites for cell integrins, thereby promoting adhesion, proliferation, and differentiation. Another effective strategy is the development of hybrid scaffolds by incorporating plant-based polymers, such as starch, cellulose, and vegetable proteins (e.g., soy and pea isolates). These hybrid biomaterials not only offer improved mechanical support but also increase the number of cellular anchor points, with 3D bioprinting emerging as a key technology for processing them into porous and stable structures. Furthermore, adjusting the scaffold's microstructure by creating thin films, increasing porosity, and enhancing surface roughness favors cell adhesion by increasing the contact area. The incorporation of plant-derived bioactive compounds, such as polyphenols and flavonoids, has also shown potential in improving the bioactivity of the scaffolds and promoting cell proliferation. In conclusion, the literature indicates that combining strategies is the most promising path to developing effective alginate scaffolds for animal-free applications. Despite considerable progress, challenges related to the standardization of methods, process scalability, and the need for long-term functional assessments in complex systems remain.

Keywords: alginate; cell adhesion; scaffolds; cultivated meat; plant-based.

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Funding: The authors would like to thank FINEP for project number 2892/22, FAPESC (Grant number 48/2021 and 2024TR002561), and CAPES (Grant number 001) for providing scholarships to students.

USE OF ALGINATE-BASED EMULSIONS AND HYDROGELS FOR THE PRODUCTION OF EDIBLE SCAFFOLDS FOR CULTURED MEAT

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ABSTRACT: Creating edible scaffolds that mimic the native extracellular matrix is an essential step for producing cultured meat. Alginate, a polysaccharide extracted from brown algae, stands out as a biomaterial for this purpose due to its biocompatibility, versatility, and non-animal origin. Manufacturing techniques like 3D printing, cryogelation, and emulsification are used to form porous microenvironments that favor cell differentiation and the development of aligned muscle fibers, a critical aspect for replicating the texture of conventional meat. This review presents current research on the use of alginate-based emulsions and hydrogels in creating edible scaffolds, focusing on strategies that avoid animal-derived inputs. The literature indicates that scaffolds formulated with alginate possess high biocompatibility along with mechanical and structural properties that can be adjusted, which is crucial for supporting the adhesion, proliferation, and differentiation of muscle and fat cells. Combining alginate with other plant-based biopolymers, such as soy and pea proteins or starch, enhances the hydrogel's properties for cell adhesion and the formation of three-dimensional tissues. The use of alginate-based hydrogels and emulsions is particularly effective because these techniques allow for precise control over the scaffold's porous microenvironment, which is essential for nutrient delivery and cell integration. As a result, studies show that these scaffolds, formulated without animal-derived components, exhibit a biological performance comparable to, and in some cases superior to their animal-derived counterparts, effectively supporting the growth of various cell types, including bovine myoblasts. In conclusion, the use of alginate-based emulsions and hydrogels presents an effective and sustainable strategy for producing edible scaffolds for the cultured meat industry. This approach successfully eliminates the need for animal-derived components, addressing key ethical and safety issues. While considerable progress has been made, overcoming the final hurdles—precisely replicating the sensory characteristics of meat, achieving industrial-scale production, and securing consumer acceptance—will be pivotal. Advancements in emulsion and hydrogel technologies are particularly crucial, as they offer unique control over scaffold porosity and nutrient distribution, which are essential for developing the complex, structured texture of conventional meat. **Keywords:** alginate; scaffolds; cultured meat; plant-based; emulsions.

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Funding: The authors would like to thank FINEP for project number 2892/22, FAPESC (Grant number 48/2021 and 2024TR002561), and CAPES (Grant number 001) for providing scholarships to students.



CROSSLINKING, DECELLULARIZATION AND EMULSIONS AMONG TECHNOLOGIES FOR OBTAINING MICROCARRIES FOR CULTURED MEAT PRODUCTION

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ABSTRACT: Conventional meat production is associated with environmental issues, including depletion of land and water, greenhouse gas emissions, inefficient nutrient conversion, and livestock-related diseases. Cultured meat has emerged as a sustainable alternative, enabling *in vitro* meat production without animal sacrifice. The process involves isolating a small number of cells from animals and expanding them in bioreactors to generate biomass. For large-scale expansion, microcarriers can be employed. These particles support adherent cell growth in suspension, typically ranging from 100–200 µm in diameter. Several fabrication techniques are reported, including crosslinking, emulsion, decellularization, and electrospray. This review aimed to summarize the main microcarrier fabrication methods, the raw materials used, and the cell types tested. Searches were performed using strategies in four scientific databases (Scopus, Web of Science, Compendex, FSTA) and two patent databases (Espacenet, Google Patents). Only experimental studies in English related to cultured meat were included, while reviews, book chapters, and chemical or health studies were excluded. Since the field is new, publication date was not restricted. After refinement, 31 scientific articles and 43 patents remained. Studies were classified according to focus: 66% addressed microcarrier fabrication, 24% cell culture conditions and 10% bioreactors or cultivation systems development. Regarding raw materials, 10% of the microcarriers were polymeric, 35% plant-based, 32% animal-based, and 23% unspecified. Plant-derived sources appeared slightly more frequent, yet animal-based matrices remain widely applied, highlighting opportunities to explore non-animal options. The most common fabrication approaches were crosslinking (27%), decellularization (5%), emulsion (7%), electrospray (7%), and combined strategies such as decellularization + crosslinking (8%). Other methods included extrusion, film formation, fermentation, and self-assembly, reflecting the diversity of available possibilities. Cell types tested included myoblasts (27%), skeletal muscle cells (7%) and stem cells (7%) from different origins. The most frequent animal sources were mouse, bovine, avian and porcine, in that order. In conclusion, a wide range of raw materials, fabrication methods, and cell types has already been investigated. Patents emphasized novel approaches, while articles applied established techniques with varied materials. Continuous refinement is essential to ensure compatibility with target cell types and to advance cellular agriculture.

Keywords: cellular meat; adherent cell expansion; bioreactor, scaffold fabrication; animal-free matrices

Funding: The authors would like to thank FINEP for project number 2892/22, Santa Catarina State Research and Innovation Support Foundation (FAPESC) for financial support for projects (2024TR001596, 2024TR002658, 2024TR002561), CNPq 303672/2024-, and for the FAPESC scholarship (No. 00001321/2025 and code 18V6MYL8).

PLANT-BASED HYDROGEL SCAFFOLDS IN CULTIVATED MEAT: A TECHNOLOGICAL LANDSCAPE STUDY

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REVIEW: Conventional meat production is associated with environmental impacts, public health challenges, and animal welfare concerns. Cultivated meat, obtained through cell culture, has emerged as a promising alternative and attracted attention from academia and industry. In this context, patent-based technological prospecting plays a strategic role by identifying innovation trends, research gaps, and protecting intellectual property [1]. The production of cultivated meat generally requires scaffolds; three-dimensional structures designed to mimic the extracellular matrix and support for cell growth [2]. Among these, hydrogels are particularly noteworthy because of their biocompatibility and high water-retention capacity. This study aimed to conduct a technological prospecting of hydrogel scaffolds applied to cultivated meat, mapping the plant-based materials used and identifying technological trends in scaffold development. Patent searches were carried out between September 22 and 23, 2025, using the Espacenet® database. The search strategy employed the following keywords: “cultivated meat,” “scaffolds,” “plant-based,” and “hydrogel”. Of the 118 patents identified, 12 were selected after screening titles and abstracts, specifically those describing plant-based scaffolds for cultivated meat applications [3–15]. At the time of analysis, all patents had been filed but not yet granted. Most filings originated from companies ($n = 10$). Israel and the United States accounting for the largest share ($n = 3$ each). Although the first cultivated meat patent was filed in 1999, filings have increased since 2020, peaking in 2024 ($n = 5$). The selected patents highlight variations in scaffold composition and fabrication methods for cultivated meat, including the use of microbial cellulose as a structural matrix and combinations of biopolymers that allow the adjustment of mechanical properties [5, 10]. Sensory-oriented scaffolds have also been developed, such as a scaffold incorporating a compound with a functional group containing a disulfide bond, designed to enhance the flavor of cultivated meat and produce a taste similar to that of conventional meat. [14]. These strategies, ranging from structural optimization to flavor enhancement, reflect the technological diversity of the sector. The growing use of plant-based hydrogels highlights the role of patent prospecting in driving research, investment, and sustainable innovations in meat production.

Keywords: Biomaterials; Cultivated meat; Patent analysis.

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Funding: The authors acknowledge the Federal University of Santa Catarina (UFSC) for providing the research infrastructure. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, the Funding Authority for Studies and Projects (FINEP) for the financial support to project 2892/22, and the Research and Innovation Support Foundation of the State of Santa Catarina (FAPESC) for the financial support to projects No. 2024TR001596 and No. 2024TR002658.

UMBILICAL CORD TISSUE FROM PURUNÃ CATTLE AS A CELL SOURCE FOR CULTIVATED MEAT

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ABSTRACT: Cells for cultivated meat mostly come from slaughtered animals or a biopsy. The umbilical cord (UC) may be a relevant and non-invasive source. We aimed to establish a protocol for collecting and isolating mesenchymal stem cells from bovine UCs obtained during natural delivery in high welfare and using least disturbance procedures, for cultivated meat and fat development. Between August 2024 and July 2025, a total of eleven samples were collected from newborn Purunã calves in an extensive pasture system, in collaboration with the Rural Development Institute of Paraná, Fazenda Modelo. Cords were washed with 70% ethanol and PBS containing 100 U/mL penicillin, 100 µg/mL streptomycin, 2,5 µg/mL amphotericin B, and refrigerated. In the lab, each cord was opened along its longitudinal axis, the interior blood was removed, and the tissue cut into ~0.5 cm² fragments and plated lumen-side down to isolate cells through explants. Cultures were maintained in DMEM-High with 15% FBS and 1% antibiotic/antimycotic, with medium changes every two days. Cord fragments were removed after 15 d. The first three umbilical cords were collected within minutes to a few hours after birth, but were contaminated with soil and mud, which led to fungal growth during culture. Cord 4, obtained from a calf that died shortly after birth, also did not yield viable cells. Cords 5, 6, 7, 9, and 10 successfully resulted in cell isolation after storage periods ranging from 24 to 96 hours. Additionally, the inner tissue of UC 6 was processed by enzymatic digestion with 0.25% trypsin for two hours, which also enabled cell isolation, though in smaller amounts. Cords 8 and 11 were also contaminated with fungi. All isolated cells displayed morphology consistent with mesenchymal stem cells. The results suggest the feasibility of minimally invasive collection after natural birth and storage of samples for up to 96 hours before processing. The explant method appears more advantageous than enzymatic digestion. The isolated cells will be further tested for their ability to differentiate into adipocytes and muscle cells, supporting their potential use in the development of cultivated meat products.

Keywords: mesenchymal stem cells; primary cells; Wharton's jelly.

Funding: NAPI Alternative Proteins - Araucária Foundation - State Government of Paraná

STORAGE AND PROCESSING CONDITIONS FOR MUSCLE CELL ISOLATION FROM PURUNÃ CATTLE FOR CULTIVATED MEAT RESEARCH

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ABSTRACT: Satellite cells derived from animal muscle are widely used for cultivated meat research and development. Storage time and conditions, as well as sample processing, are crucial for the successful isolation of cells. We aimed to isolate cells from the ribeye (*Longissimus dorsi*) muscle of adult Purunã cattle stored and processed under different conditions. The work was conducted in collaboration with Fazenda Modelo of the Rural Development Institute of Paraná. Twenty muscle samples were collected from ten animals at a commercial slaughterhouse: ten within minutes to 2 h post-slaughter, and ten from the same animals 48 h later. Upon collection, samples were washed with 70% ethanol and PBS containing 1% antibiotics and antimycotics and refrigerated. In the lab, ~5 g of muscle tissue were finely minced with a scalpel and digested with 0.25% trypsin for 2 h. The digested material was centrifuged and filtered through 100-µm and 70-µm cell strainers. Ten samples were subjected to the pre-plating technique after digestion, and other ten samples were additionally processed using the explant technique. All cultures were maintained in DMEM-High supplemented with 15% FBS and 1% antibiotic/antimycotic. After two weeks, all samples collected on the day of slaughter and processed by enzymatic digestion yielded cells with an elongated morphology. Among the samples collected two days post-slaughter, seven produced cells with similar characteristics, although in lower numbers. Explant cultures were not successful. In pre-plating, cells were detected in both the original plate and the plate containing the transferred supernatant. Passages were standardized at a density of 4,000–6,000 cells/cm². Oldest cultures are currently active, at passage

13. Some cells were seeded in 24-well plates and induced to differentiate in medium with 2% horse serum, exhibiting morphological signs of myotube formation. The results demonstrated that viable cells can be isolated from cattle ribeye up to 48 h post-slaughter, although yield decreased with time. Overall, a simple enzymatic digestion protocol combined with adequate storage offers a feasible strategy for sourcing bovine muscle cells with differentiation potential for cultivated meat development. Further cell characterization and refinement of the differentiation protocol are currently under investigation.

Keywords: *Longissimus dorsi*; sample storage; satellite cells.

Funding: NAPI Alternative Proteins - Araucária Foundation - State Government of Paraná

***Spirulina* CULTIVATION IN BRACKISH AND MARINE WATERS: STRATEGIES FOR PROTEIN PRODUCTION**

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REVIEW: Global food security is under increasing pressure from population growth, projected to reach 9.2 billion by 2050¹, and from the impacts of climate change, which compromise the availability of food, arable land, and water resources^{1, 2}. In this context, the search for alternative protein sources with lower environmental impact and a reduced carbon footprint has intensified³. Microalgae stand out due to their nutritional value, rapid growth, ability to utilize non-arable land, and alternative sources of water and nutrients. Among them, the microalga *Spirulina* has a protein content ranging from 50 to 70% of dry biomass, surpassing that of conventional sources such as soybeans⁴. To reduce production costs and ensure sustainable biomass generation, cultivation in brackish or marine waters has emerged as a promising alternative, as it dispenses with the need for freshwater ¹. However, saline stress can alter metabolic pathways, requiring cultivation strategies that preserve protein synthesis⁵. This study aimed to review cultivation strategies for *Spirulina* using unconventional water sources (brackish groundwater and seawater), with an emphasis on protein production. Cultivation of *Spirulina platensis* in seawater, either diluted or undiluted, supplemented with nitrogen, phosphorus, and iron resulted in biomass with protein contents ranging from 59.9 to 67%⁶. Similarly, the protein content of *Spirulina* sp. LEB 18 cultivated in seawater supplemented with nitrogen, phosphorus, iron, and EDTA was reduced by only 7.8% compared with the control⁷. With respect to brackish groundwater (SSG), cultivation of *Spirulina* sp. LEB 18 in SSG supplemented with 25% of the nutrient concentration of Zarrouk medium presented ~42% protein content⁸. Furthermore, *Spirulina* sp. LEB 18 cultivated in SSG supplemented with 25% of carbon, nitrogen, phosphorus, iron, and EDTA in Zarrouk medium presented biomass with 27.8% protein, compared with 50.1% in the control. Despite the 44.5% reduction in protein content, the maximum biomass productivity (0.17 g L⁻¹ d⁻¹) remained the same as that of the control culture⁵. Thus, *Spirulina* demonstrates robustness to saline environments and highlights the potential of using alternative water sources to reduce production costs and freshwater demand, thereby strengthening its application as a sustainable protein source.

Keywords: Alternative protein; *Spirulina*; Sustainability.

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Funding: The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) (Finance Code 001).

STRUCTURAL FUNCTIONS OF THE SYMBIOTIC KOMBUCHA CULTURE "SCOBY" IN CELL CULTURE AND CULTIVATED MEAT - A REVIEW

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REVIEW: Cultured meat emerges as a promising alternative to meet the growing global demand for sustainable protein sources, but its production still faces substantial challenges related to cell expansion and biomass generation on an industrial scale. These challenges arise primarily from the limited cell proliferative capacity and sensory and nutritional reproduction of conventional meat (Martins et al., 2024). In this context, Symbiotic Culture of Bacteria and Yeast (SCOBY)—a biofilm formed during kombucha fermentation—has gained scientific interest due to its properties and applicability as a biomaterial. Composed of a cellulose matrix, SCOBY presents non-toxic, edible, and biocompatible characteristics and has been used in cell culture as a scaffold in its natural form or after reengineering processes, such as 3D printing, providing physical support for cell adhesion, growth, and differentiation into myogenic, adipogenic, and osteogenic lineages (Dugdale et al., 2018; Kim et al., 2017; Lorthongpanich et al., 2024; Sodré et al., 2025). We aimed to analyze the potential uses of SCOBY in the production of cultured meat. A systematic review was conducted, and the sample comprised 19 articles retrieved from the Scopus and Web of Science databases. The results indicated the biological potential of SCOBY. Among these, antibacterial activity was observed against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Candida albicans*, and *Saccharomyces* spp., suggesting potential applications in environments at risk of contamination (Sharma et al., 2020; El-Fallal et al., 2023; Sknepnek et al., 2024). Furthermore, attributes such as biocompatibility, moisture retention, and adaptability were highlighted (Yakaew et al., 2022; Yan et al., 2023), along with cytocompatibility, cell viability, and adhesion (Pillai et al., 2021; Candra et al., 2023). Collectively, these findings establish SCOBY as a promising biomaterial for tissue engineering applications, with particular emphasis on the valorization of agro-industrial byproducts that combine functionality, biodegradability, and ecological appeal. Considering that the SCOBY is produced in parallel with the beverage, this process reduces costs and, at the same time, generates revenue from kombucha and biomaterial for cultured meat.

Keywords: Kombucha Tea, Scoby, Tissue Scaffolds, In Vitro Meat.

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Funding: This work was supported by Coordination for the Improvement of Higher Education Personnel – Brasil (CAPES) - (CAPES: 88887.919621/2023-00) for scholarship.

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CORN STEEP LIQUOR AS A SUBSTRATE FOR CHLORELLA VULGARIS CULTIVATION AIMING AT PROTEIN-RICH BIOMASS PRODUCTION FOR CULTIVATED MEAT MEDIA

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ABSTRACT: Corn steep liquor (CSL) is an agro-industrial by-product rich in nitrogen ($\approx 3.4\%$ N, equivalent to 21% protein), representing a low-cost and promising source for microalgal cultivation. This study evaluated the use of CSL for the growth of *Chlorella vulgaris*, focusing on protein-rich biomass production. In preliminary tests, CSL at 10 g L^{-1} stood out, yielding biomass of $0.93 \pm 0.04 \text{ g L}^{-1}$ and protein content of $60.2 \pm 0.2\%$. At 0.5 g L^{-1} of CSL, the biomass concentration was 0.35 g L^{-1} with 55.5% protein, while at 20 g L^{-1} of the CSL medium, the biomass remained similar (0.92 g L^{-1}) but protein dropped to 37.1%. CSL combined with BG11 medium did not improve performance, with protein between 36.5 and 50.7%. The BG11 medium alone gave the lowest results, with biomass of $0.28 \pm 0.01 \text{ g L}^{-1}$ and protein of $48.2 \pm 1.0\%$. These findings highlight the viability of CSL as an independent medium for *C. vulgaris*. A central composite rotational design (CCRD) was then applied, considering pH and CSL concentration as variables. The model was significant ($R^2 = 92.15\%$) and indicated an optimal region around pH 7.0–7.5 and $4.0\text{--}5.0 \text{ g L}^{-1}$ of CSL, where protein reached $\approx 61\%$, confirming response robustness. Validation in 1-L Erlenmeyer flasks ($n = 8$) yielded biomass of $0.93 \pm 0.17 \text{ g L}^{-1}$ on day 10, with protein content of $63.1 \pm 0.6\%$. Growth curve analysis showed an exponential phase until day 3, with a specific growth rate (μ) of 0.85 day^{-1} and maximum biomass productivity (P_{max}) of $0.36 \text{ g L}^{-1} \text{ day}^{-1}$. These results demonstrate that CSL supports efficient growth of *C. vulgaris*, producing biomass with high protein content and confirming its potential as a sustainable input for bioprocesses. The process is currently at the scale-up stage (6-L Erlenmeyer flasks), aiming to obtain sufficient biomass for subsequent enzymatic hydrolysis with Alcalase, physicochemical characterization, and peptide analysis, toward developing protein inputs applicable in culture media for cultivated meat as an alternative to fetal bovine serum.

Keywords: *Chlorella vulgaris*; corn steep liquor; protein hydrolysates; cultivated meat; fetal bovine serum substitution

Funding: CAPES, CNPQ, FAEPEX and FAPESP.

COMPOUNDS UEMUFPR001 AND UEMUFPR002 AS POTENTIAL SUPPLEMENTS FOR CELL CULTURE IN CULTIVATED MEAT

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ABSTRACT: The optimization of culture media devoid of animal-derived inputs is crucial for improving cell proliferation and viability in ethical cultivated meat production. Our objective was to evaluate the potential of the compounds UEMUFPR001 and UEMUFPR002 as supplements for FBS-free culture media. The pH of all samples was adjusted with NaOH to physiological range (7.2–7.4). Cytotoxicity and viability assays were performed using an immortalized cell line of murine myoblasts (C2C12). Cells were cultured in DMEM-High supplemented with 10% FBS and 0.5% antibiotic/antimycotic for 24h. For the cytotoxicity assay, the culture medium was replaced by DMEM-High containing 10% FBS, 10% resazurin 10x, and dilutions of both compounds in logarithmic scale (100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%). Fluorescence was measured at Ex: 560 nm and Em: 590 nm, and cytotoxicity was calculated relative to untreated controls. For the viability assay, after the initial 24 h, cultures were treated with UEMUFPR001 (5%, 2.5%, 1%, and 0%) and UEMUFPR002 (5%, 2.5%, 1%, and 0%) in media containing decreasing serum concentrations (10%, 5%, 2.5%, and 0% FBS). In serum-free conditions, UEMUFPR001 did not sustain cell viability 24h later; in contrast, UEMUFPR002 increased viability to 89.2% at 5% and 76.3% at 2.5%. In addition, a dose-dependent decline was observed, with viability dropping to 44.4% at 1% UEMUFPR002, which was close to the untreated control (42.1%). This suggests that higher UEMUFPR002 contents provide additive bioactive molecules in concentrations that can increasingly support cell metabolism in the absence of FBS. In medium with 10% FBS, the effects of UEMUFPR002 supplementation were less evident. Our preliminary results indicate that compound UEMUFPR002 is a potential supplement in serum-free media, contributing to the reduction of animal-derived input use and suggesting an important role in cellular agriculture. Further studies are needed to assess whether cells remain in adequate conditions for long-term proliferation and functionality, and to identify the specific relevant UEMUFPR002 molecules.

Keywords: Animal-free inputs; Bioactive compounds; C2C12 myoblasts; Viability.

Funding: NAPI Alternative Proteins - Araucária Foundation - State Government of Paraná; AFLORABEM - FINEP.

PLANT-BASED HYDROGEL SCAFFOLDS FOR CULTIVATED MEAT: A REVIEW

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REVIEW: Cultivated meat, produced via cell culture without animal slaughter, represents a sustainable alternative to conventional production. Despite approvals in Singapore, Israel, and the United States, large-scale expansion remains limited by scalability and cost [1; 2]. A key limitation lies in the fabrication of scaffolds, three-dimensional structures that mimic the extracellular matrix, guide cell adhesion, and support cell growth [3]. Scaffolds are generally classified into three categories: porous, fibrous, and hydrogel-based. Hydrogels stand out due to biocompatibility, tunable mechanical properties, and high-water retention [4; 5]. This review aims to explore the biomaterials employed in the production of hydrogels for cultivated meat. Traditionally, scaffolds have been composed of animal-derived materials, including collagen and gelatin. The novelty of this review lies in its focus on studies that replace animal-derived materials with plant-based alternatives, supporting sustainable approaches aligned with the principles of cultivated meat. A literature search was conducted from September 22 to 23, 2025, across PubMed, ScienceDirect, Scopus, SpringerLink, and Web of Science. The search strategy used keywords in the title, abstract, and keyword fields: “cultivated meat,” “cultured meat,” “scaffolds,” “plant-based,” and “hydrogels,” combined with Boolean operators AND/OR. The search retrieved articles in PubMed ($n = 624$), ScienceDirect ($n = 409$), Scopus ($n = 6$), SpringerLink ($n = 383$), and Web of Science ($n = 783$). Studies in the biomedical field were excluded, considering only articles published in the last 5 years that employed plant-based hydrogels as scaffolds for cultivated meat. The selected studies converged on the use of plant proteins, particularly soy protein, as the primary raw material for scaffold fabrication [6-11]. Despite employing diverse strategies, such as crusting fabrication [6], dual crosslinking with edible agents [8], or combination with other plant proteins and polysaccharides [7; 11], the studies share common objectives: developing three-dimensional, biocompatible, porous, and mechanically stable matrices capable of supporting cell adhesion, proliferation, and differentiation. The importance of scaffold biodegradability and edibility is also emphasized, as these properties are essential for food applications. These advances highlight the potential of plant proteins as sustainable and functional alternatives in cultivated meat development.

Keywords: Biomaterials; Natural polymers; Plant proteins; Synthetic polymers; Tissue engineering.

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Funding: The authors acknowledge the Federal University of Santa Catarina (UFSC) for providing the research infrastructure. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, the Funding Authority for Studies and Projects (FINEP) for the financial support to project 2892/22, and the Research and Innovation Support Foundation of the State of Santa Catarina (FAPESC) for the financial support to projects No. 2024TR001596 and No. 2024TR002658.

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STRATEGIES FOR BIOFABRICATION OF HYDROGELS FOR USE IN CULTURED MEAT CELL CULTIVATION

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ABSTRACT: Hydrogels have been widely studied as functional matrices for scaffolds applied to cultured meat cell cultivation, due to their ability to mimic the extracellular matrix (ECM) and provide adequate support for cell adhesion, proliferation, and differentiation. Among various desired properties, such biomaterials must exhibit biocompatibility and mechanical stability in order to sustain cell development throughout cultivation. In this study, formulations based on chia mucilage, cow-pea protein, and gellan gum (GG) were developed, exploring properties such as gelation, water retention, and structural reinforcement. With the introduction of gellan gum, the aim was to form a thermally more stable and irreversible gel, obtained through crosslinking with CaCl_2 at a concentration of 0.180 M. The addition of the hydrocolloid at a concentration of 0.2% resulted in a significant increase in mechanical properties, raising the maximum compressive stress from 10019 ± 368.19 (Pa) to 14271 ± 1192.37 (Pa) and the Young's modulus from 13.26 ± 10.01 (Pa) to 47.16 ± 11.36 (Pa), evidencing greater resistance and rigidity of the material. With this modification, greater structural stability of the hydrogel containing GG was observed over 7 days at 37 °C in PBS++ solution. The formulation without GG showed progressive loss of the interlinked anisotropic network and structural collapse by the seventh day, characterized by disintegration of the porous matrix, rupture of polymeric connections, and dispersion of fragments in the solution. In contrast, the GG-containing sample maintained its structural integrity, preserving the continuity of the porous network, anisotropy, and pore interconnectivity, without occurrence of fractures or particle release. Maintaining these characteristics is fundamental during cell cultivation to ensure homogeneous nutrient distribution and efficient gas diffusion within the scaffold. Thus, the objective of this work was to ensure the maintenance of the hydrogel's structural features and mechanical stability under cell cultivation conditions, aiming to mimic the extracellular matrix and provide a scaffold functionally suitable for cell adhesion, proliferation, and differentiation.

Keywords: Hydrogel; Polysaccharides; Gellan gum; Mechanical Properties.

Acknowledgments: The authors acknowledge the support of CNPq and FAPESP (National Council for Scientific and Technological Development, Brazil and São Paulo Research Foundation) for the financial support of this study.

RESEARCH AND DEVELOPMENT OF STRUCTURED CULTIVATED MEAT WITH PLANT-BASED SCAFFOLDS

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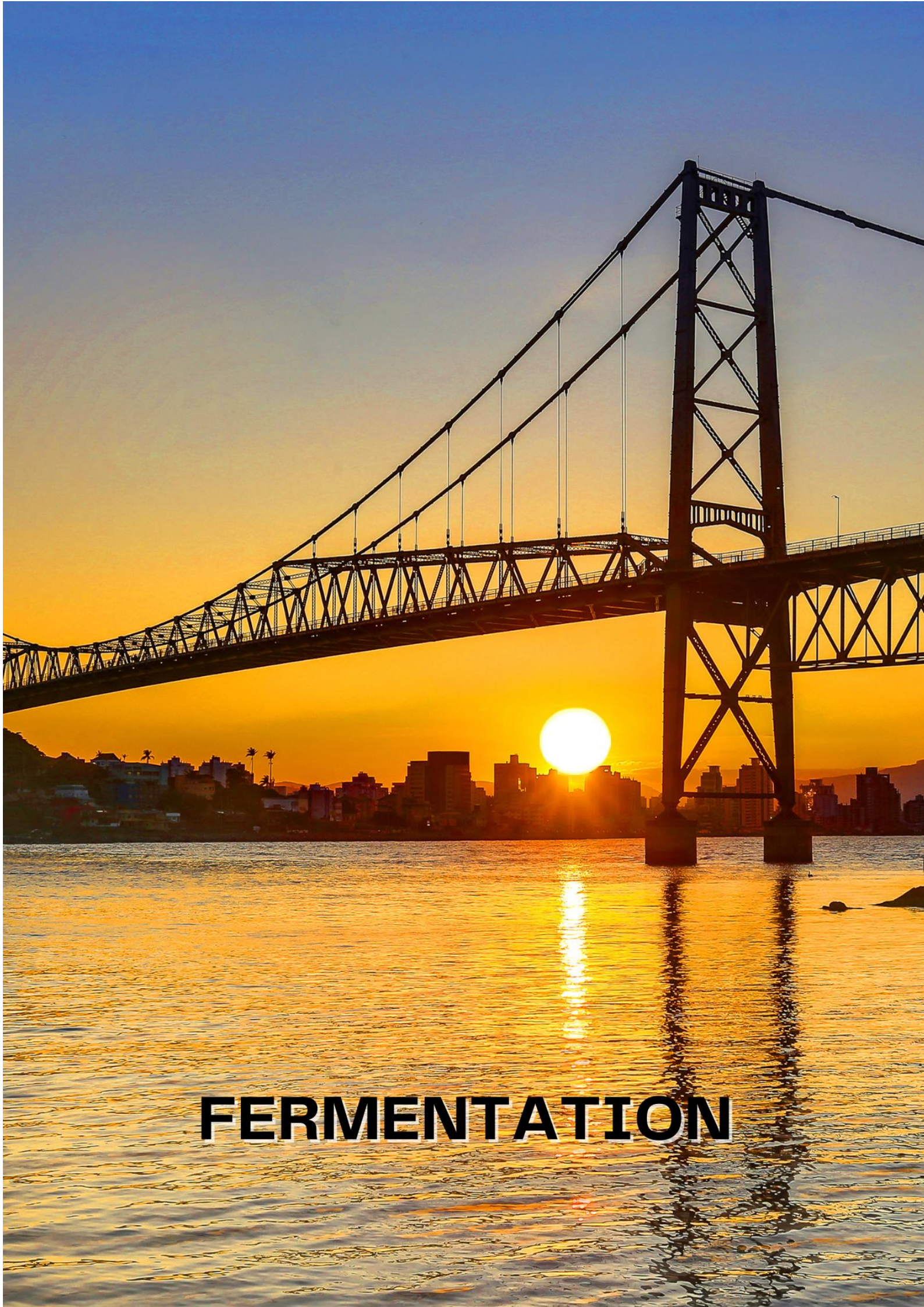
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ABSTRACT: The increasing global demand for meat and the environmental, ethical, and social impacts associated with intensive livestock farming drive the search for sustainable alternatives, such as cultivated meat. In this context, plant-based scaffolds emerge as a promising strategy, combining low cost, biodegradability, sustainability, and cell compatibility, while also reducing dependence on animal-derived inputs. This study aimed to develop scaffolds from decellularized Fuji apple and evaluate their structural, physicochemical, and biological properties for application in cultivated meat production. Plant tissues were subjected to decellularization protocols using Sodium Dodecyl Sulfate (SDS) at 1% and 2%, with or without polymeric coating, and characterized through DNA quantification, scanning electron microscopy (SEM), sterilization assays, and cell viability analysis by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) using bovine cells. DNA quantification indicated residual values below 50 ng/μL (46.85 ng/μL for 1% SDS and 46.45 ng/μL for 2% SDS), although with signs of protein, phenolic, and salt contamination. Micrographs revealed three-dimensional porous structures, with scaffolds treated with 2% SDS being more open and interconnected, while those treated with 1% SDS exhibited smaller and flatter pores. The cell viability assay suggested that the 1% SDS concentration was more favorable for maintaining initial metabolic activity, whereas 2% showed evidence of cytotoxicity. The sterilization protocol demonstrated high efficiency, ensuring aseptic handling of the material. Overall, the results indicate that decellularized apple scaffolds represent a viable and sustainable alternative for cultivated meat production, particularly those treated with milder SDS concentrations, although complementary methodologies are still required to validate biocompatibility.

Keywords: plant-based *scaffold*; cultivated meat; decellularization; sustainability.

Funding: The authors would like to thank FINEP for project number 2892/22, FAPESC (Grant number 48/2021 and 2024TR002561), and CAPES (Grant number 001) for providing scholarships to students.



FERMENTATION

ASPERGILLUS ORYZAE AS A CELL FACTORY FOR THE PRODUCTION OF BOVINE ALBUMIN AND GROWTH FACTORS BY PRECISION FERMENTATION

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ABSTRACT: Filamentous fungi of the genus *Aspergillus* spp. are widely employed for recombinant protein production and well-established as cellular factories. Among these, *Aspergillus oryzae* is particularly used in the food industry due to its safety and classification as Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration (FDA). The versatility of *A. oryzae* in producing recombinant proteins makes it a promising solution to meet the growing demand for alternative proteins in the food industry, while addressing environmental challenges associated with conventional meat production. Cultured meat cell requires a medium containing two groups of components: (i) carbon sources and (ii) specific components, including growth factors and albumin from fetal bovine serum. This study aimed to engineer an *A. oryzae* strain for the production of recombinant *Bos taurus* serum albumin (rAlbumin) and three growth factors Epidermal growth factor (EGF), Fibroblast growth factor 2 (FGF-2) and Insulin-like growth factor 1 (IGF-1), using CRISPR- Cas9-based genetic engineering. To achieve this, an expression platform was developed using the *A. oryzae* strain ORY7, which contains a specific integration site (COSI). Codon-optimized sequences for albumin (*bsa*) gene (AF542068.1), EGF (Q866M9), FGF-2 (P03969) and IGF-1 (F2X2F2) were inserted into this site under the control of the *glaA* inducible promoter and the *trpC* terminator. Starting with the *bsa*, a CRISPR-Cas9::sgRNA vector was constructed for transformation. Transformants were selected on MMG medium supplemented with X-Gluc and three mutants were confirmed by diagnostic PCR. For expression analysis, mutants were cultivated for 72 hours in maltose-inducing medium, and secreted proteins analyzed by 12% SDS-PAGE. No detectable secretion of albumin was observed compared to the parental ORY7 strain. Mass spectrometry (Synapt G2-Si) identified only glucoamylase (65 kDa). However, RT-qPCR confirmed gene transcription, suggesting that the absence of secretion may result from translational errors, degradation, or misfolding. In conclusion, this work demonstrates the successful CRISPR/Cas9 mediated gene integration in *A. oryzae*; though further optimization is needed for effective recombinant protein secretion.

Keywords: alternative proteins; filamentous fungi; CRISPR/Cas9.

Funding: CAPES and FAPESP.

YEASTS ISOLATED FROM BRAZILIAN ECOSYSTEMS AS SOURCES OF PROTEINS FOR HUMAN DIETS

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ABSTRACT: Single-Cell Proteins (SCPs) have re-emerged in the global scenario amid the growing demand for sustainable and vegan protein sources. They are composed of essential amino acids and can be produced from agro-industrial residues, even in environments hostile to plant-based sources. This study aimed to evaluate the potential of yeasts isolated from Brazilian ecosystems for SCP production as a protein supplement for human consumption. A total of 290 yeast strains were obtained from the culture collections of LAPROBQI-UFV, FERMICRO-UFV, and the Culture and Cell Collection of UFMG. Safety analyses included tests for the production of biogenic amines, hydrogen sulfide (H₂S), gelatinase, and hemolytic activity. Carbohydrate assimilation was assessed on agar supplemented with soybean molasses or acid whey, enriched with mineral salts or YP medium. Nitrogen source assimilation was tested in YCB medium supplemented with 1% w/v of diammonium phosphate, peptone, yeast extract, or ammonium sulfate. Growth was evaluated in media containing 20% and 50% w/v glucose, 10% w/v NaCl, or 8% v/v ethanol, as well as at temperatures of 20, 30, 37, and 40 °C. Protein quantification was performed using the Bradford method, and the five highest protein levels were expressed per wet biomass. Of the 290 strains, 32.8% (95) produced H₂S. Among the remaining 195, 11.3% (22) exhibited gelatinolytic activity, while none produced biogenic amines or showed hemolytic activity. Of these, 84.9% (147) utilized soybean molasses as a carbon source, and only 54.9% (95) grew in salt medium with whey, increasing to 70.5% (122) in the presence of more complete nutrient sources (YP). All 147 selected strains assimilated the tested nitrogen sources and grew in 20% glucose; however, only 69.4% (102) grew in 50% glucose, 62.6% (92) in 10% NaCl, and 55.1% (81) in the presence of ethanol. Temperatures of 37 and 40 °C were limiting for 24.5% (36) and 46.3% (68) of the strains, respectively. Five *Wickerhamomyces anomalus* (WA) isolates presented the highest protein contents, ranging from 0.32 to 0.36 g per 100 g of biomass, highlighting their biotechnological potential for SCP production from agro-industrial substrates, with significant yields of protein-rich biomass.

Keywords: Single-Cell Protein; microorganisms; protein enrichment.

Funding: CAPES, CNPq, FAPEMIG.

CHALLENGES ON THE USE OF YEASTS AS PROTEIN SOURCES FOR HUMAN NUTRITION

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REVIEW: The use of microorganisms as an alternative protein source has resurged in the past decade as a strategy for food security, sustainability, and innovation in functional ingredients. Although the concept dates back to the 1960s and 1970s, with initiatives such as the Brazilian consortium UNICELPE [4], only recently has the international market renewed its interest: in 2022, Solar Foods received regulatory approval to commercialize SCP-based food products in Singapore, positioning itself as a pioneer in this new generation of foods [5]. Yeasts stand out due to their high cell productivity, protein profiles suitable for human nutrition, absence of toxins in primary metabolism, and ability to grow under diverse conditions, including on lignocellulosic substrates and effluents. Species like *Kluyveromyces marxianus*, *Cryptococcus aureus*, *Wickerhamomyces anomalus*, and *Trichosporon coremiiforme* are recognized for efficiently converting waste into high-value protein [4]. However, applying yeasts to food requires more than nutritional and biotechnological performance. Sensory acceptability, as well as economic and commercial feasibility, are also essential. The organoleptic profile must be controlled, particularly regarding bitterness, metallic aftertaste, and undesirable coloration—often linked to sulfur compounds, polyphosphates, and pigments. Moreover, yeasts have a relatively high nucleic acid content (5–8%), and if not properly reduced, may lead to health concerns such as hyperuricemia and related nephropathies [9, 10, 11]. Their cell walls also show limited digestibility. To address these challenges, enzymatic degradation, as well as thermal and mechanical treatments, have been explored, though they introduce additional costs and affect biomass stability. Furthermore, labeling, toxicological assessments, and technical regulations for yeast-based ingredients remain unclear in regions like Latin America and the European Union. Despite these barriers, pilot studies incorporating yeast SCPs into bread, pasta [6, 7], fermented beverages, nutritional bars, and meat substitutes have shown promising results in terms of digestibility, texture retention, and essential amino acid content. Thus, the use of yeasts for SCP production represents not only a technically viable solution but also a strategic opportunity to reshape the global protein supply chain.

Keywords: alternative protein; single-cell protein; biotechnology.

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Funding: CAPES, CNPq, FAPEMIG.

APPLICATIONS OF RECOMBINANT PROTEINS IN CULTURED MEAT PRODUCTION

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REVIEW: Cultured meat is a sustainable alternative to conventional animal protein, showing potential for addressing food security challenges for a growing global population. However, its path to large-scale production is impeded by critical obstacles. These challenges range from developing suitable, edible cell growth scaffolds, to enhancing the nutritional profile to match traditional meat. The most significant barrier is the cost of cell culture media, which can account for over 99% of production expenses¹. This high cost is largely driven by supplements like fetal bovine serum, which introduce economic and ethical concerns due to its association with animal slaughter. This review examines how recombinant proteins, produced via precision fermentation, can offer viable solutions to these challenges. This technology leverages microorganisms as efficient “cell factories” to produce specific, high-value recombinant proteins and molecules. It is an essential tool for generating key components with greater purity and quality compared to animal-derived counterparts. These proteins include growth factors, hormones, and serum proteins for culture media², as well as structural proteins like collagen and gelatin for scaffolds, sensory proteins such as heme, and nutritional components like vitamin B12. The review also details the use of various expression hosts to accomplish this, from bacteria like *E. coli* to more complex yeasts and fungi that perform necessary post-translational modifications. In conclusion, while significant hurdles in scaling the process and minimizing costs remain, precision fermentation is positioned as a pivotal strategy by addressing the primary barriers of cost, ethics, and product quality that currently limit cultured meat.

Keywords: Cellular agriculture; Cultivated meat; Culture media; Expression hosts.

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Funding: Brazilian institutions CNPq, FAPESP from UNICAMP, CAPES and FAPESP.

SELECTING FUNGAL STRAINS FOR MYCELIUM PRODUCTION ON SUSTAINABLE CARBON AND NITROGEN FEEDSTOCKS

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ABSTRACT: This research investigates the potential of fungal strains for biotechnological applications through the evaluation of their growth performance in varied culture media compositions, focusing on carbon and nitrogen sources, including agroindustrial residues. The main objective was to assess the specific growth rates of 20 selected fungal strains under different nutrient conditions to identify promising candidates for sustainable biomass production using low-cost, complex substrates. The methodology involved cultivating each fungal strain in multiple media formulations containing both defined and complex carbon and nitrogen sources, including agroindustrial wastes such as lignocellulosic residues and protein-rich by-products. The growth kinetics were monitored by dry biomass measurements and optical density over time, and specific growth rates (μ) were calculated using exponential growth models. Results revealed significant variation among strains and substrates, with complex media compositions derived from agroindustrial residues consistently promoting higher growth rates. Specifically, strains cultivated on media containing a combination of starch-based and proteinaceous agroresidues exhibited μ values ranging from 0.15 h^{-1} to 0.32 h^{-1} , significantly higher ($p < 0.05$) than those in defined synthetic media, which showed μ values between 0.08 h^{-1} and 0.18 h^{-1} . Statistical analysis using ANOVA confirmed the influence of both carbon and nitrogen complexity on biomass productivity. The discussion highlights the adaptability of filamentous fungi to nutrient-rich, heterogeneous substrates, suggesting that enzymatic versatility and substrate accessibility play crucial roles in enhancing fungal proliferation. These findings align with the growing interest in using fungal biotechnology for valorizing agroindustrial by-products, reducing environmental impact, and supporting circular bioeconomy strategies. In conclusion, the study demonstrates that several of the tested fungal strains have high bioconversion potential when cultivated in complex, waste-derived media, validating their application in sustainable production platforms. Future work will focus on optimizing culture conditions and scaling up fermentation using selected high-performing strains.

Keywords: Mycelium; Growth-rate; Agroresidues.

Founding: The Good Food Institute USA; CAPES PRONEX

"HARNESSING MUSHROOM MYCELIA FOR SUSTAINABLE HYBRID MEAT PRODUCTION: A REVIEW OF POTENTIAL AND CHALLENGES"

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REVIEW: The urgent need to develop sustainable food systems in response to global population growth, climate change, and health concerns is related to the traditional meat production transition model. This study aims to evaluate the potential of mushroom mycelia as alternative protein sources in hybrid cell-cultured meat products. Edible fungi, particularly from the *Pleurotus* genus, offer high protein content, essential amino acids, and beneficial bioactive compounds while requiring minimal environmental resources for cultivation. This review outlines how fungal mycelia can be cultivated through submerged or solid-state fermentation, using optimized culture media that comprise carbon, nitrogen, and nutrient sources—including agro-industrial biowaste—to reduce environmental impact. The mycoprotein derived from these fungi exhibits a high nutritional profile and structural similarity to muscle meat, making it suitable for use in meat analogs. We further explore cellular agriculture, distinguishing between tissue engineering and fermentation-based approaches for cultivating animal-derived products without the use of livestock. Hybrid meat analogs, which combine plant, fungal, and animal cell components, are highlighted as viable solutions to address challenges in meat production, texture, flavor, and sustainability. Key obstacles include scaling production, consumer acceptance, regulatory frameworks, and cost. The integration of fungal mycelia can enhance the sensory properties and nutritional content of cultured meat, especially when combined with plant proteins and flavor enhancers. This study concludes that while the use of mushroom mycelia in hybrid meat products presents a promising avenue for sustainable food production, further research and development are essential to address technical, regulatory, and market challenges. Effective communication, accurate labeling, and adherence to regulatory standards will be critical to mainstreaming these innovations and meeting global food security needs.

Keywords: Mycelium; Cultivated meat; Hybrid meat.

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Funding: The Good Food Institute (GFI-USA) and CAPES/PRONEX

EVALUATION OF FUNGI GROWTH SPEED IN YEAST EXTRACT AND RICE BRAN

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ABSTRACT: Yeast extract is widely used in fermentation processes because it is a good source of carbon and nitrogen, which promotes cell growth. Rice bran is a lignocellulosic residue rich in nutrients such as starch, proteins, non-starch polysaccharides, and polyphenols. The objective of this study was to evaluate the influence of the addition of yeast extract (LE) and rice bran (RF) to potato dextrose agar (PDA) on the growth rate of the fungi *Pleurotus albidus*, *Ganoderma australe*, and *Hericium erinaceus*. The fungi were grown in 10.5 cm Petri dishes in PDA medium, PDA medium supplemented with 10 g/L of LE (PDA-LE), and in parallel PDA medium supplemented with 20 g/L of RF (PDA-RF), both maintained in an incubator at 28 °C for 15 days. Growth was measured with a caliper in two perpendicular directions, obtaining the mean diameter of the strains at 24-h intervals. The mean growth rate was calculated by dividing the mean diameter by the growth time. The fungi *P. albidus* and *G. australe* showed marked growth on the LE and RF-enriched media, with *P. albidus* reaching maximum radial growth in 8 days. However, *H. erinaceus* demonstrated slow growth and greater adaptability to PDA-LE. The mean growth rates obtained for PDA medium ranged from 0.20 to 0.50 cm day⁻¹, for PDA-LE, from 0.30 to 0.70 cm day⁻¹, and for PDA-RF, from 0.20 to 0.75 cm day⁻¹. Based on the results, it was found that PDA-LE and PDA-RF media demonstrated increased growth rates for both fungi when compared to the medium containing only PDA, being more effective for *P. albidus*. Furthermore, changes in the visual appearance of the mycelia were observed with the addition of LE and RF, resulting in a denser layer on the plate surface. These results may have been influenced by the greater availability of carbon and nitrogen in the medium and may also be associated with the presence of essential amino acids, whose synthesis is facilitated when available from organic carbon sources.

Keywords: Enrichment; Growth; Average speed.

Funding: Santa Catarina State Research and Innovation Support Foundation – FAPESC

MYCELIUM PRODUCTION FOR FOOD PURPOSES: ADVANCES, CHALLENGES AND PERSPECTIVES

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REVIEW: The demand for alternative protein sources has driven the exploration of sustainable biotechnologies, notably the production of edible mycelium. Mycelium represents a promising protein biomass, rich in fiber and essential amino acids, and with low environmental impact, positioning itself as a relevant candidate in the transition to sustainable systems (Ritala et al., 2017; Finnigan et al., 2019). This review aims to present the scientific and technological advances related to mycelium production for food use, highlighting its nutritional importance, cultivation strategies, and the main technical challenges faced by this emerging segment. Edible mycelium production is based on fungal fermentation using substrates rich in carbon and nitrogen, with emphasis on species such as *Fusarium venenatum*, *Neurospora intermedia*, and *Aspergillus oryzae* (Nielsen et al., 2022). These fungi are cultivated in bioreactors under controlled pH, temperature, and oxygenation conditions, producing biomass with high protein digestibility (up to 90%) and an amino acid profile comparable to that of animal sources (Smetana et al., 2021). The mycoprotein derived from mycelium also has functional advantages, such as a fibrous texture similar to meat and the ability to be molded into various food formats (Wiebe, 2002). However, significant challenges still limit its widespread adoption: production costs, the need for specific regulatory approval (such as classification as a "novel food" in Europe), and sensory barriers such as aftertaste and consumer acceptance (Ritala et al., 2017). Mycelium production for food purposes represents an innovative frontier in food biotechnology, combining nutritional, environmental, and technological attributes. Despite promising advances, overcoming technical and regulatory challenges is essential for its consolidation in the market. Investments in applied research, process optimization, and consumer communication strategies will be crucial to the success of this alternative in the future of global food systems.

Keywords: Mycoprotein; Biomass; Fermentation.

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Funding: Santa Catarina State Research and Innovation Support Foundation – FAPESC

CO-FERMENTATION OF PLANT INGREDIENTS AND MICROALGAE FOR FUNCTIONAL PROTEIN-RICH FOODS

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REVIEW: The growing interest in sustainable and health-promoting foods has led to innovative approaches combining plant-based matrices and microalgae through co-fermentation. Microalgae such as *Spirulina platensis*, *Chlorella vulgaris*, *Dunaliella salina*, and *Tisochrysis lutea* are rich in high-value nutritional and functional compounds, including proteins, polyunsaturated fatty acids, vitamins, minerals, carotenoids, and fibers^{1,2,3,5}. These compounds make microalgae promising substrates for fermentation with lactic acid bacteria and yeasts, resulting in food products with enhanced nutritional and functional properties^{1,3,6}. Co-fermentation enhances the bioavailability of antioxidant compounds such as polyphenols and carotenoids and promotes the release of bioactive peptides with antidiabetic, immunomodulatory, and anti-inflammatory activities⁶. Microalgae-based fermentation has also shown potential to reduce antinutritional factors and improve digestibility^{3,5}. Additionally, microalgae exhibit prebiotic activity, promoting the growth and viability of probiotic strains during fermentation^{1,2}. A recent study have shown that co-fermenting *Spirulina* with recombinant *Saccharomyces cerevisiae* resulted in the bioconversion of canthaxanthin into astaxanthin, a high-value carotenoid with potent antioxidant activity⁴. Co-fermentation of dairy, cereal-based, and plant-based beverages with microalgae and selected microbial strains has led to enhanced sensory attributes, improved texture, higher nutritional value, and greater product stability^{1,3,6}. Moreover, the integration of microalgae into fermentation platforms is increasingly explored in food technology, driven by their alignment with circular economy principles and environmental sustainability^{1,2}. Despite current limitations, such as regulatory challenges, sensory acceptance, and standardization of microalgal biomass^{2,3}, co-fermentation stands out as a promising biotechnological tool to develop next-generation protein-rich, functional, and sustainable foods^{1,3,6}. Its potential includes emerging applications such as 3D food printing, personalized nutrition, and the development of hybrid foods that integrate health benefits, safety, and consumer appeal^{1,2}. This co-fermentation approach aligns with the growing demand for clean-label, plant-based products that not only support human health but also contribute to reducing environmental impact^{1,2}.

Keywords: co-fermentation; microalgae; functional foods; bioactive compounds; protein enrichment.

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Funding: The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support (Finance code 001).

EFFECT OF INOCULUM CONCENTRATION WITH *Rhizopus oryzae* ON SOLID FERMENTATION APPLIED TO DIFFERENT SUBSTRATES TO OBTAIN FLOURS WITH PROTEIN AVAILABILITY

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ABSTRACT: The search for sustainable ingredients for aquaculture feed has increased interest in plant-based sources such as sorghum, maize, and soybean [1]. However, the presence of antinutritional compounds and the low protein bioavailability limit their use. In this context, solid-state fermentation with *Rhizopus oryzae* is proposed as a strategy to enhance its nutritional value [2, 3]. This study aimed to evaluate the effect of this fermentation on the soluble protein content of each substrate and their mixture, with a view toward application in sustainable aquaculture diets. Three inoculum levels (1%, 3%, and 5%) were tested, and the most favorable conditions were selected using a bifactorial design and a kinetic fermentation analysis based on soluble protein, pH, sugars, and phenolic compounds. Treatment with a 5% inoculum in sorghum increased the soluble protein content by 3.5-fold compared to the unfermented control. In the case of maize and soybean, the application of a 3% inoculum led to 8.2 and 5.5-fold increases, respectively. Based on these results, a mixture was formulated for aquaculture feeding, which showed a 6.2-fold increase in soluble protein content, a 1.92-fold increase in total phenolic compounds, and a 1.04-fold increase in fat content, compared to the unfermented mixture. It is concluded that fermentation with *R. oryzae* improves the nutritional quality of these plant-based ingredients and that their combination into a fermented mixture represents a promising alternative to the use of fishmeal and fish oil in aquaculture diets.

Keywords: Fermentation; Nutritional enhancement; Animals; Aquaculture feed.

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FERMENTED BEVERAGE FROM RICE AND PEA PROTEINS WITH UVAIA PULP (*EUGENIA PYRIFORMIS*): A SUSTAINABLE APPROACH FOR THE PLANT-BASED FOOD MARKET

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ABSTRACT: The growing demand for plant-based proteins has stimulated the development of innovative functional foods, particularly fermented beverages that combine nutritional value with probiotic benefits. In this study, we investigated the production and *in vitro* gastrointestinal digestion of a fermented plant-based beverage (FPBB) formulated with pea protein concentrate (PPC) and rice protein concentrate (RPC), incorporating *Lactocaseibacillus rhamnosus* GG (Lr) as the fermentative culture. Uvaia pulp was added at different concentrations, 0% (FPBB-C), 5% (FPBB-5), and 10% (FPBB-10), to assess its influence on probiotic survival and bioactive compound bioaccessibility. The *in vitro* digestion assay evaluated probiotic viability, total phenolic compounds (TPC), and antioxidant activity before and after gastrointestinal simulation. After 48 h of digestion, high viability of *L. rhamnosus* GG was maintained in all treatments, with survival rates above 100%. Specifically, FPBB-C (106.89%) and FPBB-5 (109.38%) outperformed FPBB-10 (102.20%), suggesting that moderate enrichment with uvaia pulp may favor probiotic protection and potential prebiotic effects. Importantly, the use of pea and rice proteins as the structural base of the beverage ensured high cell counts ($>9 \log \text{CFU g}^{-1}$) throughout digestion, highlighting their functionality to the probiotics. Bioactive compound analysis revealed a significant increase in TPC after digestion across all formulations. Antioxidant activity was also enhanced, as determined by DPPH (4.06–8.44 mg TEAC mL⁻¹), ABTS (10.28–11.97 mg TEAC mL⁻¹), and FRAP (863.87–1983.23 mg TEAC mL⁻¹). Thirteen compounds were identified and quantified in uvaia pulp by HPLC-DAD-ESI-MS, particularly epigallocatechin gallate, quercetin-3-rhamnose, and quercetin-3-glucoside. Isorhamnetin was the main phenolic compound detected in the colon, presumably due to the conversion of quercetin-3-glucoside by the probiotic cells. Overall, FPBB formulations based on pea and rice proteins demonstrated strong potential as sustainable vehicles for probiotic delivery and bioactive compound release. Their ability to combine plant protein matrices with fruit-derived phytochemicals reinforces their role as promising systems for next-generation functional foods.

Keywords: Beverage fermented; Probiotic; Prebiotic; Antioxidant activity.

Funding: The authors are thankful to the National Council for Scientific and Technological Development (CNPq-Brazil), grant number [402338/2023-5], and Coordination for the Improvement of Higher Level Personnel (CAPES-Brazil) for the financial support and scholarships (Code 001). Callebe Camelo-Silva and Guilherme Dallarmi Sorita acknowledge the financial support from CNPq (grant number 174880/2023-5 and 150281/2024-2).

ADVANCING A SUSTAINABLE PROTEIN FUTURE: BUSINESS DETERMINANTS AND STAKEHOLDER VIEWS ON MARKETABILITY IN THE EUROPEAN ALTERNATIVE PROTEIN SECTOR

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ABSTRACT: Introduction: Alternative protein (AP) products have the potential to support a more sustainability agri-food system, by generating significantly lower carbon emissions and requiring lower land and water use compared to animal-based products. AP companies actively contribute to advancing the protein transition and shaping the future of food system. While the AP market has expanded substantially in Europe, the determinants driving this growth remain underexplored. Objectives: The present study aims to look at AP industry by (1) identifying key determinants of business performance of AP companies and (2) exploring stakeholders' perspectives on the marketability of AP products. Methodology: The methodological approach includes two steps. The first step employed semi-structured interviews with 37 AP companies to identify barriers and enablers to business performance. Interviews were analyzed through a qualitative content analysis with NVIVO 12. In the second step, Q methodology was employed to investigate stakeholders' perspective on marketability. 35 experts ranked 34 statements according to their level of agreement, and principal component analysis identified five factors representing shared viewpoints. Results and discussion: Findings revealed that critical determinants of marketability and business performance include product-related features such as price, taste and ultra-processed classification. Performance is further enhanced by having a highly skilled team, investment in R&D to improve products characteristics, and benefiting from increased retailer commercialization of AP products. Limiting factors include financial and technological challenges to scaling production, uncertainties regarding market growth, lack of level playing field, and regulation hurdles. The findings underscore the dynamic and innovative nature of the AP industry, while also emphasizing the need for multi-level strategy to support its continued market growth. Greater efforts to advance knowledge on technology and ingredient applications, especially on less well-known plant and fermented proteins, should arrive from joint actions of companies and research centers, backed by NGOs and government. Policy interventions are needed to address the existing price gap and to ensure a level playing field between animal-based and AP products. Conclusion: The findings highlight key issues of the European AP market and offer insights into unlocking the full potential of APs as a cornerstone of a more sustainable, resilient agri-food system.

Keywords: plant-based protein; fermentation; sustainable food; marketability; business development.

Funding: This manuscript is funded with NRRP ex M.D. 118/2023 resources and by the "From niche to main to stream: alternative proteins for everybody and everywhere (LIKE-A-PRO)" project, which has received funding from the European Union's Horizon Europe research and innovation program under grant agreement no. 101083961 LIKE-A-PRO. The content of this paper reflects only the authors' views, and the European Commission is not liable for any use that may be made of the information it contains.

SOLID FERMENTATION AS A STRATEGY FOR ENHANCEMENT OF SOYBEAN MEAL AND PRODUCTION OF ALTERNATIVE PROTEINS

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REVIEW: Solid-state fermentation (SSF) of plant substrates emerges as a promising alternative for developing new protein sources. Given the growing unsustainability of animal protein production and population growth, it is essential to search for alternative proteins that meet society's nutritional demands ^[1]. The application of SSF to substrates such as soybean meal can generate products with higher added value, improved nutritional quality, and potential for use in meat alternatives, representing an innovation strategy in the food sector ^[2]. The objective of this review was to identify how solid-state fermentation modifies soybean meal, focusing on protein enrichment and its impact on nutritional quality. The literature analysis revealed that, although studies exist with microorganisms of the genus *Bacillus*, there is a lack of research focused specifically on soybean meal, indicating an opportunity for future research. The results varied considerably depending on the microorganism species and process conditions. In fermentation with *Bacillus amyloliquefaciens*, the protein isolate yield was dependent on the extraction temperature, reaching 130.9% at low temperature and 41.9% at high temperature ^[3]. On the other hand, for specific strains of *B. subtilis*, crude protein enrichment increased from 1.7% in fermentation at a constant high temperature of 55 °C to 2.8% in a process that simulated natural temperature variation (between 37 °C and 50 °C). The lower temperature may have favored less protein degradation and release ^[4]. Considerably, another study with *B. subtilis* achieved a 6.6% increase in crude protein, but also increased soluble protein by 16.5%. This increase in solubility is important because it indicates that microbial enzymes degrade high-molecular-weight insoluble proteins into smaller compounds, such as peptides and free amino acids, resulting in a possible direct improvement in bran digestibility ^[5]. It is concluded that FES modifies the nutritional structure of soybean meal, not only increasing its protein content but also improving its functionality. Therefore, FES represents a viable and sustainable technology for the use of soybean residue, enabling the development of new, innovative, and attractive alternative protein products for consumers.

Keywords: solid fermentation; soybean meal; protein enrichment.

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Funding: The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) (Finance Code 001). CNPQ Grant number PQ 306194/2025

EVALUATION OF THE EFFECT OF SOLID-STATE FERMENTATION WITH *Pleurotus albidus* ON THE PROTEIN CONTENT OF RICE BRAN

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ABSTRACT: Rice bran is an excellent source of nutrients, with a significant protein content (approx. 18%) and high potential in terms of quality and functionality for the development of innovative and nutritious foods. Despite this potential, the application of rice bran in the food industry is limited due to its rapid deterioration, mainly caused by lipolytic enzymatic activity [1]. For this reason, it is predominantly used as a raw material for the extraction of edible oil, which results in the underutilization of its protein and functional value [2]. The present study aimed to evaluate the solid-state fermentation kinetics of rice bran using the fungus *Pleurotus albidus*, analyzing its effect on the substrate composition and the potential for transforming rice bran into a promising ingredient for the development of new food products. The experiments were conducted in fixed-bed bioreactors, with initial moisture adjusted to 50% using a mineral solution and a temperature of 27 °C. The fermentation process was carried out over 11 days, with samples collected every 2 days to monitor biochemical changes. The results demonstrated a 46.69% increase in protein content after 11 days of fermentation, accompanied by a progressive decrease in pH and variations in phenolic content throughout the process. These outcomes indicate that solid-state fermentation with *P. albidus* is an effective strategy for valorizing rice bran by enriching its nutritional profile. Furthermore, the findings highlight the potential of fermented rice bran as a functional ingredient for the development of more nutritious and sustainable foods.

Keywords: Fermentation; Alternative Protein; New Food; By-products; *Pleurotaceae*

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Funding: Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina – FAPESC

MASS BALANCE OF PROTEIN EXTRACTION FROM MYCELIAL BIOMASS

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ABSTRACT: The alternative protein sector has been standing out due to the development of ingredients derived from mycelial fungal biomass, a sustainable source of proteins, fibers, and bioactive compounds. This study aimed to perform the mass balance of protein extraction from mycelial fungal biomass (MFB), evaluating the recovery of the protein fraction and the yield of the obtained concentrate. Protein extraction was conducted in two trials of Extraction by Heating and Agitation (EHA). In test 1, the MFB was ground in an industrial blender, and in test 2, in a bench mill. In both cases, the biomass (40 g) was suspended in distilled water (150 g), alkalinized to pH 11.0 with NaOH (0.1 mol L⁻¹), and subjected to agitation (550 rpm) and heating (55–60 °C) for 60 minutes. After filtration, the cake and the crude extract were obtained; the extract was then precipitated to pH 2.7 and centrifuged, resulting in the protein concentrate. Protein content was determined by the Kjeldahl method. In test 1, the initial biomass contained 4.91 g of protein. The obtained fractions were cake with 1.88 g, supernatant with 0.78 g, and concentrate with 1.53 g, totaling 4.19 g of protein, equivalent to 85.3% recovery, with estimated losses of 0.72 g. In test 2, also starting with 4.91 g of protein, the fractions were: cake with 2.26 g, supernatant with 0.79 g, and concentrate with 1.53 g, resulting in 4.58 g of protein, corresponding to 93.3% recovery, with 0.33 g losses. The results indicate that grinding in a bench mill (test 2) promoted higher extraction efficiency compared to the blender (test 1). The application of the EHA process proved to be an efficient strategy for extracting proteins from mycelial fungal biomass, with recovery above 85% and significant concentration in the protein precipitate. The study highlights the potential of fungal biomass as a sustainable source of alternative proteins, reinforcing the feasibility of this protein in food applications. It is concluded that the improvement of pretreatment conditions can further increase yields, strengthening the industrial application of this bioproduct.

Keywords: Alternative proteins; Fungal biomass; Protein recovery; Bioprocessing

Funding: Cnpq, Typical

RICE BRAN AND RICE HULLS AS ALTERNATIVE PROTEIN SOURCES VIA SOLID-STATE FERMENTATION WITH *Rhizopus oryzae*

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ABSTRACT: Rice is one of the most cultivated and consumed cereals in the world, and its polishing removes its outer layers, generating byproducts such as husk and bran. Rice husk is the outermost layer of the grain, removed during milling, and represents 20% of paddy rice. Rice bran is the outer layer of the endosperm, representing 11% of milled rice. The valorization of these byproducts holds promise for the sustainable production of alternative protein ingredients. This study aimed to evaluate the effect of fermentation by *Rhizopus oryzae* on the nutritional composition of white rice bran and rice husk. The substrates were fermented at 30 °C for 96 h in bioreactors containing 4×10^6 spores g⁻¹ and a nutrient solution with 22.5 g L⁻¹ (KH₂PO₄ 2 ; MgSO₄ 1 ; NH₂CONH₂ 1.8), with humidity adjusted to approximately 50%. The substrates and fermented biomasses were characterized for moisture, ash, lipids, proteins, crude fiber, and carbohydrates (by difference) according to the AOAC (2012) methods. The analyses were performed in triplicate, and the data were evaluated by Student's t-test ($p < 0.05$) using Statistica 12.5. Solid-state fermentation significantly modified the nutritional composition of the evaluated substrates. In white rice bran, ash increased from $5.89 \pm 0.17\%$ to $12.15 \pm 0.37\%$, lipids from $12.81 \pm 0.63\%$ to $21.61 \pm 0.29\%$, proteins from $10.96 \pm 0.62\%$ to $17.69 \pm 0.55\%$ and crude fiber from $4.01 \pm 0.38\%$ to $10.31 \pm 0.65\%$, while carbohydrates decreased from 66.33% to 38.24%. In rice husk, no significant difference was observed in ash content ($20.08 \pm 0.13\%$ to $19.36 \pm 0.74\%$), but lipids increased from $3.95 \pm 0.16\%$ to $5.24 \pm 0.01\%$, proteins from $0.94 \pm 0.07\%$ to $2.68 \pm 0.05\%$ and crude fiber from $49.48 \pm 0.09\%$ to $50.22 \pm 0.30\%$, while carbohydrates decreased from 25.55% to 22.50%. Solid-state fermentation with *Rhizopus oryzae* proved to be highly effective in enriching the protein content of rice processing co-products. The reduction of carbohydrates in all substrates indicates that the fungus utilized these compounds as its main energy source, thus concentrating proteins and minerals in the biomass. The significant increase in protein, especially in rice hulls, demonstrates the potential of these fermented materials as alternative protein sources for food and feed applications.

Keywords: Nutritional composition; Agro-industrial co-products; Filamentous fungi.

EFFECT OF TEMPERATURE ON THE FERMENTATION OF CASHEW NUT EXTRACT BY *Lactocaseibacillus paracasei* AND *Streptococcus thermophilus*

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ABSTRACT: Fermentation of plant-based beverages is emerging as an alternative to dairy products, contributing to desirable sensory properties and product stability through the action of Lactic Acid Bacteria (LAB). These bacteria reduce pH, produce bioactive compounds, and contribute to the product's sensory and microbiological quality. Cashew nut concentrate, a readily available option in the Brazilian market, represents a favorable matrix for LAB cultures due to its nutritional profile and acceptance potential. This study aimed to evaluate the fermentation of this concentrate using *Lactocaseibacillus paracasei* (L.p.), *Streptococcus thermophilus* (S.t.), and their association (L.p. + S.t.) at two different temperatures 37 °C and 45 °C. Titratable Total Acidity (TTA), pH, and microbiological counts were monitored over 24 hours of fermentation. Among the individual BAL, a significant difference ($p < 0.05$) in pH values at 24h was observed: 4.89 (37 °C) and 4.97 (45 °C) for L.p., and 4.32 (37 °C) and 4.75 (45 °C) for S.t. However, when associated (L.p. + S.t.), the cultures showed no significant differences ($p < 0.05$) in final pH values, maintaining an average of 4.37. This suggests that the interaction between species maintained acidification capacity regardless of temperature, though acid profile or concentration may vary. No significant differences ($p < 0.05$) in TTA were observed for both conditions. Regarding growth kinetics, the initial growth (0–4 h) was equivalent at both temperatures. However, despite a brief increase at 37°C between 4 and 12 hours (reaching $8.25 \log \text{CFU} \cdot \text{g}^{-1}$), this temperature led to a $0.37 \log \text{CFU} \cdot \text{g}^{-1}$ drop by 24 hours. Conversely, 45 °C ensured consistent growth, increasing from 7.56 to $8.36 \log \text{CFU} \cdot \text{g}^{-1}$ in 14 hours, and maintained better viability until the end of fermentation ($8.05 \log \text{CFU} \cdot \text{g}^{-1}$). Overall, the cashew nut beverage proved to be a highly adaptable matrix for LAB, allowing for rapid acidification and acid production without long fermentation periods, a desirable characteristic for the development of fermented plant-based drinks.

Keywords: Fermented plant-based beverage; Fermentation kinetics; Cashew nut.

Funding: The authors are thankful to Financiadora de Estudos e Projetos (FINEP 2892/22), Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC 2022TR002005), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 402338/2023-5 303672/2024-2) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/Proex) for the financial support.

NUTRITIONAL VALORIZATION OF *ATTALEA TESSMANNII* (COCÃO) VIA PROTEIN ENRICHMENT THROUGH SOLID- STATE FERMENTATION WITH *RHIZOPUS ORYZAE*

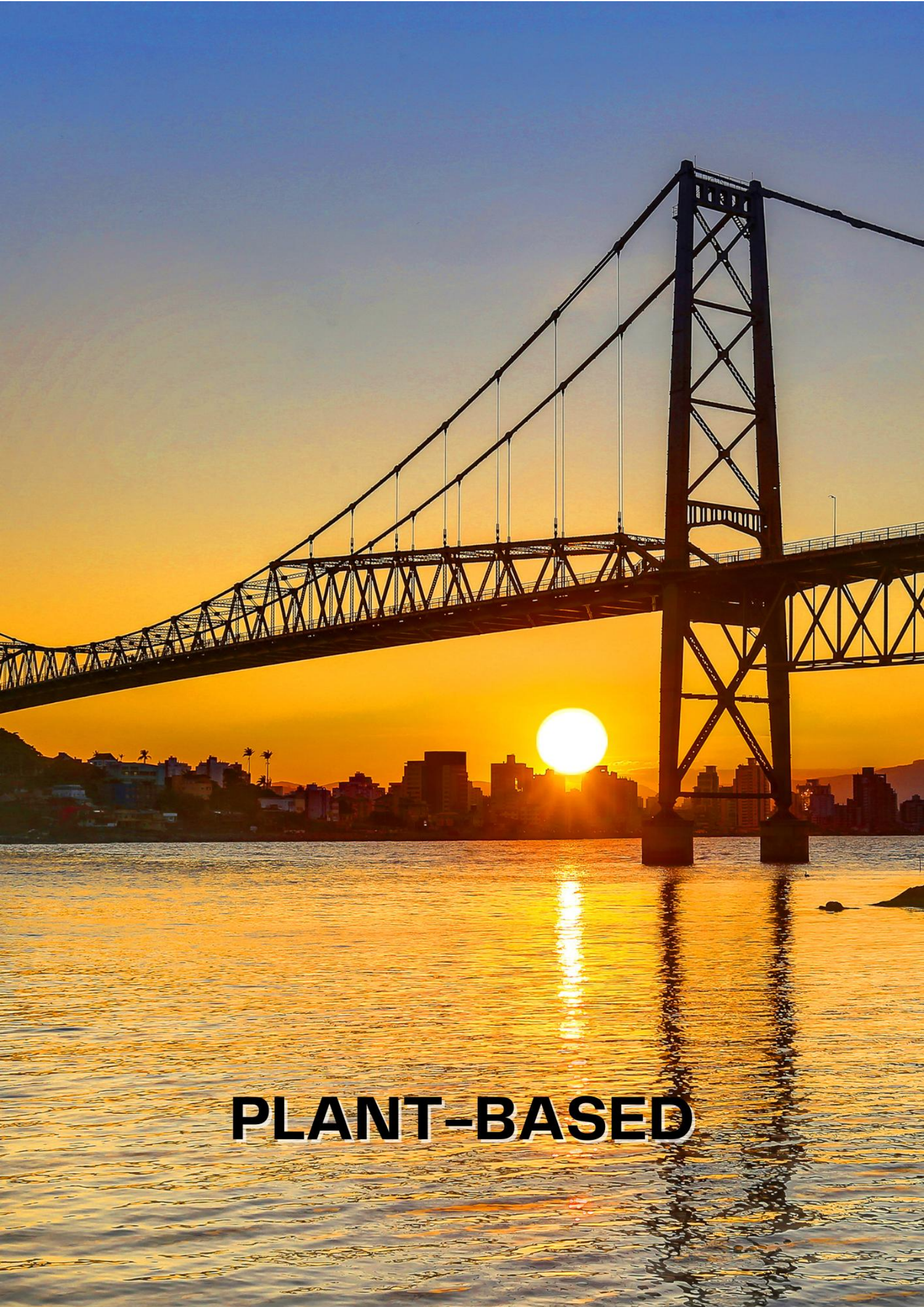
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ABSTRACT: Brazilian biomes present a high diversity of plant species, especially the Amazon Rainforest. *Attalea tessmannii*, a palm tree native to the region, is primarily used for extracting oil from its almonds. This process produces the mesocarp, endocarp, and epicarp, which represent approximately 90% of the fruit. The use of biotechnological processes emerges as a strategy to valorize this residue. Therefore, this study aims to evaluate the effect of fermentation by *Rhizopus oryzae* on the nutritional composition of *Attalea tessmannii*. The mixture containing 26% endocarp, 48% mesocarp, and 26% epicarp of *A. tessmannii* was fermented at 30°C for 96 h in bioreactors containing 4×10^6 spores g⁻¹ and a nutrient solution containing 22.5 (g L⁻¹): KH₂PO₄ (2); MgSO₄ (1); NH₂CONH₂ (1.8), with a moisture content of 50%. The coconut mixture and the obtained biomass were physicochemically characterized in terms of moisture, ash, lipids, proteins, crude fiber, and carbohydrates (by difference) according to the AOAC (2012) methods. The analyses were performed in triplicate, and the data were statistically analyzed using Student's t-test ($p < 0.05$) using Statistica 12.5 software (StatSoft). The unfermented mixture and biomass obtained lipid contents of $1.84 \pm 0.14\%$ and $4.08 \pm 0.06\%$, ash of $2.75 \pm 0.01\%$ and $1.72 \pm 0.02\%$, protein of $4.51 \pm 0.01\%$ and $5.72 \pm 0.05\%$, and carbohydrates of $90.96 \pm 0.05\%$ and $88.47 \pm 0.02\%$, respectively. A significant increase in the protein content of the biomass was observed compared to the unfermented mixture, corresponding to an increase of 27.96%. This increase demonstrates the efficiency of solid-state fermentation with *R. oryzae* as a nutritional enrichment strategy, since the resulting biomass presented greater protein potential for use in food formulations. Furthermore, fermentation promoted an increase in lipid content (121.74%) and a reduction in carbohydrates (2.49%), explained by the microorganism's utilization of carbohydrates as its primary carbon source. The results demonstrate that solid-state fermentation by *Rhizopus oryzae* significantly contributed to the protein enrichment of *Attalea tessmannii* residue. This increase highlights the potential of biomass as an alternative food ingredient with superior nutritional value.

Keywords: Nutritional composition; Agro-industrial residue valorization; Alternative protein ingredient.



PLANT-BASED

POTENCIAL OF PLANT PROTEINS IN 3D FOOD PRINTING: A REVIEW

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REVIEW: The technology of 3D food printing represents a promising innovation in the personalization of nutrition, reduction of food waste, and development of functional products. Within this context, plant proteins emerge as sustainable and versatile alternatives to animal-derived proteins. The objective of this study was to conduct a review of the scientific literature regarding the use of plant proteins in 3D food printing. A search was carried out in the Scopus, Web of Science, and ScienceDirect databases using the descriptors “Food 3D Printing” and “Vegetable Proteins.” The inclusion criteria comprised articles published between 2019 and 2025 that explicitly addressed the application of plant proteins in the formulation or performance of 3D-printed foods. A total of 30 articles were selected, of which 10 were excluded for not explicitly addressing the intended topic. Among the 20 articles included in the review, 9 were published in 2025, highlighting the current relevance and promising nature of this research field, in which many discoveries remain to be made. The main proteins used as printing ink are pea and soy proteins, both demonstrating excellent structure-forming capabilities. Plant proteins, oils, and water are the primary components in the formulation of 3D printing inks. However, proteins alone are often insufficient for achieving the desired texture, necessitating the incorporation of additional ingredients such as polysaccharides and polyphenols. Physical modifications—such as heating, microwave treatment, and ultrasound—are strategies employed to enhance the gelation properties of plant protein isolates and thereby facilitate the printing process. The appropriate selection of ingredients and formulations enables the production of meat analogues that closely resemble the microfibrillar and anisotropic structure of animal meat. Thus, 3D food printing emerges as a promising approach for the application of alternative proteins in food production. The growth of research focused on the incorporation of alternative proteins in 3D printing is expected in the coming years, engaging professionals from various fields within food science and technology.

Keywords: Alternative proteins; vegan food; food innovation; sustainability; hidrocoloids

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Funding: Federal University of the Jequitinhonha and Mucuri Valleys and the Institute of Science and Technology for institutional and financial support (grant n° 23086.001699/2022-41. National Council for Scientific and Technological Development (CNPq) for financial support (grant n° 421777/2021-4) and The Research Support Foundation of the State of Minas Gerais (FAPEMIG) for the scholarships granted to T.M.S. (#77597).

ENGINEERING PLANT-BASED MEAT ANALOGUES: STRUCTURAL OPTIMIZATION OF SOY-GLUTEN SYSTEMS VIA MOISTURE-DEPENDENT THERMOPLASTIC EXTRUSION

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ABSTRACT: The growing demand for sustainable protein sources has stimulated the development of plant-based meat analogues. This study investigated the structural and functional characteristics of meat analogues produced via high-moisture (HM) and low-moisture (LM) thermoplastic extrusion. Soy protein isolate (SPI) and vital gluten (VG) were used as protein matrices due to the complementation of amino acid profile and fibrillar structure development, and optimized formulations (OP) were compared with control samples (CT) in terms of protein solubility, total amino acid composition, and conformational changes assessed through FTIR. The raw materials exhibited high protein contents, measured at 88.53% (SPI-HM), 91.04% (SPI-LM), and 77.01% (VG). VG showed a gluten index of 77.12%. Protein solubility varied significantly across treatments and solvent systems ($P < 0.10$; $R^2 > .80$). In phosphate buffer (PB), solubility was recorded at 10.15% for OP-HM and 13.20% for CT-HM. In PB+SDS, solubility reached 36.99% and 39.46%, respectively. The highest solubility was observed in the β -mercaptoethanol + urea system, with values of 53.26% (OP-HM) and 51.18% (CT-HM), indicating enhanced disulfide bond formation. Amino acid profiling confirmed that all formulations complied with the FAO reference pattern for adults. Lysine (6.09–6.63 g/100g protein), leucine (8.02–8.31g/100 g), and valine (5.24–5.33g/100 g) were present in adequate amounts. Nonetheless, significant reductions in thermolabile amino acids were observed, particularly in cysteine (0.89–1.03g/100 g) and tryptophan (0.94–1.06g/100 g), with losses up to 67% and 39%, respectively, under high-shear, high-temperature conditions. FTIR analyses demonstrated notable alterations in protein secondary structure, with reduced absorbance in the Amide I (1650cm^{-1}) and II (1540cm^{-1}) bands for extruded samples (0.31–0.57), compared to raw materials (0.90–1.20). These spectral changes are indicative of extensive protein denaturation, especially under LM extrusion, which was associated with greater mechanical energy input and limited water-mediated lubrication. It was concluded that the incorporation of VG into SPI-based matrices promoted fibrous structuring through the synergistic action of disulfide bonding, hydrogen bonding, hydrophobic, and ionic interactions. The optimization of extrusion parameters was shown to be crucial for achieving desirable textural attributes and preserving the nutritional integrity of plant-based meat analogues.

Keywords: Disulfide cross-linking; Nutritional enhancement; Texturization

Funding: UFVJM, ICT, CNPq, FAPEMIG, and CAPES.

WATER-SOLUBLE EXTRACT FROM GERMINATED *Lupinus albus*: A PROMISING ALTERNATIVE PLANT PROTEIN SOURCE

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REVIEW: The search for alternative and sustainable sources of plant-based protein has intensified interest in legumes such as *Lupinus albus* (white lupin), recognized for its high protein content and agronomic adaptability. Since the germination increase the availability of free amino acids and alters the solubility of proteins [1], this study aimed to evaluate the effect of germination under varying conditions on protein solubility, with the objective of producing a water-soluble protein extract suitable for use as a functional ingredient in plant-based food applications. Germination conditions were optimized using a Central Composite Rotational Design (DCCR) comprising 12 experimental assays, in which germination time (24 to 96 hours) and temperature (14 °C to 32 °C) were systematically varied. Following germination, seeds were processed to obtain a water-soluble extract, and the concentration of soluble proteins was determined using the Kjeldahl method, expressed in g/L. Results indicated that although none of the germinated samples surpassed the protein concentration of the non-germinated control (27.48 g/L), germination imparted beneficial characteristics to the extract. Notably, the central point condition (60 h at 23 °C) yielded the highest soluble protein concentration among the treatments (26.33 g/L). Both germination time and temperature significantly influenced protein yield, with extended durations and extreme temperatures leading to greater reductions in soluble protein content, as observed in trial 6 (96 h at 23 °C), which yielded only 18.73 g/L. In this case, the decrease in protein content may be related to its use in the protein synthesis of the root [2]. Despite the slight decrease in soluble protein concentration compared to the raw seeds, germination is known to reduce antinutritional factors and enhance nutrient digestibility and bioavailability, which may offset the quantitative protein loss. Consequently, the water-soluble extract from germinated *Lupinus albus* demonstrates promising potential as a functional, plant-based protein ingredient. Future investigations will focus on characterizing the amino acid profile, sensory properties, and technological stability of the extract for its application in food formulations.

Keywords: plant-based food; bioconversion; legumes.

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Funding: CAPES (Coordination of Superior Level Staff Improvement) (code 001), CNPq (Nacional Council for Scientific and Technological Development) for the scholarships of C.V.D.M (#173116/2024-8), E.R.S.S (#173015/2024-7), G.C.A (#172881/2024-2) and M.A.A (#88887.990587/2024-00), UFVJM (Federal University of Jequitinhonha e Mucuri Valleys) for the scholarship of L.K.J (institutional quota).

STRUCTURING PLANT-BASED MEAT ANALOGUES: OPTIMIZING EXTRUDED SOY-GLUTEN EXTENDERS FOR EMULSIFIED SYSTEMS

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ABSTRACT: The growing demand for plant-based foods has driven the development of meat analogues and their derivatives, requiring formulations that combine technological performance with nutritional value. Among these products, cooked emulsions such as mortadella present challenges when meat is replaced by plant proteins, especially regarding emulsion stability, texture, and protein digestibility. In this study, the technological performance of meat extenders from soy protein isolate (SPI) and vital gluten (VG), obtained through high-moisture (HM) and low-moisture extrusion (LM), was evaluated in model emulsified meat analogues. The extenders were produced in a co-rotating twin-screw extruder under distinct conditions: HM with 88% SPI, 12% VG, 58% moisture, and barrel temperatures ranging from 50 to 100 °C; LM with 95% SPI, 5% VG, 18% moisture, and temperatures from 60 to 135 °C. A mixture design (simplex-centroid) was employed to vary the replacement of meat (0–100%) by extruded extenders. The dependent variables included emulsion stability (ES), water activity (aw), instrumental color (L*, a*, b*), texture profile (hardness, adhesiveness, cohesiveness, gumminess, and chewiness), moisture content, and in vitro protein digestibility. The control sample showed ES of 72.26%, aw of 0.9742, L* = 71.57, a* = 8.67, b* = 11.90, and hardness of 12.90 N. In the optimized formulation (37% meat + 63% HM), values of ES = 100%, aw = 0.9743, L* = 70.21, a* = 6.50, b* = 19.25, hardness = 15.19 N, adhesiveness = -0.12 N·s, cohesiveness = 0.67, and chewiness = 8.26 N were achieved. When only LM was used, a reduction in shear force (1.70 N), darker color (L* = 58.80), and increased yellowness (b* = 25.41) were observed. Protein digestibility reached 94.48±1.79% in the control and 85.23±2.52% in the optimized formulation, with reduction attributed to residual antinutritional factors in soy, partially inactivated by extrusion. It was demonstrated that extruded plant proteins could replace up to 63% of meat without compromising the technological quality of the emulsion. The HM extender provided better results than LME, particularly in instrumental texture attributes. These findings support the application of thermoplastic extrusion in producing plant-based emulsified analogues with favorable structural and functional characteristics.

Keywords: Protein functionality; Soy protein; Gluten vital; Texturization.

Funding: UFVJM, ICT, CNPq, FAPEMIG, and CAPES.

TAILORING FUNCTIONAL PROPERTIES OF PLANT-BASED MEATS THROUGH PROTEIN COMPOSITION AND EXTRUSION PARAMETERS

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ABSTRACT: The increasing demand for plant-based protein products has driven the development of meat analogues with physicochemical and sensory characteristics comparable to animal meat. In this context, the technological properties of meat analogues formulated with soy protein isolate (SPI) and varying concentrations of vital gluten (VG) were investigated. The products were produced through low-moisture thermoplastic extrusion and evaluated using a Central Composite Rotational Design (CCRD) with three independent variables: VG content (0–20%), conditioning moisture (CM; 16–30%), and temperature of the third and fourth extruder zones (T3,4; 80–140 °C). A set of dependent variables was measured, including instrumental color parameters (L^* , C^* , h^*), water absorption capacity (WAC), water solubility index (WSI), compression force (CF), water hydration capacity (WHC), specific volume (SV), oil absorption capacity (OAC), protein dispersibility index (PDI), and specific mechanical energy (SME). Statistically significant effects ($p \leq 0.10$) of the independent variables were found for all responses except C^* . Maximum WAC (600.16%) was observed with 4% VG and 19% CM, while minimal CF (3.57 N) and maximal SV (11.95 mL·g⁻¹) were achieved under low VG and CM with T3,4 at 135 °C. The PDI ranged from 12.09 to 18.61%, reflecting varying degrees of protein denaturation, and SME ranged from 72.00 to 115.56 kW·kg⁻¹, increasing under conditions of lower VG, CM, and T3,4. ANOVA confirmed high predictive accuracy of the regression models ($R^2 > 0.87$; $p < 0.001$). These optimal conditions also yielded lighter products ($L^* = 58.51$) with improved water retention and fibrous texture. The interactions between SPI and VG, combined with controlled processing parameters, significantly influenced product structuring, hydration properties, and protein network formation. Thus, the partial substitution of SPI by VG under tailored extrusion conditions can be considered a promising strategy to improve the quality, functionality, and consumer appeal of plant-based meat analogues designed to emulate the texture and performance of animal-derived products.

Keywords: Protein functionality; Soy protein; Gluten vital; Sustainability.

Funding: UFVJM, ICT, CNPq, FAPEMIG, and CAPES.

RECENT TRENDS IN ALTERNATIVE PROTEINS: THE POTENTIAL OF CHICKPEA AND LENTIL AS PLANT-BASED PROTEIN SOURCES

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REVIEW: The growing demand for sustainable protein sources has driven significant advances in research on alternative proteins, particularly those of plant origin. In this context, chickpea (*Cicer arietinum*) and lentil (*Lens culinaris*) stand out as promising raw materials due to their high nutritional value, balanced protein profile, and broad technological applicability. These legumes have attracted attention not only for their functional properties but also for their potential in innovative food products such as meat analogues, plant-based formulations, and protein-enriched snacks [1–3]. This study aimed to analyze the scientific output regarding chickpea and lentil in the alternative protein field from 2020 to 2024. Searches in the ScienceDirect database retrieved 63 articles, comprising 8 reviews and 55 original research papers. Meanwhile, the Web of Science database returned 17 articles, including 1 review and 16 original research papers. Data were organized and visualized through the VOSviewer software. An increasing trend in publications was observed until 2023, with a slight decline in 2024, likely reflecting incomplete data availability for the current year. Term co-occurrence analysis revealed prominent thematic trends. Keywords such as “plant-based proteins,” “protein isolates,” and “functional properties” demonstrate a growing focus on the extraction, characterization, and application of these proteins in novel food systems. The recurrence of terms like “emulsifying activity,” “water-holding capacity,” and “foaming capacity” emphasizes techno-functional attributes essential for product stability, texture, and performance in processed formulations [2,4]. Additionally, expressions including “extrusion,” “texture,” “sensory evaluation,” and “snacks” point to advances in industrial applications, particularly extruded and ready-to-eat products, along with increased attention to sensory acceptance and consumer preferences [1,3,4]. The frequent presence of “digestibility” and “nutritional quality” underlines the emphasis on health benefits and bioavailability, reinforcing an integrated approach that values both nutritional and technological aspects [5]. In summary, chickpea and lentil have established themselves as strategic ingredients in alternative protein research, significantly contributing to the development of sustainable, technologically feasible, and nutritionally balanced food products aligned with evolving industry and consumer demands.

Keywords: protein isolates; emulsify activity; extrusion technology; nutritional quality; sensory evaluation

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Funding: The authors acknowledge the Federal University of Jequitinhonha and Mucuri Valleys and the Institute of Science and Technology for institutional support, financial assistance (#23086.001699/2022-41), and the institutional scholarship granted to L. K. J. The authors also thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for financial support (#001) and the scholarship awarded to M. A. A. (#88887.990587/2024-00). Finally, the authors acknowledge the Research Support Foundation of the State of Minas Gerais (FAPEMIG) for the scholarship granted to B. R. M. (#7640).

PLANT PROTEINS IN CHEESE ANALOGUE DEVELOPMENT: A REVIEW

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REVIEW: The growing demand for healthy, sustainable, and ethically aligned foods has driven the development of plant-based cheese analogues. These alternatives have been widely explored as substitutes for conventional dairy cheeses due to their lower carbon emissions, reduced environmental impact, and nutritional benefits. In this context, the aim of this study is to conduct a literature review on the use of plant proteins in the development of cheese analogues. Plant-based cheeses are designed to replicate the sensory attributes of dairy cheeses using non-dairy matrices, with an emphasis on plant-based protein sources. Among these, legumes such as chickpeas, lentils, and soybeans; oilseeds such as cashew nuts and almonds; and pseudocereals like quinoa stand out. Plant proteins play a crucial role in the formation of the protein matrix, being responsible for key characteristics such as texture, firmness, and water retention. However, replicating the physicochemical and sensory properties of milk caseins remains a technological challenge due to structural differences, solubility, gelling capacity, and emulsifying properties of plant proteins. Consequently, recent research has focused on optimizing formulations, improving technological processes, and applying emerging technologies; such as artificial intelligence, machine learning, and 3D printing; to overcome these limitations and enhance the functional and sensory properties of the products. Although challenges persist in reproducing complex attributes such as flavor, aroma, and texture, scientific advances have been promising. Thus, the development of plant-based cheese analogues represents not only a response to environmental demands, dietary restrictions, ethical concerns, or preferences for plant-based diets but also a promising field for technological and scientific innovation.

Keywords: dairy alternatives; plant-based cheese; vegan cheese.

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Funding: Federal University of the Jequitinhonha and Mucuri Valleys and the Institute of Science and Technology for institutional support. The Research Support Foundation of the State of Minas Gerais (FAPEMIG).

USE OF THE RESIDUE OBTAINED FROM THE EXTRACTION OF LICURI OIL (SYAGRUS CORONATA) FOR THE PREPARATION OF PLANT-BASED PRODUCTS

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ABSTRACT: In pursuit of health and environmental responsibility, the global population has been seeking a diet that includes plant-based products instead of animal-based ones. With this trend, the development of plant-based products is one of the alternatives found by the food industry. Based on this, the residue obtained from the extraction of licuri oil (*Syagrus coronata* mart. becc), a species native to the Caatinga, was evaluated for its physical-chemical composition and technological properties. The licuri residue was processed and evaluated for particle size, performing analyses of centesimal composition, color, and water and oil absorption capacity according to official methods. The licuri residue presented a high lipid content (41.64 ± 5.30), followed by carbohydrates (31.16 ± 5.34) and proteins (19.06 ± 0.04). The highest particle retention of the licuri residue remained on the 12-mesh sieve ($1.40 \mu\text{m}$). The residue also showed water absorption (5.53 ± 0.36) and oil absorption (7.07 ± 0.38). The color of the residue was closer to light, with an L value of 55.54, with a tendency toward red and yellow. The licuri residue had a higher lipid, carbohydrate, and protein content, as well as a lower moisture content, compared to flours from different types of yam studied in another study. The adequate particle distribution of the residue contributes to the consistency of the product, favoring water absorption and influencing mixing time. The ability to absorb oil and water may be associated with the high protein and carbohydrate content, as well as with protein denaturation during processing. The results obtained with the licuri residue indicate the need for other technological processes to reduce lipids and carbohydrates and concentrate proteins, so that this residue can be used as a base for the production of products analogous to animal protein. Thus, it is concluded that it is possible to develop a flour from the residue of licuri oil extraction, supporting the sustainable development of traditional communities in the semi-arid Northeast and using it in the production of plant-based products, with future prospects for the development of new products for the food sector.

Keywords: circular economy; meat analogues; plant-based proteins.

Funding: Financiadora de Estudos e Projetos (FINEP).

ALTERNATIVE SOURCES OF BRAZILIAN PLANT-BASED PROTEINS: TECHNOLOGICAL AND NUTRITIONAL POTENTIAL OF LICURI, BABAÇU, BARU, AND CATOLÉ

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ABSTRACT: The growing demand for sustainable alternative proteins has driven the search for new plant-based sources that combine nutritional value with techno-functional properties suitable for application in the food industry. Brazilian biomes are home to promising yet underexploited species, such as licuri (*Syagrus coronata*) and catolé (*Syagrus cearensis*), typical of the Caatinga, and babaçu (*Attalea speciosa*) and baru (*Dipteryx alata*), characteristic of the Cerrado. Considering the plant-based potential of these fruits, this study aimed to compare the nutritional composition and techno-functional properties of protein concentrates obtained from these oilseeds. A systematic review of articles published between 2020 and 2025 was conducted in the PubMed, SciELO, and Science Direct databases, using the descriptors "Brazilian biomes," "plant-based," and "functional foods," in Portuguese and English. Twelve articles met the inclusion criteria. Among the results, baru stands out as the oilseed with the highest protein content (24–28 g/100 g), followed by licuri, catolé, and babaçu—the latter with a protein content 64.29% lower than that of baru. In addition to quantity, protein quality was highlighted in some studies, particularly for baru and licuri, with amino acid profiles compatible with human nutrition. The techno-functional properties of the concentrates, such as solubility, emulsifying capacity, and water retention, were satisfactory for application in plant-based products. Babaçu had the highest content of lipids considered healthy, although licuri, catolé, and baru also excelled in this regard. Regarding fiber, babaçu and baru had the highest levels, followed by licuri and catolé. In the mineral fraction (Fe, Zn, and Mg), baru was the richest oilseed, followed by licuri, catolé, and babaçu. Regarding bioactive compounds, baru demonstrated high antioxidant activity; licuri, a significant presence of phenols; and babaçu, high levels of vitamin E and tocopherols. The energy value was similar among the fruits, from highest to lowest: babaçu, licuri, catolé, and baru. It is concluded that these oilseeds have complementary nutritional and functional profiles, with great potential for application in the plant-based food industry, such as supplements and meat analogues, energy bars, bakery products, and vegetable pastes. These native ingredients represent viable and sustainable alternatives for innovation in functional foods.

Keywords: dietary proteins; edible plants; food industry.

Funding: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

RHEOLOGICAL AND PHYSICOCHEMICAL CHARACTERIZATION OF SACHA INCHI (*PLUKENETIA VOLUBILIS* L.) PRESS CAKE WITH HIGH AND LOW FAT CONTENT

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ABSTRACT: This study presents a comprehensive analysis of sacha inchi (*Plukenetia volubilis* L.) press cakes with high and low fat content, obtained after the oil extraction process. Their chemical, functional, thermal, and rheological properties were evaluated to explore their potential use in protein-rich food formulations. The samples analyzed were A1 (low fat content, 0.2430g), A2 (high fat content, 0.2520g), and A3 (high fat content, 0.2474g). Using differential scanning calorimetry (DSC), differences were observed in the thermal parameters among the samples, particularly in peak temperature (T_p) and enthalpy (ΔH), indicating that fat content and type influence the material's thermal stability. Viscosimetric analysis (RVA) revealed significant differences in peak viscosity, shear resistance, and retrogradation among the cakes, with higher values in the high-fat samples (A2 and A3), suggesting greater interaction between components in the presence of fat. Microstructural analysis using scanning electron microscopy (SEM) allowed the observation of differences in morphology and particle size, as well as structural integrity and fiber presence. These results reflect how fat content modifies the cake's structure, affecting its functional properties. Additionally, pH and moisture content were evaluated, showing slightly acidic values and low moisture levels, which are favorable for product stability and preservation. Overall, the obtained data demonstrate that sacha inchi cake, especially the defatted version, is a promising source of proteins and bioactive compounds, suitable for use in functional and sustainable food products. This study highlights the importance of optimizing extraction and post-processing methods to enhance the nutritional and functional value of oily seed by-products, contributing to the development of innovative and healthy food ingredients.

Keywords: Sacha inchi, press cake, thermal characterization, rheological properties, alternative proteins.

PROSPECTIVE USE OF FAVA D'ANTA (*DIMORPHANDRA MOLLIS BENTH.*) FRACTIONS ON PLANT-BASED FOOD FORMULATIONS

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ABSTRACT: Fava d'anta (*Dimorphandra mollis* Benth.), a native little explored in terms of its nutritional composition, particularly regarding protein content across its different parts. This work presents an unprecedented study of the centesimal composition, lipid profile, and bioactive compounds extracted from the husk, seed, and berry fractions of the fruit. The samples were dried, crushed, and subjected to ethanolic extraction using a Soxhlet apparatus. The analyses were performed in triplicate and the experimental data were presented as mean $\pm$ standard deviation. The differences among samples were evaluated by analysis of variance (ANOVA), with a significance level of $p < 0.05$. The results of the centesimal composition showed that the seed stood out for its protein content, 19.28 ± 2.85 %, significantly higher ($p < 0.05$) than the other parts. The protein content of seeds is similar to other legumes such as chickpea and soy, which evidences its potential as a plant protein source. Furthermore, the seed also presented the highest lipid content (3.03 ± 0.95 %) and lowest fiber content (18.35 ± 3.08 %), unlike the husk, which was the fraction richest in fiber (20.94 ± 1.66 %). The berry had the highest carbohydrate (63.97 ± 1.13 %) and moisture ($11.60\% \pm 0.07$ %) content. Analysis of the lipid profile revealed four main fatty acids, being palmitic acid (12.92 ± 0.01 %) and oleic acid ($8.94\% \pm 0.04$ %) the predominant, both with concentrations higher in the seed. For the bioactive compounds, the berry presented the highest concentrations of phenolics, flavonoids, and flavonols, 65.82 ± 0.30 mg GAE/g, 74.62 ± 2.20 mg QUE/g, and 63.34 ± 2.59 mg QUE/g, respectively. On the other hand, in the evaluation of antioxidant activity using the FRAP method, the husk stood out, presenting 937 ± 1.69 μ mol ET/g, possibly due to the phenolic compounds with greater reducing capacity and the high fiber content present in the peel. Therefore, this characterization of the parts of fava d'anta indicates that this underused resource can be completely valorized to develop ingredients for the food industry. The seed stands out as a source of protein, lipids, and bioactive compounds with potential applications in plant-based food formulations.

Keywords: nutritional composition; extraction; plant-based protein.

Funding: CAPES.

IMPACTS OF DRYING METHODS ON THE BIOACTIVITY AND DIGESTIBILITY OF TRUB WASTE PROTEIN

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ABSTRACT: Trub is a by-product of beer production and is mainly composed of residual malt grains, proteins, and polysaccharides that precipitate during wort boiling and clarification. Traditionally discarded, trub has recently attracted interest as an alternative source of bioactive compounds, particularly proteins, with high nutritional and functional value. In this context, the aim of this study was to evaluate the effects of convective drying and freeze-drying on the bioactivity and digestibility of trub. For this purpose, trub obtained from Pilsner beer processing was homogenized with distilled water for 20 min at 25 °C and then centrifuged. The solid fraction was dried by convection (50 °C for 12 h) or freeze-dried (−55 °C under vacuum for 48 h). Digestibility was assessed using an in vitro gastrointestinal digestion model with pepsin and pancreatin enzymes. The antioxidant capacity was determined using spectrophotometric methods based on ABTS and DPPH radical scavenging assays. Compared with the freeze-dried sample, convection-dried trub showed a reduction in antioxidant activity of 5.3% for ABTS and 15.7% for DPPH. Regarding digestibility, the freeze-dried sample showed an 8.7% increase compared that with of the convection-dried sample. These differences may be related to the denaturation of peptides and other bioactive compounds due to prolonged heat exposure. Moreover, freeze-drying may open the structure of the material, increase the surface area, and enhance the availability of compounds for enzymatic digestion and/or antioxidant activity. Therefore, freeze-drying appears to be the most suitable method to preserve the bioactivity and protein digestibility of trub.

Keywords: brewing waste; sustainable protein; upcycling; nutrition.

Funding: CAPES and CNPq.

INFLUENCE OF TIME ON ENZYMATIC HYDROLYSIS OF BREWING PROTEIN RESIDUE (TRUB)

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ABSTRACT: Often discarded food processing residues can serve as an excellent source of protein. Hot trub, a solid material formed during the boiling of brewer's wort, may contain up to 70% protein. However, this protein fraction has low solubility, which limits its techno- functional properties, bioactivity, and bioavailability. Under optimal conditions, enzymatic hydrolysis using proteolytic enzymes has emerged as a sustainable and effective strategy to enhance and modify the functional properties of protein-rich materials. The aim of this study was to evaluate the degree of hydrolysis (DH) of trub proteins over time using an enzymatic cocktail composed of Alcalase, Flavourzyme, and Protamex. For this purpose, trub obtained from Pilsner beer production was freeze-dried prior to hydrolysis. Enzymatic hydrolysis was carried out at 55 °C in a solution containing 5% protein (w/v) and 2% enzyme mixture (g enzyme/100 g protein), with equal parts of the three enzymes. The pH was maintained at 8.0 through the addition of NaOH. Aliquots were collected at 60, 120, and 180 min and analyzed for DH using the Lowry method. The DH values were 49.5% $\pm$ 2.5, 69.9% $\pm$ 3.6, and 69.3% $\pm$ 1.45 at the respective time points. This pattern suggests intense enzymatic activity within the first 120 min, which effectively cleaves peptide bonds in trub proteins. After this period, DH plateaued, likely due to enzyme site saturation, reduced substrate availability, or possible inhibition by hydrolysis products (e.g., peptides and free amino acids). Therefore, these findings indicate that in a complex enzymatic system for trub hydrolysis, 120 min is sufficient to achieve an efficient degree of protein hydrolysis.

Keywords: waste recovery; degree of hydrolysis; peptides; sustainable process.

Funding: CAPES, CNPq, and FAPERGS.

WHAT ARE THE PROTEIN SOURCES IN PLANT-BASED MEAT ANALOGUES SOLD IN BRAZIL?

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ABSTRACT: In recent years, the market has seen a rise in plant-based products, which aim to replace animal products, particularly with the goal of benefiting both planetary health and public well-being. Additionally, various innovative methods are being developed to better meet consumer expectations. The aim of this study was to investigate which proteins are used in the manufacture of plant-based meat substitutes sold on the Brazilian market. The labels were collected nationally by photographing all sides of the label. The products were entered into the Brazilian Food Label Database, using the barcode as an identifier. The list of ingredients was used to determine the main protein source. The information on the labels of 339 plant-based meat analogues was analyzed. Soy was the main source of protein in the foods (51.3%, n=174), followed by peas (16.5%, n=56). However, almost 12% of the foods had no protein source. The other proteins found were chickpeas, beans, lentils and protein blends (soy, peas and chickpeas). Soy is the main ingredient used in Brazil and around the world to make plant-based meat analogues. It was also noted that textured protein was the most used, both in soy and peas, followed by protein in grains. Manufacturers can use proteins in the form of flour, grains, concentrates, isolates or texture proteins. However, the choice of ingredients has important technological functions, such as the use of textured protein, which aims to mimic the fibrillar structure of meat. Thus, the food industry faces challenges in obtaining products that are similar to meats in terms of texture, color, flavor and nutritional value, as well as looking for alternatives with regional ingredients that are affordable for the population. It can therefore be concluded that plant-based foods marketed in Brazil are made with soy, especially textured soy protein.

Keywords: Plant-based; meat analogues; source protein; label.

Funding: The Good Food Institute Brasil.

VALORIZATION OF SOY PROTEIN CONCENTRATION BY-PRODUCTS VIA ENZYMATIC HYDROLYSIS

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ABSTRACT: The search for alternative sources of plant-based peptides and amino acids, without compromising the human and animal food chains, justifies the investigation of industrial by-products for higher value-added applications. Soy protein, widely used in food production, generates a solid by-product during its concentration process, which retains a high protein content and can be exploited as a substrate for enzymatic hydrolysis aimed at producing hydrolysates suitable for cosmetic formulations. In this context, the present study aimed to investigate the enzymatic hydrolysis process of the solid by-product generated during soy protein concentration and to characterize the resulting amino acid profile. The by-product was homogenized as a proportional blend, presenting an initial protein content of $44.33\% \pm 0.74$. The hydrolysis process was conducted using a substrate-to-distilled water ratio of 1:10 (w/v), with the addition of 2% Alcalase enzyme (w/w relative to the substrate), pH adjusted to 8.0 with 20% sodium hydroxide solution, under orbital agitation (180 rpm) at 45 °C for 1 hour. After hydrolysis, the material was filtered using cloth filters, centrifuged, and the resulting liquid was dried in an oven for yield determination and subsequent amino acid profile analysis by high-performance liquid chromatography (HPLC). A parallel control trial was conducted without enzyme addition for comparative purposes. The protein content of the hydrolysate obtained after enzymatic treatment was $65.65\% \pm 0.37$, indicating significant protein concentration in the final product. Process yield reached $49.23\% \pm 0.01$ in the enzyme-assisted trial, compared to $9.06\% \pm 0.01$ in the control, confirming the efficiency of Alcalase in the substrate hydrolysis. The total amino acid profile showed a predominance of glutamic acid (16.03%), aspartic acid (10.21%), leucine (6.35%), arginine (5.98%), and lysine (5.49%). Regarding free amino acids, leucine (0.85%), phenylalanine (0.66%), arginine (0.55%), and lysine (0.30%) were identified. The results demonstrate the technical potential of the soy protein concentration by-product as a raw material for the production of protein hydrolysates suitable for cosmetic formulations, particularly as a source of peptides and free amino acids. The technological feasibility of the process and the functional profile of the obtained fractions support their applicability as bioactive ingredients in the cosmetic industry.

Keywords: Alcalase; Protein hydrolysate; Amino acid.

Funding: The authors thanks URI Erechim and FAPERGS (Grant number 24/2551-0002182-5) for their support.

EFFECT OF DEFATTING ON THE DETERMINATION OF PROTEIN CONTENT IN BAMBOO LEAVES

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ABSTRACT: Proteins play a crucial role in the nutritional value and functionality of foods. To ensure reliable measurement of protein content in food matrices, laboratory analyses must be precise and accurate. In this context, steps such as defatting are often employed to remove lipids, thereby improving the efficiency of protein extraction and quantification. However, this process may interfere with protein content, especially in complex plant matrices. This study aimed to evaluate the impact of defatting on the determination of protein content in bamboo leaves using the Kjeldahl method, to assess potential losses associated with the process compared to the control sample (without defatting). Bamboo leaves from *Dendrocalamus asper* species were collected, transported, selected, and sanitized using a 1% (v/v) sodium hypochlorite solution. Subsequently, the leaves were dried in a forced-air oven at 60 °C for 12 hours, ground using a knife mill, sieved through a 45-mesh screen (350 µm) to standardize particle size, and stored at -18 °C. For the preparation of the defatted leaf sample, the dried and ground material was subjected to agitation for 1 hour with a chloroform-methanol mixture (2:1, v/v) at a ratio of 1:10 (w/v; leaf/solvent), followed by centrifugation at $2,422 \times g$ for 5 minutes. The solid residue was dried in a forced-air oven at 40 °C, and the protein content was determined using the Kjeldahl method using a nitrogen-to-protein conversion factor of 6.25. Upon analysis of the results, it was observed, through the *t*-test ($p < 0.05$), that the protein content found for the control sample, without defatting, showed no statistically significant difference ($18.68 \pm 0.17\%$) when compared to the result observed for the defatted bamboo leaves ($19.03 \pm 0.18\%$). It can be concluded, therefore, that the defatting process does not compromise the determination of protein content in bamboo leaves using the Kjeldahl method.

Keywords: protein content, Kjeldahl, defatting.

Funding: CNPq/MCTI/FNDCT N° 22/2024 (Process: 444890/2024-6), CNPq/PQ2 (Process: 307668/2022-3 and 308602/2022-6), CNPq/PIBIC and Coordination for the Improvement of Higher Education Personnel (CAPES; Finance Code 001).

ANALYSIS OF SOLVENT EFFICIENCY IN LIPID REMOVAL AS A PRELIMINARY STEP IN BAMBOO LEAF PROTEIN EXTRACTION

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ABSTRACT: Demand for plant-based proteins is steadily rising worldwide, driven by changing dietary habits, health awareness, and sustainability concerns. This study proposes bamboo leaves as a potential new protein source (20% protein d.b.), given their abundance in Brazil and sustainable characteristics. Since protein extraction requires upstream lipid removal, the objective of this work was to compare the effectiveness of hexane and the chloroform:methanol mixture (2:1, v/v) to defat bamboo leaves (*Dendrocalamus asper*). Bamboo leaves were collected, sanitized with 1% sodium hypochlorite, and dried at 60°C in a forced-air oven for 12 hours. Subsequently, they were ground using a knife mill and sieved with a 45-mesh screen, resulting in an average particle diameter of less than 350 µm. Extractions were carried out separately for each solvent, consisting of agitation in a homogenizer for 1 hour in 50 mL polypropylene centrifuge tubes containing (i) 3 g of leaf in 30 mL of hexane and (ii) 2 g of leaf in 20 mL of chloroform:methanol. The tubes were then centrifuged at $2,422 \times g$ for 5 minutes. To evaluate the efficiency of the processes, lipid content was determined using the Bligh and Dyer method, aiming for levels below 3%. According to the method used, a single wash with the chloroform:methanol mixture is effective in removing the lipids (residual lipid of $2.60 \pm 0.14\%$). In contrast, three hexane washes resulted in a value that did not meet the established parameters ($5.52 \pm 0.19\%$ of residual lipids). Based on this, it was found that the chloroform:methanol mixture had greater lipid removal efficiency, with statistically significant difference ($p < 0.05$), as determined by the t-test. Thus, it is concluded that for lipid removal from bamboo leaves, the use of the chloroform:methanol mixture is more effective and more suitable for subsequent protein extraction.

Keywords: *Dendrocalamus asper*; defatting; Bligh and Dyer.

Funding: CNPq/MCTI/FNDCT Nº 22/2024 (Process: 444890/2024-6), CNPq/PQ2 (Process: 307668/2022-3 e 308602/2022-6), CNPq/PIBIC and Coordination for the Improvement of Higher Education Personnel (CAPES; Finance Code 001).

RHEOLOGICAL AND TEXTURAL CHARACTERIZATION OF 3D INK AND PRINTED CHEESE ANALOGUES FORMULATED WITH RICE AND PEA PROTEINS

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ABSTRACT: The vegan diet excludes all types of animal-derived products, while the vegetarian diet eliminates meat but may include eggs, honey, and dairy products. In contrast, the plant-based diet prioritizes plant-derived foods, with a total or partial exclusion of animal products. Other variations, such as flexitarian and pescatarian diets, are also recognized. In these dietary patterns, proteins from cereals and legumes are crucial for ensuring adequate protein intake. However, the development of foods that replace animal proteins with plant-based proteins remains a challenge, highlighting 3D food printing as an innovative technology capable of creating customized edible structures, allowing precise control over texture and nutritional profiles. This study aimed to evaluate the rheological and technological properties of 3D printing inks for cheese analogues (CAs), as well as the technological properties of the final product. The CAs were formulated with purple corn flour (rich in anthocyanins), xanthan gum, freeze-dried nopal mucilage, sodium chloride, and fat, using either rice protein (RP) or pea protein (PP) as the protein source. The results were subjected to multivariate analysis ($p < 0.05$). The inks were found to be similar in terms of cohesiveness, viscosity index, and consistency index (k). However, the inks containing RP were firmer, lighter, and yellower. The CAs made with PP exhibited higher values in texture profile analysis when compared to those with RP. On the other hand, no significant difference was observed in the water activity of the samples. Multivariate analysis indicated that the texture parameters of the CAs showed variable importance in projection (> 1.0) values higher than those of instrumental color and rheological properties. Principal component analysis revealed functional and structural differences between PP and RP, with PP being distinguished by its gelling and textural properties. At the same time, RP exhibited a more consistent texture profile and greater lightness. These findings were confirmed through heatmap analysis and Pearson correlation. PP demonstrated a greater gelling capacity than RP, resulting in inks with superior printability characteristics. These distinctions are crucial for selecting the most suitable plant protein for food formulations, depending on the desired sensory and functional attributes of the final product.

Keywords: Vegetable proteins; Food printing; Texture; Ink rheology.

Funding: UFVJM, ICT, CNPq, FAPEMIG, EPAMIG and CAPES

EFFECT OF pH SHIFTING ON THE MODIFICATION OF PEA PROTEIN ISOLATE FOAMING PROPERTIES UNDER ACIDIC OR ALKALINE CONDITIONS

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REVIEW: Projections made by the UN indicate that the global population will reach 9.8 billion people by 2050. This scenario presents challenges related to animal welfare, disease spread, ecological footprint, and risks of food insecurity, all exacerbated by conventional production systems that rely heavily on the intensive use of natural resources. In this context, plant-based proteins have been studied as more sustainable and nutritionally viable alternatives compared to conventional sources. Among them, pea stands out due to its balanced nutritional quality, lower water and land requirements, reduced greenhouse gas emissions, and lower allergenic potential compared to soy. Furthermore, its broad availability in different parts of the world supports its application as an alternative protein source. However, there are limitations related to taste and processing methods, the second compromising solubility and, consequently, techno-functional performance. To overcome these limitations, non-thermal technologies involving pH shifting have been explored to promote structural modifications capable of improving performance of this ingredient. This technology consists of exposing the protein to extreme pH conditions, either acidic or alkaline, followed by a return to neutral pH. In this context, the current study aimed to evaluate the effects of acidic (pH = 2) and alkaline (pH = 12) pH shifting on the foaming properties of commercial pea protein isolate (PPI), in its native, modified, and intermediate forms during the modification process (pH: 2, 2–7, 12, 12–7, and native). Foam formation and stability, microstructure, turbidity, and Zeta potential were assessed. The results demonstrated that alkaline pH shifting significantly increased ($p < 0.05$) foam formation ($62.0 \pm 2.82\%$), the absolute value of the Zeta potential, and reduced turbidity, indicating greater stability. On the other hand, the acid treatment showed inferior performance, altering the size and uniformity of the bubbles after foam formation. These findings highlight that alkaline pH shifting can be used as an effective strategy for modifying PPI, with the potential to enhance its foaming properties and suitability for industrial applications.

Keywords: alternative proteins; vegetable protein; structural modification; foam

Funding: We gratefully acknowledge the Brazilian funding agencies CNPq, Fapemig and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, for the financial support.

GLOBAL TRENDS IN FOOD PRINTING: A BIBLIOMETRIC ANALYSIS ON THE USE OF PEA PROTEINS (*Pisum sativum* L.)

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REVIEW: Three-dimensional (3D) printing, also known as additive manufacturing, is one of the key technologies driving the Fourth Industrial Revolution^{1,2}. Its ability to design customized structures has propelled the productive sector, expanding the portfolio of applications within the food industry^{3,4}. In this context, the development of edible inks has been explored to achieve specific characteristics, including high fluidity during the extrusion process and the recovery of viscosity and elasticity after printing^{5,6}. To address this challenge, various ingredients have been evaluated as the basis for food matrices, including pea-based materials. This plant source is highly relevant due to its sustainability, nutritional quality, accessibility, lower allergenicity, and technological properties suitable for the production of food gels and emulsions^{3,7,8,9}. Therefore, this study presents a bibliometric analysis of the advances and trends in 3D food printing based on pea proteins. The study was conducted in three phases: (1) search, (2) selection, and (3) data analysis. A total of 45 scientific articles were gathered from the SCOPUS database, published between 2014 and 2024, using Boolean operators. Articles were filtered based on title relevance to the topic and the information provided in the abstracts. The analysis of the knowledge network among authors, countries, citations per document, and keywords was performed using R software 4.4.1, VOSviewer 1.6.20.0, and Microsoft Office Excel 2016. The results show that the international knowledge network is primarily composed of China, the United States, and Canada. In this scenario, 23 out of 73 authors form a collaborative network connected by a central author. Among the topics covered, the effects on rheological, textural, and structural properties are recurrent^{10,11,12}. Non-thermal technologies, such as ultrasound, pH shifting, enzymatic treatments, and plasma, have contributed to the improvement of these properties^{4,6,8,13}. Additionally, high internal phase emulsions and Pickering emulsion gels combined with fruit-derived polysaccharides, such as banana and mango paste and flaxseed gum, stand out for the development of products with improved texture and nutritional value, particularly for groups such as infants, the elderly, and individuals with dysphagia^{1,2,4,12,14,15}. Despite the advances in the development of complex materials, consumer sensory perception remains underexplored.

Keywords: 3D food printing; alternative protein; personalized nutrition.

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Funding: We gratefully acknowledge the Brazilian funding agencies CNPq, Fapemig and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, for the financial support.

BLACK BEAN PROTEIN AS EMULSION STABILIZER FOR FOOD APPLICATIONS

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ABSTRACT: Emulsions are thermodynamically unstable systems commonly applied in the food industry. To improve its stability, emulsifying agents are used to prevent phase separation, flocculation, and coalescence of droplets. These agents act at the interface, reducing interfacial tension and forming a protective layer around the droplet. Currently, there are different types of emulsifiers, but from a sustainability perspective, plant-based proteins are excellent alternatives as they are safe, biodegradable, and biocompatible. Plant proteins can be extracted from a variety of sources. Among these sources, black beans are interesting due to their high protein content (18-26%) in their matrix compared to other vegetable sources. Thus, the aim of the present study was to use black bean protein as an emulsifying agent to stabilize emulsions for food applications. The black beans protein was extracted using an alkaline solution (pH 9.5), followed by precipitation at the isoelectric point (pH 4.1). The protein concentrate presented 69.9% protein, 5.9% moisture, 7.7% ash, 4.4% lipids and 15.0% total carbohydrates. For emulsion preparation, different concentrations of protein concentrate (1–5% w/w) and chia oil (30–50% w/w) were tested. The emulsions were characterized in terms of phase separation index, droplet size distribution, emulsion morphology, and rheology. The phase separation index ranged from 7–35%, showing greater stability for the emulsion with 5% protein. The droplet sizes ranged between 2.4 μm and 7.1 μm , with smaller droplets observed in emulsions with higher protein concentrations. Additionally, it was observed that higher concentrations of protein and oil led to an increased consistency index (k), and consequently, higher emulsion viscosity. It was found that increasing protein concentration contributed to the formation of stable droplets. In addition, the increased viscosity of the system reduced droplet mobility and delayed phase separation processes. Therefore, black bean protein may be a promising emulsifying agent alternative to improve emulsion stability.

Keywords: vegetable protein; emulsifier; black beans.

Funding: I would like to thank the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES), the São Paulo Research Foundation (FAPESP) and the National Council for Scientific and Technological Development (CNPq) for their support and financing of the project.

COWPEA PROTEIN FRACTIONS ATTRIBUTES OBTAINED USING DRY FRACTIONATION AND HYBRID PROCESSING

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ABSTRACT: Cowpea (*Vigna unguiculata*) protein has received increasing attention due to its high nutritional value and potential as an ingredient for plant-based formulations. This study aimed to evaluate the techno-functional properties of cowpea protein isolate (CPI) recovered by a hybrid process combining dry fractionation by air classification and conventional aqueous extraction, in comparison with cowpea protein concentrate (CPC) obtained exclusively by dry processing. The protein fractions were characterized in terms of solubility, emulsifying and foaming properties, oil and water-holding capacities, and gelation ability. For CPI production, the CPC was dispersed in alkaline water (pH 9.0) at a solid-to-liquid ratio of 1:10 (w/v), stirred for 1 h at 50 °C, and the extract was acidified to pH 4.5 to induce isoelectric precipitation (pI). The precipitated proteins were recovered by centrifugation, neutralization, and freeze-drying. Both CPC and CPI exhibited minimal solubility (<2 %) in the pH range of 4.0-5.0, corresponding to their pI, with solubility increasing markedly under alkaline conditions, reaching approximately 90 % at pH 9.0. CPI demonstrated a two-fold increase in emulsifying activity and oil-holding capacity compared to CPC, likely due to the enhanced solubility and increased exposure of hydrophobic regions, facilitating oil entrapment and interfacial stabilization. Similarly, CPI exhibited higher water absorption ($1.8 \text{ g} \cdot \text{g}^{-1}$ vs. $1.2 \text{ g} \cdot \text{g}^{-1}$), attributed to the exposure of polar side chains following the disruption of weak interactions during isolation. The foaming capacity was greater in CPI (70.0 %) than in CPC (53.3 %), likely due to proteins unfolding with increased molecular flexibility. Despite the CPC showing higher initial foam stability (85 % at 10 min), its stability decreased more sharply over time, while CPI maintained higher foam retention after 1 h (52.5 % vs. 47.0 %), suggesting the formation of more resilient interfacial films. Both fractions exhibited the same critical gelation concentration ($0.1 \text{ g} \cdot \text{mL}^{-1}$), indicating comparable gel-forming abilities. In conclusion, CPI exhibited enhanced functional properties due to structural modifications introduced by the isolation process, while CPC obtained via a simpler and scalable solvent-free route, displayed relevant functionality and can serve as a suitable ingredient for specific applications such as baked products, aerated systems, and meat analogues.

Keywords: Black-eyed pea; plant-based protein; air classification; hybrid extraction; functionality.

Funding: This study was supported by the GFI Brazil (The Good Food Institute Brazil).

IMPACT OF DEHULLING ON PROTEIN AND PROTEIN-RELATED ANTINUTRITIONAL FACTORS OF BRAZILIAN COMMON BEAN (*Phaseolus vulgaris* L.) FLOURS

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ABSTRACT: Common beans (*Phaseolus vulgaris* L.) are a vital source of dietary protein, particularly in underdeveloped countries with limited access to animal protein. However, whole bean meals contain antinutritional factors (ANFs), concentrated mainly in husks, such as phenolics, tannins, phytates, saponins, and trypsin inhibitors. ANFs can impair nutrient absorption and cause undesirable effects like bitterness, astringency, and flatulence. This study evaluated the effectiveness of soaking and dehulling in reducing ANFs in flours from three common bean varieties, namely black bean (BB), carioca bean (CB), and white kidney bean (WB). Whole beans were soaked in distilled water for 1 h (1:4 w/v), hand-dehulled, dried (55 °C, overnight), milled, and sieved (20 mesh) into whole bean, cotyledon, and husk flours. Phenolic compounds, tannins, phytic acid, trypsin inhibitor activity (TIA), and total saponins were quantified and expressed as mg·g⁻¹ dry matter. Despite the higher final protein content of dehulled WB (24.6%), BB and CB protein contents (22.5% and 23.4%, respectively) increased by 15% in dehulled flours, a two-fold increase compared to WB. Dehulling impaired the highest reductions in phenolic compounds and tannins in CB (↓92.6% phenolics), and BB (↓70.2% tannins), with WB showing lower concentrations than colored grains. The phytic acid content in whole beans (15.25-24.7 mg·g⁻¹) was reduced after dehulling by 42.8% (BB), 23.8% (WB), and 17% (CB), which is attributed to the high concentration of phytic acid in husks (19.9-24.3 mg·g⁻¹). Regarding saponins, only dehulled BB content was reduced (↓52.6%), since the husks concentrated more saponins (20.4 mg·g⁻¹) than the grains. Conversely, CB (5.17 mg·g⁻¹) and WB (3.97 mg·g⁻¹) saponin contents increased in dehulled flours. As expected, TIA was higher in dehulled flours (0.023-0.028 mg·g⁻¹) as this protein ANF interacts with protein and fiber in the cotyledons, and requires further thermal treatment for inactivation. Soaking and dehulling were effective low-cost strategies for reducing ANFs in common bean flours, especially in pigmented varieties such as black beans. This simple treatment can be applied in processing facilities to yield flours with higher protein content and reduced ANF load, offering a base for the production of protein concentrates.

Keywords: bean protein; phenolic compounds; phytic acid; trypsin inhibitor activity; saponins.

Funding: This study was supported by the GFI Brazil (The Good Food Institute Brazil).

NUTRITIONAL ASPECTS OF COWPEA PROTEIN INGREDIENTS OBTAINED BY AIR CLASSIFICATION AND HYBRID PROCESS

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ABSTRACT: Cowpea (*Vigna unguiculata*) is a protein-rich pulse crop of global importance, with potential for diversifying the alternative protein market. Dry fractionation is a solvent-free and potentially less costly alternative to wet fractionation for obtaining protein-rich ingredients. However, for the development of high-quality cowpea-based ingredients, it is important to characterize the nutrient composition and quantify the antinutritional factors (ANFs), which may affect protein digestibility and nutritional quality. This study evaluated the main nutritional aspects of a cowpea protein concentrate (CPC) obtained by air classification, and a protein isolate (CPI) resulting from hybrid processing (dry fractionation followed by alkaline extraction and isoelectric precipitation of the protein). The proximate composition, concentration of phytic acid, trypsin inhibitor activity (TIA), and total tannins were evaluated in both protein products. In addition, the nutritional aspects related to *in vitro* protein digestibility (IVPD) and the amino acid (AA) profile were comparatively assessed between CPC and CPI. CPC and CPI exhibited high protein contents of 52% and 83%, respectively, with a slightly higher IVPD for CPI (85.8%) compared to CPC (82.5%). Both products presented a similar AA profile, mainly consisting of glutamic acid and aspartic acid (non-essential AAs), and leucine, lysine, valine, and isoleucine as the main essential AAs. The AA profile aligns with the FAO/WHO nutrition standards, except for sulfur-containing AAs methionine and cysteine, which are limiting in cowpea. Still, the corrected IVPD by the AA score was 66% (CPC) and 70% (CPI), surpassing those reported for lentil protein concentrate, fava bean flour, and pea flour. The ANFs naturally present in pulses can bind to protein, interfering with protein digestion and decreasing its nutritional value. Soaking CPC in water for 16 h significantly ($p < 0.05$) reduced the contents of phytates (70%), tannins (49%), and TIA (11%) compared to untreated CPC. On the other hand, untreated CPI had a 1.14 times higher tannin content than raw CPC, which is attributed to the tannins- protein complexation. Cowpea protein concentrates obtained through dry fractionation by air classification exhibit a valuable nutritional profile comparable to that of protein isolates obtained by sequential wet-based processes, representing a promising route for obtaining cowpea protein ingredients.

Keywords: *Vigna unguiculata*; dry fractionation; antinutritional factors; essential amino acids; protein digestibility.

Funding: This study was supported by the GFI Brazil (The Good Food Institute Brazil).

INFLUENCE OF ULTRASOUND AND SOLVENT EXTRACTION ON OFF- FLAVOR REDUCTION AND TECHNO-FUNCTIONAL PROPERTIES OF COWPEA PROTEIN

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ABSTRACT: The growing interest in alternative plant-based protein sources has stimulated the valorization of pulses such as cowpea (*Vigna unguiculata*). However, one of the main challenges in using pulse proteins in food formulations is the presence of volatile compounds associated with off- flavors. Therefore, this study aimed to evaluate the effect of different processing treatments, focusing on mitigating off-flavors and improving techno-functional properties of cowpea concentrated protein (55%). Two types of treatment were investigated: (i) solvent extraction, using distilled water, 40% ethanol, and 80% ethanol 1:5 (w/v), at 30 °C for 1 h; and (ii) ultrasound processing at two power levels (165 W and 330 W), using the same solvents and conditions. The volatile compounds were extracted by solid-phase micro-extraction (SPME) and analyzed by gas chromatography coupled with mass spectrometry (GC-MS). Techno-functional properties such as solubility at pH 7, water holding capacity (WHC), oil holding capacity (OHC), emulsification activity index (EAI), foam capacity (FC), and foam stability (FS) were evaluated. In general, hydroalcoholic solvents were more efficient in reducing off-flavor compounds. The 40% ethanol treatment stood out as the most effective, achieving expressive reductions in major off-flavor compound classes with minimal formation of undesirable new volatiles. Conversely, washing with distilled water proved less effective and, in some cases, even contributed to the formation of aldehydes and ketones with negative odor potential. Ultrasound treatment, particularly at high power (330 W), yielded more complex results: although it promoted the removal of some volatiles, it also led to the formation of new undesirable compounds such as acetoin, terpenes, and hydrocarbons. The ultrasound treatment at 165 W using 80% ethanol as the solvent presented the highest values of solubility (82%) and EAI (10 m²/g), while using water as the solvent showed the highest values of FC (100%), FS (75%), WHC (1,90 g/g) and OHC (2.72 g/g). In conclusion, 40% ethanol washing without ultrasound proved to be the most effective and consistent treatment for mitigating off-flavors, while the ultrasound processing resulted in the best techno-functional properties. The findings highlighted the importance of carefully selecting process conditions depending on the desired quality and application of the final product.

Keywords: pulse protein; volatile compounds; protein functionalization; ultrasound; solvent washing.

DUCKWEED: A PROMISING SOURCE OF PROTEIN

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ABSTRACT: Duckweed is a free-floating aquatic plant capable of reaching up to 45% protein in its dry matter. It also contributes to water purification and atmospheric CO₂ fixation. These characteristics, along with its high growth rate, make duckweed a promising alternative protein source. However, studies on protein extraction from duckweeds are limited, and none have focused on *Landoltia punctata*. Therefore, this study aimed to evaluate the protein concentrate obtained from this species using a solubilization process followed by isoelectric precipitation. Protein extraction from duckweed biomass was conducted by solubilization in deionized water (1:25 w/v) at pH 6.5 under mechanical stirring for 1 h at room temperature. Proteins were precipitated at pH 4.0 (isoelectric point), followed by centrifugation, neutralization, and freeze-drying. The protein content and the foaming and emulsifying properties were evaluated to characterize the functional properties of the extract. The resulting protein concentrate exhibited a protein content of 47.4 ± 1.5 % (dry basis), corresponding to a protein yield of 6.1 %, based on an initial crude protein content of 27.6 ± 0.2 %. Foaming capacity and foam stability were 133.3 % and 46.4 ± 5.0 %, respectively. Emulsifying activity and stability indices were 8.3 ± 0.1 m².g⁻¹ and 18.1 ± 0.4 min, respectively. Notably, the 71.8 % increase in protein concentration surpassed the 61.3 % previously reported for *Lemna gibba* under similar extraction conditions, despite its higher crude protein content. Although the foaming properties were lower than those achieved in other duckweed studies, the mild extraction conditions employed may have contributed to preserving the native protein structure. The emulsifying activity index, however, remained below the threshold values typically observed for commercial pea protein. Nevertheless, the protein concentrate obtained from *L. punctata* may offer a promising and sustainable protein source for food and feed systems. These findings contribute to the growing body of research supporting duckweed as a viable and sustainable source of plant-based proteins for future food systems.

Keywords: plant-based ingredients; protein recovery; leaf protein concentrate; functional properties.

Funding: This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Proc. 153229/2024-1) and Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC, Proc. 2024TR002188).

PLANT-BASED TASTE PEPTIDES: ADVANCES IN SCREENING, PURIFICATION, AND DEBITTERING STRATEGIES

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REVIEW: Taste peptides (umami, salty, sweet, bitter, kokumi) are oligopeptides that intensify the taste of food. Animal sources are widely studied, vegetables, edible mushrooms and yeast extracts are alternative sources [1]. The growth of the plant-based market is driving the screening of taste peptides from plant sources (e.g., soy, pea, gluten, peanuts). A significant challenge in using plant sources to obtain taste peptides is the presence of off-flavors associated with the formation of hydrophobic peptides. The aim of this review is to address the literature on the screening of taste peptides from plant sources, with a survey of downstream technologies and debittering strategies explored recently (2020-2025). Screening for umami peptides is more widely explored and has established steps. Enzymatic hydrolysis and fermentation are recurrent extraction processes. Enzymatic processes (γ -glutamylolation and cross-linking), fermentation and the Maillard reaction have been applied as strategies to attenuate bitter peptides [2, 3]. Membrane technologies, gel filtration chromatography and others are generally applied as purification methods. Sensory panels and the increasing use of electronic tongues guide the choice of fractions with greater umami intensity during purification, which are then subjected to methods for identifying the peptide sequences present. Bioinformatics tools, such as machine learning predictors and molecular docking, are widely used in the virtual screening of umami peptides. Selected peptides can be synthesized for flavor confirmation by sensory analysis. Despite the widespread exploration of umami peptides, salty and kokumi peptides have been gaining increasing attention due to the growing interest in these flavors [4]. Virtual screening of salty and kokumi peptides is still limited, while studies on sweet peptides are scarce. The screening of plant taste peptides is advancing with the support of bioinformatics tools, purification technologies and bitterness reduction. Umami peptides are the most studied, while salty, kokumi and sweet still require investigation. Overcoming bitterness and broadening predictors of other flavors are essential for applying these peptides as sensory modulators.

Keywords: Taste peptides; Plant sources; Debittering; Virtual Screening.

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FUNCTIONAL POTENTIAL OF ORA-PRO-NÓBIS (*Pereskia aculeata*) GEL AS A PLANT-BASED PROTEIN SOURCE FOR MEAT ANALOGUES

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ABSTRACT: This study investigates the potential of ora-pro-nóbis (*Pereskia aculeata*) as a plant-based protein source with functional properties suitable for application in food technology, particularly in the development of meat analogues. Due to the increasing demand for sustainable and nutritious alternatives to animal proteins, non-conventional edible plants such as ora-pro-nóbis have gained attention for their high protein content and versatility in processing. The objective of this research was to quantify and compare the protein content in both the raw leaves and a gel obtained from them, assessing the feasibility of using this gel as a functional food ingredient. The ora-pro-nóbis leaves were collected from a plant cultivated in Rio Grande city, located in the south of Rio Grande do Sul state (Brazil). The gel was prepared by hot aqueous extraction, where crushed leaves were mixed with water at a 1:2 ratio (w/w) and heated to 50 °C, simulating a mild processing method that preserves bioactive compounds. Protein content in both samples was determined using the official Kjeldahl method, which quantifies total nitrogen and converts it into protein values. The fresh leaves presented a protein content of 18.4% ($\pm 0.14\%$), while the gel showed 17.5% protein ($\pm 0.31\%$). These results demonstrate a minimal reduction in protein concentration after extraction, indicating strong retention of nutritional value. The gel not only preserves significant protein levels but also offers promising functional characteristics, such as texture and moisture retention, making it suitable for replacing animal fat in plant-based meat formulations. The high protein concentration in the gel underscores its potential as both a nutritional and technological ingredient. In conclusion, ora-pro-nóbis leaves and their derived gel represent a valuable resource for plant-based food innovation, with the gel form maintaining a high protein content while offering added functionality for use in the formulation of healthier, more sustainable meat alternatives.

Keywords: Alternative proteins; Gel extraction; Sustainable food.

Funding: FAPERGS. Edital 14/2022 ARD/ARC, Auxílio Recém-Doutor ou Recém-Contratado, Termo de Outorga 23/2551-0000951-0.

ORA-PRO-NÓBIS FLOUR AS A PLANT-BASED PROTEIN SOURCE: A REVIEW OF NUTRITIONAL POTENTIAL FOR INNOVATION IN SUSTAINABLE FOOD SYSTEMS

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REVIEW: This review study investigates the potential of ora-pro-nóbis flour (OPNF) (*Pereskia aculeata*) as a plant-based protein source with functional properties for food applications. With the increasing demand for more sustainable proteins, unconventional food plants, such as ora-pro-nóbis (OPN), have gained notoriety for their high protein content and technological applicability. In addition to being consumed raw, OPN can be used as flour in baked goods and meat products. The objective of this research was to compare the protein content of OPNF, assessing its viability as a functional food ingredient. Four scientific articles that quantified OPNF's protein content were evaluated. In these studies, the flour was obtained from the plant's leaves, dehydrated in an oven with air circulation at 60 °C for 12-24 hours, and then ground. Protein content was determined using the official Kjeldahl method, which quantifies total nitrogen and converts it into protein values. The leaf flours presented protein contents of 28.99% ($\pm 0.59\%$)^[1]; 23.45% ($\pm 0.66\%$)^[2]; 21.08% ($\pm 0.56\%$)^[3]; 18.29% ($\pm 0.60\%$)^[4]. The results demonstrate variability in the protein content of OPNF, influenced by factors such as growing conditions and harvest stage. Despite the observed differences, the values confirm the potential of FOPN as an alternative source of vegetable protein and a functional ingredient with applicability in the food industry. In addition to its expressive protein content, OPNF also stands out for its high dietary fiber content, which makes it a viable option for the nutritional enrichment of meat products, contributing to their texture and stability. In conclusion, OPNF represents a valuable resource for innovation in plant-based foods, offering additional functionality for use in healthier and more sustainable meat formulations.

Keywords: Functional Food Ingredient; *Pereskia aculeata*; Unconventional Food Plants.

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Funding: FAPERGS. Edital 14/2022 ARD/ARC, Auxílio Recém-Doutor ou Recém-Contratado, Termo de Outorga 23/2551-0000951-0.

OBTAINING PROTEIN HYDROLYSATES BY ALKALINE ENZYMATIC HYDROLYSIS OF PEA FLOUR (*Pisum sativum* L.)

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ABSTRACT: The growing appreciation of plant-based protein sources as an alternative to animal proteins has driven the use of protein-rich raw materials, especially in the food sector. Among legumes, peas (*Pisum sativum* L.) stand out for their high protein content and widespread availability, representing a promising option to produce protein hydrolysates. Enzymatic hydrolysis in an alkaline medium facilitates the cleavage of polypeptide chains, releasing peptides with potential applications in the cosmetics and nutraceutical industries. In this context, the present study aimed to investigate the alkaline enzymatic hydrolysis of pea flour and evaluate its yield. The raw peas were initially dried in an oven with air circulation at 35°C until they reached constant weight and then ground in a knife mill to obtain a flour with an average particle diameter of 0.3 mm, presenting an initial protein content of 16.64% ± 0.11. Hydrolysis was carried out using a substrate to distilled water ratio of 1:10 (w/v), with the addition of 0.05% and 1% of the enzyme Alcalase (w/v in relation to the substrate). The pH was adjusted to 8.0 with a 20% sodium hydroxide solution, and the process occurred under orbital shaking (180 rpm) at 45 °C for 1 hour. After the reaction, the material was filtered through tissue, centrifuged, and the supernatant dried at 80 °C in an oven to quantify the yield. The hydrolysates obtained had protein contents of 23.31% ± 0.01 and 36.26% ± 0.56 for the assays with 0.05% and 1% enzyme, respectively. The process yield reached 31.41% ± 0.05 for 0.05% enzyme, and 56.23% ± 0.01 for 1% enzyme. These results demonstrate that alkaline enzymatic hydrolysis of pea flour is effective in increasing protein content and hydrolysate production yield. This indicates that pea flour could be a good raw material for creating bioactive ingredients used in the cosmetic and nutraceutical industries.

Keywords: Enzymatic hydrolysis; Protein hydrolysate; Pea Flour.

Funding: The authors thanks URI Erechim, CAPES and FAPERGS for their support.

VALORIZATION OF CUPUAÇU SEED: A STUDY ON THE PROTEIN RECOVERY

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ABSTRACT: Plant-based products (alternatives derived from plants) are gaining space and relevance in the modern diet. Cupuaçu is one of the most important fruits of the Amazon and is widely found throughout the Brazilian Amazon basin and neighboring countries. As a member of the *Sterculiaceae* family, its pulp can be used to produce chocolate powder and tablets. However, its seeds contains significant amounts of protein, fatty acids, and fiber. These features make it a promising protein source for plant-based product development, helping diversify and enrich sustainable food options. Therefore, this research aimed to fully utilize the nutritional potential of cupuaçu seed by developing a scalable laboratory process to recovery protein. The extraction was conducted using 50g of cupuaçu seed flour suspended in distilled water at solid-to-liquid ratio of 1:5 or 1:8. The suspensions were adjusted and kept to pH 10 using sodium hydroxide solution (1 mol/L) and the process was carried at 50 °C under constant stirring (120 rpm) for 60 min. The assays were performed in triplicate. The protein content on cupuaçu seed flour and each extract were determined by Kjeldahl method (AOAC 954.01) with $N \times 6.25$. The percentage of recovered protein was determined considering the mass yield and protein concentration of extracts. Was observed that the solid-to-liquid ratio 1:5 recovered 35.85% of protein present in plant matrix, which was significantly higher ($p < 0.05$) than the ratio 1:8, 50.60%. Both ratios resulted in protein concentration of about 30%. The better protein recovery using the higher solid-to-liquid ratio is probably related to facilitating the diffusion of proteins from within the plant matrix into the liquid phase. This occurs because the concentration gradient between the solid (seed) and the liquid (solvent) is more pronounced, which enhances the mass transfer process. These findings suggest that higher solvent ratios can significantly improve protein recovery from cupuaçu seeds, highlighting their potential as a valuable plant-based protein source.

Keywords: Product valorization; sustainability; alternative proteins; Amazonian fruit.

Funding: National Council for Scientific and Technological Development (CNPq).

COMPARATIVE EVALUATION OF PROTEIN EXTRACTION METHODS FROM PEQUI ALMOND (*CARYOCAR BRASILIENSE*)

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ABSTRACT: The growing demand for proteins is driving the search for sustainable alternative sources, particularly plant-based options that offer high biodiversity and a lower environmental impact. In this context, the utilization of agro-industrial side stream represents a promising solution, aligning conventional protein production with circular economy principles by promoting the valorization of by-products. The study aimed to investigate the potential of the pequi almond as a protein source for hydrolysate production. The pequi fruit comes from the *Caryocar brasiliense*, a tree native to the Brazilian Cerrado. The research compared two different extraction methods to evaluate their yield and efficiency. The first method was based on chemical extraction, with pH adjustment using NaOH and HCl (0.1 mol/L) solutions, promoting the solubilization of proteins at pH 9.0 and precipitation at pH 4.5. The second method was based on enzymatic extraction, using commercial enzymes (Alcalase® 2.4 L, Flavourzyme®, Corolase® 8000, and cellulase), both associated with physical techniques (ultrasound bath and homogenization with ultraturrax). Physical treatment alone yielded better results with ultraturrax (0.45%), due to its greater shear capacity. When enzymes were added, Flavourzyme achieved the highest protein yield (1.20%), likely due to its combined endo and exopeptidases activity, which enhances peptides solubilization. In the chemical method, alkaline extraction at pH 9.0 followed by acid precipitation at pH 4.5 showed the best yield (27.36%). However, the combination of alkaline



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extraction with proteases (Alcalase and Flavourzyme) reduced the protein yield, obtaining values of 21.36% and 11.59%, respectively. The association of cellulase, ultraturrax, alkaline extraction, and protease also achieved a similar yield (21.87%) with Alcalase, suggesting that factors such as high pH may inhibit enzymatic action or limit access to the substrate. Overall, the results indicate that simpler methods, such as conventional chemical extraction methods, may still be more effective in terms of gross protein yield, while enzymatic methods offer potential functional advantages. The choice of the ideal technique will depend on the final objectives, also considering the economic viability and the sustainability of the processes.

Keywords: Brazilian savanna; plant protein; chemical extraction; enzymatic extraction; physical methods.

Funding: Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) – Process APQ- 04336-23, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Financing Code 001 e Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) - Process: 132217/2023-6, 307787/2022-2 e 404432/2024-7. UFMG, PRPq, UFSJ, EPAMIG, GEPEQF.

Realization



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PROTEIN SOLUBILITY AND YIELD IN RICE-BASED SUPPLEMENTS: EVALUATION OF ALKALINE-ETHANOLIC EXTRACTION ACROSS COMMERCIAL BRANDS

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ABSTRACT: The growing interest in plant-based protein supplements has driven the formulation and commercialization of products containing isolated or combined proteins, primarily from sources such as rice, soy, and pea. However, data on the composition of these protein isolates are scarce. Considering the need to characterize the protein profile of these supplements, this study aimed to extract and quantify the proteins from rice-based isolated supplements using an alkaline-alcoholic extraction method. Three commercial brands (A, B, and C) were subjected to extraction using aqueous and 70% ethanolic solutions, with the pH adjusted to 12. The samples were homogenized and centrifuged at 1500 *xg*, 4 °C, for 20 minutes, followed by three steps of washing, neutralization, and lyophilization of both the precipitates and supernatants. Protein quantification was performed using the Kjeldahl method with a conversion factor of 6.95, expressed on a dry basis. The precipitates from brands A, B, and C showed protein contents of 96.2 g, 66.9 g, and 84.0 g/100 g, respectively. Alkaline-alcoholic extraction resulted in an increase in protein concentration for brands A and B, by 20 g and 3 g, respectively, while brand C showed a reduction of 18 g in protein content. The lyophilized supernatants exhibited, on average, one-third of the protein concentration found in the precipitates. Although plant proteins are more soluble in alkaline media, the higher protein concentration in the precipitates indicated a greater proportion of insoluble proteins in rice-based isolated protein supplements. This may be due to interactions with other nutrients in their composition, such as starch. The results of this study demonstrate that the extraction method used was effective in concentrating proteins from rice-based isolated protein supplements, enabling future characterization of the protein or amino acid profiles present in these supplements.

Keywords: Rice protein; Plant-based; Hydrogen potential.

Funding: FAPEMIG, CNPq e Capes.



November 12, 13, and 14th, 2025 | Florianópolis, SC | UFSC Culture and Events Center

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FUTURE OF
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PROTEIN EXTRACTION FROM PLANT-BASED PROTEIN SUPPLEMENTS: A STUDY ON SOLUBILITY AND ISOELECTRIC POINT BEHAVIOR

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ABSTRACT: The use of rice-based protein supplements has gained prominence as a viable alternative for daily protein supplementation, especially among consumers seeking plant-based sources. This study aimed to evaluate the efficiency of protein extraction from commercial rice supplements through induction to the proteins' isoelectric point. The protein supplements were homogenized in distilled water and subjected to a pH variation from 4 to 9, then homogenized and centrifuged at 1500 *xg*, 4 °C, for 20 minutes. The protocol included successive steps of centrifugation, washing, and pH adjustment. Protein content was determined using the Kjeldahl method with a conversion factor of 6.95, expressed on a dry basis. The highest extraction yields were obtained at pH 4 (35%) and pH 7 (33%), indicating that isoelectric precipitation is an efficient method compatible with the nature of rice proteins, whose isoelectric points range between pH 4.5 and 6. Although the literature indicates that plant proteins tend to exhibit higher solubility and extraction in alkaline media, the data obtained showed the opposite behavior, suggesting particularities in the protein composition of rice. The correspondence between the pH levels with the highest extraction yields and the proteins' isoelectric points indicates that the method is suitable for this type of raw material. The findings of this study contribute to the optimization of extraction processes and to ensuring the quality and reliability of protein sources used in the formulation of commercial supplements.

Keywords: Rice protein; Performance; Hydrogen potential.

Funding: FAPEMIG, CNPq e Capes.

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SUSTAINABILITY AND FOOD SECURITY: PROTEIN EXTRACTION FROM FRUIT BY-PRODUCTS

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REVIEW: Brazilian fruit production is internationally recognized for its wide diversity of cultivars, generating significant economic impact ^[1]. However, agro-industries produce large amounts of residues such as bagasse, peels, and seeds, which can represent up to 50% of the total fruit weight. These residues have polluting potential, leading to environmental problems and additional costs for agro-industries, since treatment is required for their proper disposal ^[2]. The technological potential present in these residues allows their conversion into by-products with high nutritional value. In this context, the present study aims to review and discuss scientific advances focusing on plant proteins extracted from fruit processing by-products. Seeds, for instance, are promising sources of storage proteins, accounting for 10% to 40% of the main proteins found in oilseeds ^[3]. This nutrient is in high demand compared to other protein sources due to its health benefits and easy digestibility ^[4]. Research addressing the isolation, characterization, and application of proteins extracted from plant sources has been gaining prominence. Reported protein concentrations include jackfruit seeds with values ranging from 18% to 37%, depending on the species ^[5-6], watermelon seeds with 16% ^[7], and cajá seeds with 5% ^[1]. Beyond protein content, these by-products also display bioactive potential, being rich in antioxidants, carotenoids, and nutraceutical compounds ^[8]. Possible applications include the production of jams, edible oils, and flour for incorporation into a wide variety of foods, such as cakes, cookies and pasta ^[9]. It is important to note that the extraction technique directly influences the results, as any change in protein charge polarity may alter its structure, making it necessary to determine the most suitable method for each specific by-product ^[3]. Therefore, obtaining proteins from fruit seeds emerges as a promising and sustainable source of protein components. Studies on different seeds show that protein content varies depending on genus and species. Despite differences in concentration and extraction methods, these by-products prove to be a viable source for protein production, with potential applications across the food industry.

Keywords: Waste recovery; Fruit seeds; Protein extraction.

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Funding: The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) (Finance Code 001).

OPTIMIZATION OF CHEMICAL PEELING AS A PRETREATMENT FOR DRYING *Erythrina edulis* SEEDS WITH A FOCUS ON DESIRABILITY INCLUDING PROTEIN CONTENT

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ABSTRACT: Pajuro (*Erythrina edulis*) is a tree that produces seeds with a high protein content; however, its production and consumption remain limited due to a lack of knowledge about its potential applications. In contrast, it has been traditionally preserved and cultivated through cultural practices in inter-Andean regions of South America, particularly in Peru and Colombia. Several challenges exist regarding its preservation and processing. Therefore, this study aimed to optimize the drying pretreatment, which consisted of chemical peeling using NaOH, through a central composite design based on temperature (25–60 °C) and NaOH concentration (5–10%), totaling 11 experiments (a 2x2 factorial design, four star points, and three central points). Drying was conducted after chemical peeling at 60 °C for 1400 minutes per treatment. Weight variations were converted to dimensionless moisture content and modeled using the Page model to determine drying constants. Seed images were analyzed using image processing techniques to obtain the gray scale index (GSI). In addition, the resulting flour was analyzed for final moisture content, soluble protein (using the Lowry method), and water absorption index (WAI) as a techno-functional property indicator. The desirability function was applied to simultaneously optimize the variability in the following responses: drying rate constant "k" (Page), GSI, final moisture content, soluble protein, and WAI. An overall desirability of ~0.73 was obtained, indicating a very acceptable compromise among responses. The optimal chemical peeling conditions were 17.75 °C and 6.65% NaOH, which are estimated to maximize drying rate, protein content, WAI, and GSI, while minimizing final moisture content. These results are promising for the development of future applications involving this seed in various processes.

Keywords: pajuro; soluble protein; optimization; drying.

Funding: CNPq

APPLICATION OF PULSED LIGHT AND ULTRASOUND FOR THE FUNCTIONALIZATION OF BLACK BEAN PROTEIN

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ABSTRACT: Black beans have been raising interest as a source of protein concentrates and isolates with high nutritional and functional properties. This study aimed to evaluate the effect of pulsed light (PL) on black bean protein (BBP) extracts and compare it with ultrasound (US) treatments, to expand the application potential of this protein. For this, an aqueous extract (TE = total extract) and an extract obtained by isoelectric precipitation (PE = precipitated extract) at a protein concentration of approximately 1 mg/mL were exposed to up to 30 pulses for PL treatment or ultrasonication at different levels (30% to 70%). The samples were characterized by techno-functional and structural properties. Despite a significant ($p < 0.05$) increase in protein content of PE (76 mg/100 g) compared to TE (48 mg/100 g), some properties, such as emulsifying capacity (EC) and foaming capacity (FC), decreased 40% and 48%, respectively, after precipitation. PL treatment allowed to improve EC by up to 21% and FC by up to 84%, primarily in the more intense assay (30 pulses). In contrast, US treatment increased EC by up to 45% and FC by up to 40%, primarily when the moderate intensity (50% amplitude) was applied on PE. Furthermore, surface hydrophobicity (H₀) increased by up to 1.33 times after PL treatment and by up to 2.5 times after US, while the free sulfhydryl groups (SH) generally decreased to 6% in PL and to 29% in US. The observed changes were accompanied by alterations in the secondary structure of BBP extracts, primarily an increase in β -sheet content and a decrease in β -turns, which correlated with the increases in PL and US intensities. The consequences of these modifications on the application of black bean protein extract may vary depending on the food product and the processing steps and parameters required for each. Nevertheless, the presented results contribute to the current knowledge on non-thermal treatments, PL and US, and support their roles as promising tools to improve the application of black bean and other pulse proteins.

Keywords: *Phaseolus vulgaris*; pulses; non-thermal treatments; techno-functional properties; structural characteristics.

Funding: Brazilian Foundation for the Coordination of the Improvement of Higher Education Personnel (CAPES).

VALORIZATION OF *MACAUBA* KERNEL BY-PRODUCTS THROUGH HIGH-PRESSURE EXTRACTION: A PATHWAY TO PLANT-BASED INGREDIENTS DEVELOPMENT

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ABSTRACT: In the pursuit of sustainable development and the exploration of new plant-based ingredients, utilizing underutilized crops and agro-industrial residues, following the concept of a biorefinery, can be a viable alternative. This study aimed to valorize the *macauba* kernel by-product as a Brazilian source of plant-based ingredients by applying high-pressure fluid extraction, in comparison with conventional methods, to obtain and characterize distinct fractions and evaluate their application in nugget analog prototypes. The high-pressure pathway was conducted applying supercritical carbon dioxide extraction (SC-CO₂) (35 g of raw material, 25 MPa, 60 °C, and a CO₂ flow of 1 kg/h), followed by PLE (solid-to-solvent ratio of 1:15 (g/mL), at 60 °C, for 15 min) using water and ethanol as the solvents, sequentially. The conventional pathway consisted of Soxhlet extraction using boiling hexane under reflux for 6 hours, followed by heating stirring extraction (HSE) (solid-to-solvent ratio of 1:15 (g/mL), at 60 °C, for 15 min) using water and ethanol as the solvent, sequentially. The high-pressure one presented the following yields: (i) 17.6% of lipid fraction, (ii) 0.78% of bioactive extract with antioxidant activity (DPPH: 1.26 µmol TE/g; ABTS: 3.64 µmol TE/g), (iii) 18.13% of protein-rich fraction with approximately 90% of protein concentration, and (iv) 62 % of fiber fraction. These yields were significantly higher ($p < 0.05$) than conventional pathway: (i) 14.8% of lipid, (ii) 0.48% of bioactive extract (DPPH: 1.07 µmol TE/g; ABTS 2.55 µmol TE/g), (iii) 10.11% of protein fraction with 70-76% of protein concentration and (iv) 74.61% of fiber. The higher efficiency of the high-pressure pathway is related to improved solvent diffusion at pressurized conditions, which increases solvation power and facilitates the rupture of the solid matrix. Additionally, the protein obtained by the high-pressure pathway was highlighted due to its good essential amino acid profile and solubility of around 70%. The obtained ingredients were applied in two plant-based nugget formulations—with and without fiber—and demonstrated potential for use in meat analogues. Therefore, high-pressure fluid extraction is demonstrated to be a promising and sustainable approach for obtaining food ingredients from *macauba* by-products, supporting plant-based innovation and circular bioeconomy strategies.

Keywords: biorefinery; plant-based protein; amino acid profile; techno-functional properties; meat analog

Funding: The Good Food Institute Brazil (GFI, Brazil).

INFLUENCE OF FAT CONTENT ON THE THERMAL (DSC) AND RHEOLOGICAL (RVA) PROPERTIES OF SACHA INCHI (PLUKENETIA VOLUBILIS L.) PRESS CAKE AS AN ALTERNATIVE PROTEIN SOURCE

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ABSTRACT: This study presents a comprehensive analysis of sacha inchi (*Plukenetia volubilis* L.) press cakes with high and low-fat content, obtained after the oil extraction process. Their chemical, functional, thermal, and rheological properties were evaluated to explore their potential use in protein-rich food formulations. The samples analyzed were A1 (low fat content, 5%), A2 (high fat content, 25%), and A3 (high fat content, 25%). Using differential scanning calorimetry (DSC), differences were observed in the thermal parameters among the samples, particularly in peak temperature (T_p) and enthalpy (ΔH); A1 showed a peak temperature (T_p) of 46.59 °C and enthalpy (ΔH) of 976.6 J/g; A2 presented T_p of 52.92 °C and ΔH of 944.0 J/g; while A3 recorded T_p of 49.48 ± 3.20 °C and ΔH of 1117.92 ± 273.35 J/g, indicating that fat content and type influence the material's thermal stability. Viscosimetric analysis (RVA) revealed significant differences in peak viscosity, shear resistance, and retrogradation among the cakes, with higher values in the high-fat samples (A2 and A3), suggesting greater interaction between components in the presence of fat. Microstructural analysis using scanning electron microscopy (SEM) allowed the observation of differences in morphology and particle size, as well as structural integrity and fiber presence. These results reflect how fat content modifies the cake's structure, affecting its functional properties. Additionally, pH and moisture content were evaluated, showing slightly acidic values and low moisture levels, which are favorable for product stability and preservation. Overall, the obtained data demonstrate that sacha inchi cake, especially the defatted version, is a promising source of proteins and bioactive compounds, suitable for use in functional and sustainable food products. This study highlights the importance of optimizing extraction and post-processing methods to enhance the nutritional and functional value of oily seed by-products, contributing to the development of innovative and healthy food ingredients.

Keywords: Sacha inchi, press cake, thermal characterization, rheological properties, alternative proteins.

PROXIMATE COMPOSITION OF SHIITAKE (*LENTINULA EDODES*) AND WHITE SHIMEJI (*PLEUROTUS OSTREATUS*) MUSHROOM FLOURS

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ABSTRACT: Shiitake (*Lentinula edodes*) and white shimeji (*Pleurotus ostreatus*) mushrooms are known for their complete amino acid profile, high levels of bioactive compounds, and low environmental impact, making them promising ingredients in the development of sustainable plant-based foods. This study aimed to determine and compare the proximate composition of flours obtained from these species, providing preliminary nutritional data to support their potential applications in food formulations. After harvest, mushrooms were dehydrated at 50–55 °C for 5 hours using an electric dehydrator, then ground into flour. Analyses were carried out on a dry basis: in triplicate for moisture, ash, protein, carbohydrates, and total dietary fiber (TDF), and in duplicate for lipids. Comparisons between samples were made using an unpaired Student's t-test, with p-values < 0.05 considered statistically significant. Shiitake flour showed significantly higher moisture ($12.24 \pm 0.34\%$) than white shimeji ($11.31 \pm 0.23\%$, $p = 0.0178$), as well as higher carbohydrate content ($60.31 \pm 0.70\%$ vs. $56.38 \pm 0.47\%$, $p = 0.0242$) and lower ash content ($5.75 \pm 0.13\%$ vs. $6.28 \pm 0.20\%$, $p = 0.0194$). White shimeji flour had higher average protein and lipid contents than shiitake, although differences were not statistically significant. TDF content was approximately 41.80% for shiitake and 33.93% for white shimeji. These compositional differences may reflect species-specific characteristics, cultivation conditions, or processing methods. The high carbohydrate and dietary fiber contents in both flours indicate potential use in fiber-enriched formulations and textural enhancement in plant-based products. Moreover, their protein content supports the inclusion of these flours as alternative protein sources, though further investigation into digestibility and amino acid bioavailability is needed. In conclusion, this preliminary analysis highlights the nutritional quality and technological potential of shiitake and white shimeji mushroom flours, reinforcing their relevance for developing innovative, sustainable plant-based food products.

Keywords: Alternative proteins; Mushrooms flour; Functional foods; Proximate composition; Plant-based ingredients

OPTIMIZING HIGH-PROTEIN, PLANT-BASED INKS FOR 3D PRINTING: SYNERGISTIC EFFECTS OF PEA PROTEIN AND *SPIRULINA* BIOMASS ON RHEOLOGY AND FUNCTIONALITY

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REVIEW: The advancement of 3D food printing for creating personalized and sustainable plant-based products is often hindered by the challenge of developing high-protein formulations with optimal printing performance. This study aimed to optimize plant-based ink formulations by investigating the synergistic effects of pea protein and *Spirulina* biomass to maximize printability, dimensional fidelity, and textural properties. The experimental methodology involved 11 formulations following a 2² RCCD design with central point triplicates, using pea protein (15-20%) and *Spirulina* biomass (0-6%), evaluated for printability (1-5 scale), print fidelity (%), hardness (g), adhesiveness (g.s), and protein concentration (%). Advanced rheological analysis and colorimetric evaluations complemented the functional assessment. The results have shown distinct contributions of the ingredients. Pea protein acted as the primary structuring agent, critically governing printability, with optimal performance achieved at 17.5-18%. *Spirulina* biomass proved to be a multifunctional additive, significantly increasing hardness in synergy with pea protein and acting as an effective natural colorant, shifting formulations from reddish-yellow tones to distinct green hues (representing a 73% shift toward green coloration). In-depth rheological analysis demonstrated that successful printing depends not solely on high structural rigidity (Storage Modulus, G') but on a balance between structural rigidity and viscoelastic recovery. Formulations with a higher Linear Viscoelastic Region (LVR) limit (>3%) showed greater resistance to fracture during extrusion, indicating that network resilience is a critical parameter for printability. The optimal formulation range was identified as 16- 18% pea protein combined with 3-5% *Spirulina*, achieving superior hardness and structural integrity ($R^2 = 0.82$). The optimized formulation, validated with 18% pea protein and 4% *Spirulina*, achieved highest results with 95.4% print fidelity and a printability score of 4.5/5.0, while elevating total protein content to 22%. This research presents an effective method for the development of high protein printed foods with controlled texture, color, and nutritional value. It establishes technical parameters for scalable, sustainable plant-based meat analog production, offering a novel approach to alternative protein systems.

Keywords: Alternative proteins, 3D food printing, plant-based meat analogs, microalgae biomass.

Funding: The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES), The National Council for Scientific and Technological Development of Brazil (CNPq), Research Support of the State of Rio Grande do Sul (FAPERGS) and Ministry of Science, Technology, and Innovation (MCTI) for the support.

DEVELOPMENT OF A PLANT-BASED SAUSAGE: EFFECT OF FAT CONTENT ON TEXTURAL AND PHYSICOCHEMICAL PROPERTIES

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ABSTRACT: The increasing consumer demand for healthier products with functional benefits has driven the development of alternative foods. In this study, five formulations of plant-based sausages were produced using a high-moisture extruded meat analog composed of 12% debittered germinated tarwi flour and 88% soy protein isolate, with a conditioning moisture content of 65.5%. The formulations differed in the ratio of meat analog to palm fat, with fat levels ranging from 10% (F1) to 25% (F5) in 5% increments relative to the total formulation. Among the formulations, F5, containing the highest fat content, exhibited the lowest values of moisture (47.09%), pH (6.87), elasticity (1.01), chewiness (57.24%), hardness (198.75 N), and adhesiveness (−0.355 N·s), resulting in a more fragile and less cohesive texture. Conversely, F1 demonstrated higher pH (6.98), lightness ($L^* = 64.58$), cohesiveness (0.758), and elasticity (0.971), characteristics favorable for sensory acceptance and physical stability. All formulations displayed water-holding capacities exceeding 75%, with F3 achieving the highest value (80.92%). Significant differences among treatments were confirmed by the Scott-Knott test ($p < 0.05$). Multivariate analysis using partial least squares- discriminant analysis (PLS-DA) identified cohesiveness, elasticity, and brightness as the most influential discriminating variables. Principal component analysis (PCA) further revealed a clear separation among the formulations, with F1 and F3 showing superior overall performance. Based on these findings, F3 was selected as the most balanced formulation in terms of texture, technological properties, and potential functionality. These results highlight the potential of germinated tarwi as a technofunctional ingredient in the development of sustainable plant-based sausages.

Keywords: Vegetable sausage; high-moisture extrusion; germinated tarwi, texture; plant protein.

Funding: This work was funded by CONCYTEC through the PROCIENCIA program, within the framework of the Financial Scheme E041-2023-02 titled “Proyectos de Investigación Aplicada” (Grant PE501082005-2023). Thanks to CNPq for the research scholarship provided to M.S. (312759/2025-8).

FRACTIONATION AND TECHNO FUNCTIONAL PROPERTIES OF BLACK BEAN PROTEIN FOR APPLICATION IN FOOD HYDROGELS

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ABSTRACT: This study investigated the impact of extraction and fractionation methods on the physicochemical, thermal, and functional properties of black bean proteins. Black bean flour was extracted by aqueous (AEP) and enzyme-assisted aqueous (EAEP) methods at a solid-to-liquid ratio of 1:10, for 60 min at 50 °C. AEP was conducted at pH 7.0, whereas the EAEP was conducted at pH 9.0 with 0.5% alkaline protease. In both cases, part of the protein extract was reserved as the concentrate, while the remainder underwent isoelectric precipitation at pH 4.5 to generate the whey (soluble proteins) and the curd (precipitate). Enzyme addition in the EAEP increased protein extractability from 62.1% (AEP) to 73.6% (EAEP). The higher extractability observed with the EAEP stems from proteolysis, which generates smaller, more soluble peptides and increases protein recovery in the extract (concentrate). Following isoelectric precipitation, protein yields in the curd fractions were similar for the AEP ($42.08 \pm 0.83\%$) compared to the EAEP ($43.52\% \pm 1.63$). In contrast, the EAEP whey fraction showed a higher protein yield than the AEP whey fraction ($31.30\% \pm 0.33$ vs. $20.50\% \pm 2.22$). Molecular weight analysis revealed phaseolin (~48 kDa) in the AEP concentrate, while the EAEP concentrate was enriched in smaller fragments (~25 kDa), consistent with proteolysis. The shift toward lower molecular mass increases solubility at pH 4.5, accounting for the higher protein recovery observed in the EAEP whey fraction. While the protein concentrates showed higher thermal stability for the AEP, the whey and curd fractions were more thermally stable for the EAEP, likely due to protein aggregation. All fractions formed hydrogels with 20% protein at pH 3.0 and 7.0. Texture Profile Analysis (TPA) showed that AEP curd hydrogels at pH 3.0 had higher hardness, while AEP whey hydrogels at pH 3.0 and 7.0 exhibited greater elasticity, possibly due to higher carbohydrate content in the whey fraction. These functional properties suggest distinct applications for AEP and EAEP protein fractions, with curd hydrogels suitable for meat analogs, whey hydrogels for fat replacers, and concentrates for fortified beverages and supplements.

Keywords: Aqueous extraction; Bean Proteins; Proteolysis; Protein Fractionation.

Funding: Supported by the USDA Agricultural Research Service Pulse Crop Health Initiative (grant number 58-3060-0-044-0) and CNPq (National Council for Scientific and Technological Development) 201146/2024-0.

RICE AND PEA PROTEINS AS A PROTECTIVE MATRIX FOR *Lactacaseibacillus rhamnosus* GG

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ABSTRACT: Techniques capable of producing probiotic microcapsules with small sizes and high encapsulation yields are of great industrial and scientific relevance. In this study, an innovative membrane emulsification system was investigated for the production of microcapsules containing *Lactacaseibacillus rhamnosus* GG[®] (Lr), sodium alginate (ALG), and plant-based proteins (rice protein concentrate (RPC) and pea protein concentrate (PPC)) as well as whey protein isolate (WPI) for comparison. The objective was to evaluate the technological performance of plant proteins as encapsulating agents and their ability to protect probiotics under adverse conditions. The microcapsules were characterized by particle size distribution, optical microscopy, encapsulation yield, morphology, water activity, hygroscopicity, thermal properties, Fourier-transform infrared spectroscopy (FTIR), and probiotic survival during *in vitro* gastrointestinal simulation. The membrane emulsification process produced microcapsules with diameters ranging from 18 to 29 μm and encapsulation yields consistently higher than 93%. Formulations containing plant proteins (RPC and PPC) displayed comparable results to WPI, with improvements in encapsulation efficiency and thermal stability. These findings confirm that rice and pea proteins can form stable biopolymer networks with alginate, ensuring structural integrity of the capsules and effective protection of probiotics. During gastrointestinal simulation, the encapsulation system effectively protected probiotics against gastrointestinal fluids, ensuring high viability in the intestinal phase ($>7.18 \log \text{CFU g}^{-1}$). In conclusion, the proposed encapsulation technology using rice and pea proteins represents a promising strategy for developing efficient probiotic delivery systems. By combining functionality and sustainability, plant-based proteins emerge as valuable alternatives to traditional encapsulating agents, reinforcing their role in the growing field of alternative proteins and functional food innovation.

Keywords: Probiotic; Encapsulation; Membrane emulsification; Plant-based proteins.

Funding: This work was financially supported by the CNPq (National Council for Scientific and Technological Development, Brazil) (420275/2018–5, 307576/2018–3, and 200246/2022–4). Callebe Camelo-Silva and Guilherme Dallarmi Sorita acknowledge the financial support from CNPq (grant number 174880/2023-5 and 150281/2024-2).

ULTRAFILTRATION AS A STRATEGY FOR DESIGNING PEPTIDE TECHNO-FUNCTIONAL PROPERTIES

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REVIEW: Peptides have attracted growing attention beyond their nutritional relevance due to their techno-functional properties, which make them promising candidates for food applications. This work reviews the use of ultrafiltration (UF) to fractionate plant-based hydrolyzed peptides into fractions with distinct properties. Emphasis is placed on techno-functional traits such as solubility, water/oil-holding capacity (WHC/OHC), emulsifying activity (EA), and foaming capacity/stability (FC/FS). UF is presented as a sustainable and selective alternative to traditional methods like acid precipitation. Literature suggests that peptide size has a significant impact on solubility. *Spirulina platensis* peptides <3 kDa exhibited markedly higher solubility (77.70%) compared to unfractionated peptides (37.81%) [1]. Similarly, fava bean peptides <10 kDa maintained solubility >90% at both pH 5 and 7, while larger peptides (>10 kDa) showed limited solubility at acidic pH, likely due to hydrophobic interactions and aggregation near the isoelectric point [2]. Peptides exhibit low WHC due to high solubility, while OHC generally increases with the increase in molecular weight (MW). Fava bean peptides >10 kDa (8.8 g oil/g protein) displayed higher OHC than <10 kDa (6 g oil/g protein), presumably owing to larger hydrophobic regions and more complex structures [2]. EA also varies with MW distribution. Soybean–corn peptides in the 2–30 kDa ranges displayed higher emulsifying activity indices than <2 kDa and 30–100 kDa peptides [3]. FC is also influenced by peptide MW, with larger peptides exhibiting higher capacity. Rice protein, unfractionated, showed a higher FC (253%) than UF peptides (171%), due to the presence of larger proteins with greater air-trapping ability. In contrast, UF peptides exhibited superior FS (21% vs. 13%), which could be attributed to their smaller size, leading to faster interfacial diffusion and the formation of cohesive films that limit bubble coalescence [4]. In conclusion, UF enables the selective fractionation of peptides by MW, thereby modulating their functional properties. Although not universal, trends indicate that smaller peptides are highly soluble, suitable for beverages and supplements, while mid- and high-mass peptides enhance OHC, EA, and FS.

Keywords: Membrane; Ultrafiltration; Sustainability; Functionality.

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Funding: The authors acknowledge the financial support from CNPq (grant numbers 150281/2024-2 and 174880/2023-5).

ULTRAFILTRATION AS A STRATEGY FOR BOOSTING THE BIOACTIVITY OF ALGAL PEPTIDES

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REVIEW: Bioactive peptides derived from algae have attracted increasing attention, not only for their nutritional value but also for their diverse spectrum of biological activities. In this context, this work reviews the use of ultrafiltration (UF) to fractionate algal-based hydrolyzed peptides into fractions with distinct biological properties, with a particular focus on their antioxidant, antidiabetic, anti-aging, and antibacterial properties for applications in functional foods and nutraceuticals. Recent studies have highlighted a tendency for low molecular weight (MW) peptides (<3 kDa) to exhibit greater in vitro antioxidant capacity (ABTS and DPPH methods) than non-fractionated peptides (NF) or > 3 kDa peptides, as observed in *Chlorella* [1], *Sargassum ilicifolium*, and *Spirulina platensis* [2], [3], e [4], possibly due to better solubility and greater exposure of redox- active amino acid residues. Low MW bioactive peptides have also presented enhanced antihypertensive activities. Peptides shorter than 3 kDa from *Chlorella* [1], and *Ulva intestinalis* [5] exhibited higher angiotensin-converting enzyme (ACE) inhibitory activity compared to high MW peptides (> 3kDa). Enhanced anti-aging effects have been associated with low-MW peptides. Peptide fractions <3 kDa from *Spirulina platensis* and *Chlorella vulgaris* exhibited significantly higher in vitro inhibition of elastase, collagenase, and tyrosinase compared to high MW peptides [4]. This trend was further supported by in vivo studies conducted on *Spirulina platensis* [3]. Following the same pattern, peptides <3 kDa displayed stronger antibacterial activity than unfractionated peptides against *L. monocytogenes*, *S. aureus*, *E. coli*, and *S. typhimurium*, as observed in *Sargassum ilicifolium* and *Spirulina platensis* [2]. A similar trend was observed in *Dunaliella salina*, where peptide fractions <3 kDa exhibited stronger antibacterial activity against *E. coli* compared to the 3–10 kDa fraction [4]. In summary, UF enables the isolation of selective algal peptide fractions, particularly those with molecular weights below 3 kDa, which tend to exhibit enhanced bioactivity. This enhancement is reflected in multiple studies, which consistently report superior antioxidant, antidiabetic, anti-aging, and antibacterial activities in peptides obtained from species such as *Chlorella*, *Sargassum ilicifolium*, *Spirulina platensis*, and *Ulva intestinalis*. Thus, UF stands out as a sustainable tool for peptide fractionation, aiming to develop functional ingredients in food and nutraceuticals.

Keywords: Membrane; Ultrafiltration; Algal bioactive peptides; Nutraceuticals.

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Funding: The authors acknowledge the financial support from CNPq (grant n° 150281/2024-2 and 174880/2023-5).

THERMAL CHARACTERIZATION OF PEA PROTEIN ISOLATE BY DIFFERENTIAL SCANNING CALORIMETRY (DSC)

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ABSTRACT: The growing global demand for sustainable alternative proteins has stimulated the development and study of functional plant-based ingredients, with emphasis on pea protein isolate (PPI), due to its balanced nutritional profile and technological potential in plant-based food formulations. This study aimed to investigate the thermal behavior of PPI, prepared in a 1:2 ratio (PPI:water), using the Differential Scanning Calorimetry (DSC) technique. The analyses were performed in triplicate, using PPI samples stored in hermetically sealed Hastelloy steel cells, subjected to heating in the Multi Cell DSC equipment (TA Instruments, USA). The procedure involved a temperature sweep from 5°C to 140°C, a heating rate of 5°C/min, and a constant nitrogen flow rate of 10 mL/min. Thermograms revealed two main endothermic events, reproducible between samples. The first event, attributed to the vicilin fraction (7S), showed an onset temperature of 56.2 ± 0.3 °C, peak at 63.3 ± 0.2 °C, and endset at 68.6 ± 1.2 °C, with an average enthalpy of 0.0171 ± 0.0017 J/g. The second event, related to legumin (11S), initiated at 72.6 ± 1.0 °C, peak at 82.5 ± 2.2 °C, endset at 89.8 ± 3.6 °C, with an average enthalpy of 0.0509 ± 0.0372 J/g. These values corroborate recent literature data for industrial PPI, although the relatively low enthalpies suggest partial denaturation or prior aggregation resulting from commercial processing. The clear separation of the two thermally distinct events indicates good structural definition of the pea protein fractions. The DSC technique proved effective in characterizing the thermal transitions of PPI, providing essential parameters for quality control and optimization of industrial heated food processes. The thermal profile presented indicates a native conformation of the proteins present in the protecia isolate. Pea protein isolate (PPI) can contribute to the implementation of innovative technologies and formulations, enhancing its use as a functional, sustainable, and competitive alternative to traditional proteins.

Keywords: pea protein isolate; differential scanning calorimetry; alternative proteins.

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INFLUENCE OF A HYBRID WHEY–PEA PROTEIN SYSTEM ON BIGEL DIGESTIBILITY

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ABSTRACT: Bigels have emerged as promising systems for mimicking saturated fat while also offering nutritional benefits through protein incorporation, which supplies essential amino acids and may improve satiety. Protein-based bigels were structured with 15% (w/v) whey protein isolate (WPI) and 20% (w/v) pea protein (PP), either individually or in combination (1:1). Protein hydrogels were prepared via pH- and heat-induced gelation. These hydrogels were incorporated into an oleogel phase (10% w/w glycerol monostearate) to form bigels at oleogel-to-hydrogel ratios of 30:70 and 70:30, which were selected to evaluate the effect of phase predominance on the structural and functional properties of the bigels. Intrinsic fluorescence analysis was performed to assess structural rearrangements in proteins induced by the processing conditions. Compared to WPI, PP exhibited lower fluorescence; however, gelation enhanced fluorescence intensity in all systems, indicating greater exposure of aromatic residues during network formation. Notably, WPI–PP bigels, particularly in gel form, showed the highest fluorescence intensity, suggesting synergistic protein–protein interactions. In addition, protein digestibility was assessed using the o-phthalaldehyde (OPA) assay, which quantifies free amino groups released during enzymatic digestion. Leucine release was used as a marker due to its abundance in proteins and its nutritional importance as an essential amino acid, providing a sensitive indicator of protein hydrolysis. PP-based bigels with a 30:70 oleogel-to-hydrogel ratio exhibited the greatest leucine release in the intestine, partly due to the higher protein content of the pea protein hydrogel (20% PP) compared with the whey protein hydrogel (15% WPI). Hybrid bigels displayed an intermediate digestion profile, combining the nutritional value of WPI with the modulatory capacity of PP, suggesting a balanced strategy for tailoring amino acid release. Overall, both protein concentration and bigel composition were shown to influence on digestion, with PP standing out as a sustainable and functional plant-based alternative, and hybrid systems offering a promising compromise between functionality and sustainability.

Keywords: gelation, hybrid system, leucin, release.

Funding: The authors thank the São Paulo Research Foundation (FAPESP) for financial support (grant #2019/27354-3) and fellowships to Marluci Palazzolli da Silva Padilha (#2023/02631-0 and #2024/11701-4).

SACHA INCHI AND CORN PROTEINS AS INNOVATIVE RAW MATERIALS FOR MEAT ANALOGUES: EVALUATION OF TEXTURE PROPERTIES

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ABSTRACT: The use of plant proteins in meat analogues addresses the growing demand for high-quality, sustainable, and environmentally friendly foods. Sacha inchi protein (SIP) is recognized to have superior nutritional value, while corn protein (CP) contributes to textural properties, which are crucial for product structure and consumer acceptance. This study investigated the partial replacement of SIP by CP and its effects on the textural properties of high-moisture extruded meat analogues. Five formulations with SIP:CP ratios were prepared: F0 (100:00), F1 (90:10), F2 (80:20), F3 (70:30), and F4 (60:40). Samples were processed in a corotational Process 11 Hygienic TSE twin-screw extruder (Thermo Fisher Scientific, Germany) at 55% moisture, and analyzed for hardness, elasticity, cohesiveness, chewiness, resilience, shear force, and work to shear using a TAXT Plus texture analyzer (MicroStable Systems, Australia). Data were statistically evaluated using the Scott-Knott test ($P < 0.05$). Partial replacement had a significant effect on all parameters ($P < 0.001$). Hardness increased with higher CP levels, ranging from 35.74 ± 1.89 N in F2 to 84.31 ± 7.67 N in F3, with F0 exhibiting intermediate values (53.20 ± 3.61 N), indicating that small substitutions reduce firmness, while higher levels promote structural compaction. Elasticity was highest in F0 ($86.22 \pm 1.40\%$) and decreased progressively to F4 ($75.54 \pm 2.09\%$), showing that sachu inchi supports structural recovery, whereas CP decreases matrix flexibility. Cohesiveness followed a similar trend, highest in F0 ($77.30 \pm 0.21\%$) and decreasing with CP content. Chewiness mirrored hardness, with F2 being the lowest (17.64 ± 2.48 N) and F3 the highest (39.47 ± 8.53 N), reflecting variability at higher substitutions. Resilience was greatest in F0 ($35.42 \pm 0.97\%$) and F3 ($33.60 \pm 1.40\%$), while F2 and F4 decreased, indicating a loss in energy recovery. Shear force and work to shear measurements confirmed these trends: F0 had higher resistance than F1, but increased CP raised shear force (up to 20.40 ± 0.48 N in F4) and work to shear (up to 23.70 ± 0.67 N·s in F4). Overall, SIP provides a resilient matrix, whereas CP enhances firmness and cutting energy, enabling the development of meat analogues with tunable texture properties. This innovative combination of plant proteins represents a viable strategy for the design of meat analogues with customizable texture, supporting the production of sustainable protein alternatives.

Keywords: High-moisture extrusion, Texture profile analysis, *Plukenetia volubilis* L., Sustainability.

Funding: The authors acknowledged the institutional support of Universidad Nacional Agraria La Molina, the Federal University of Jequitinhonha and Mucuri Valleys, and the Institute of Science and Technology. Financial support was granted by CAPES (#001), including the scholarship awarded to M. A. A. (#88887.990587/2024-00); by FAPEMIG, through the scholarship granted to B. R. M. (#7640); and by CNPq, through the research productivity scholarship granted to M. S. (#312759/2025-8). This study was carried out within the project “Strengthening the Food Safety, Security, and Sustainability Laboratory (LISSA-FIAL-UNALM) for the development of meat analogs with proteins and fibers from Andean grains with nutritional and functional potential, aimed at preventing and reducing national malnutrition and undernutrition and mitigating environmental impacts,” which was funded by CONCYTEC-PROCIENCIA (Contract No. PE501086016-2023- PROCIENCIA-BM).

ENZYMATIC STRATEGIES AT DIFFERENT TEMPERATURE RAMP AND TIMES FOR IMPROVEMENT OF PROTEIN EXTRACTION IN OAT BEVERAGE PROCESSING

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ABSTRACT: The search for alternatives to animal-based foods has driven the growth of the plant-based beverage market. Oat is a nutrient-rich grain with the potential to strengthen this sector. However, current extraction methods are still limited, resulting in low protein recovery in the liquid phase, which reduces the nutritional value and hinders the market's ability to reach new consumers. Therefore, strategies must be adopted to enhance the enzymatic hydrolysis used during production, aiming to increase protein extraction and solubility in the beverage. This study aimed to determine the conditions of temperature, enzyme addition time, and hydrolysis duration that lead to the highest protein extraction from oat flour during plant-based beverage production. Twenty enzymatic hydrolysis conditions were tested using oat flour-to-water ratios of 1:10 and 1:5, with temperature ramps ranging from 50 °C to 90 °C. The enzymes α -amylase, β -glucanase, and protease were added either together or separately at different stages of the temperature ramps. Controls were performed without enzymes. Protein quantification was carried out using the Lowry method. A standard process used by a private oat beverage company was also tested. The highest protein concentration (0.29 ± 0.04 mg/mL) was obtained under an alternative condition (60 °C/60 min; 50 °C/60 min; 90 °C/10 min) when protease was added at 60 °C. This value was higher than the standard process (0.13 ± 0.02 mg/mL). The alternative method resulted in a shorter hydrolysis time and produced an extract with a higher concentration of soluble proteins, possibly due to the synergistic enzymatic action on breaking down complex protein aggregates. In the standard process, interactions between reducing sugars and amino acid residues may have contributed to decreased thermal stability of proteins. Combined with prolonged exposure to high temperatures, this likely led to protein structural modifications and lower concentrations. Therefore, the alternative approach may reduce industrial costs and improve the nutritional use of oats, as milder time and temperature conditions can achieve higher protein extraction.

Keywords: Oat; Enzymatic Hydrolysis; Protein; Plant-based; Beverage.

Funding: CAPES; SL Administração e Participações.

TASTING THE FUTURE: HOW CONSUMERS PERCEIVE PLANT-BASED FOODS

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ABSTRACT: Studies indicate that the global population will increase significantly by 2050, which will drive greater demand for alternative food sources. In this context, proteins that substitute traditional animal-based foods have emerged as viable options for building more sustainable food systems and are increasingly being adopted by consumers. Cultivated meats, algae, edible insects, and plant-based products have surfaced as alternatives to expand food supply and meet this growing demand^[1]. The objective of this study was to explore consumer perceptions regarding plant-based products, highlighting the key factors that determine acceptance and rejection, as well as their implications for the industry. A literature review was conducted using the CAPES Periodicals database, focusing on articles published in the last five years, utilizing the keywords "plant-based" and "consumer." Overall, studies indicate that consumers consider three main factors when adopting plant-based products: sustainability, nutritional aspects and sensory characteristics. Regarding sustainability, consumers are increasingly aware of the negative environmental impacts associated with animal farming. Therefore, they tend to seek products with a lower carbon footprint, reduced greenhouse gas emissions and respect for animal welfare,^[2]. Concerning nutritional aspects, it is observed that, depending on the consumer profile, doubts still exist about the nutritional quality of plant-based products available on the market. In general, there is a preference for foods that are low in cholesterol and sodium, rich in fiber and minerals, and that provide clear information about their ingredients, especially regarding the types of protein sources used^[3]. However, the primary challenge faced by the plant-based industry relates to sensory characteristics, as replicating the appearance, texture, flavor, and mouthfeel of animal-derived products remains a significant challenge, particularly in meeting the expectations of omnivorous and flexitarian consumers^[4]. Although plant-based products hold growing potential in the market, their widespread acceptance will depend not only on technological advancements to enhance sensory attributes but also on effective education and communication strategies that align nutritional value, sustainability, and eating pleasure with consumer expectations.

Keywords: food innovation; *plant-based* food; consumer; sustainable diets; alternative proteins

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Funding: SDI/MAPA and CAPES.

THERMAL AND VISCOAMYLOGRAPHIC CHARACTERIZATION OF GLUTEN FOR APPLICATION IN PLANT-BASED COOKIES INTENDED FOR 3D PRINTING

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ABSTRACT: The growth of the plant-based food market has stimulated the development of products for 3D printing. Wheat gluten (*Triticum spp.*), composed of gliadins and glutenins, forms macromolecular networks with unique viscoelastic properties, fundamental to the quality of baked products. Gluten characterization becomes essential to understand its application in new food systems. In this work, thermal analyses by differential scanning calorimetry (DSC) and viscoamylographic analyses using Rapid Visco Analyser (RVA) were performed to evaluate gluten behavior during processing and its application in plant-based cookie formulations intended for 3D printing. For RVA analysis, the RVA 4500 equipment was used with 2.90g of gluten diluted in 25mL of distilled water. In DSC analysis, samples were weighed in aluminum capsules and subjected to a temperature scan from 5°C to 145°C, with a heating rate of 10°C/min under nitrogen atmosphere. The gluten concentrate presented low peak viscosity (224 MPa·s), evidencing reduced starch presence. The decrease to holding viscosity (159 MPa·s) indicates partial rupture of the protein structure, with moderate breakdown (65 MPa·s). The high final viscosity (279 MPa·s) and increase during cooling (120 MPa·s) demonstrate strong protein gelification capacity. The high pasting temperature (89.6°C) suggests residual protein-starch interactions. DSC analysis revealed three distinct endothermic events: the first at 42.52°C (TI 34.63; TF 50.59°C; $\Delta H = 0.1258$ J/g), related to bound water mobilization; the second at 68.91°C (TI 59.05; TF 83.83°C; $\Delta H = 0.1082$ J/g), indicative of intermediate protein denaturation; and the third at 97.61°C (TI 87.99; TF 109.66°C; $\Delta H = 0.24$ J/g), corresponding to denaturation of the main protein fractions of gluten. The results indicate that gluten possesses adequate thermal and rheological characteristics for application in formulations intended for 3D printing, presenting good gel formation capacity and thermal stability. These properties are fundamental to ensure dimensional precision and structural quality of 3D printed products.

Keywords: gluten; 3D printing; plant-based foods; DSC; RVA

NUTRITIONAL AND TECHNOLOGICAL PERSPECTIVES OF PLANT-BASED PROTEINS

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REVIEW: For health and sustainability reasons, plant-based proteins have gained increasing prominence, being used as alternatives to animal proteins due to their health benefits and functional properties^{1,2}. Various plant sources can be employed as raw materials in the formulation of products, including legumes, grains, cereals, and algae³. In this context, it is relevant to review recent evidence on the nutritional quality and technological functionalities of plant proteins applied in foods. Plant proteins used as meat substitutes provide high protein quality and help reduce cholesterol and saturated fat intake. However, they still face important sensory challenges and require improvements in appearance, texture, and flavor⁴. Plant-based beverages, mainly produced from soy, peanuts, nuts, and coconut through different technological processes, represent an alternative to animal-derived milk, especially for individuals with milk allergy or lactose intolerance⁵. Plant protein powders are widely used as nutritional supplements, applied to increase the protein content of foods while also enhancing structural and flavor characteristics⁶. Beyond their protein contribution, plant sources are rich in bioactive peptides, present in nutraceutical foods due to their specific physiological functions, as well as antioxidants, phenolic compounds, and flavonoids^{7,8}. Plant proteins therefore represent a promising alternative to animal proteins for the development of sustainable and innovative foods. Advances in research and processing are essential to overcome sensory barriers and expand their potential in both industry and health.

Keywords: foods; sustainability; bioactive peptides.

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EMULSION GELS IN 3D FOOD PRINTING

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REVIEW: Three-dimensional (3D) food printing, or additive manufacturing, has emerged as an innovative technology for fabricating customized food products layer by layer. This technique can provide advantages, such as personalization, low production costs, and operational flexibility to meet consumer demands for tailored shape, texture, and flavor. For successful printing, the material must flow during extrusion and maintain structural integrity after deposition. Thus, the printing “ink” requires thixotropic behavior. Among the different matrices explored, emulsion gels have gained prominence as “inks”. Their hybrid structure, emulsion droplets dispersed within a gel matrix, provides tunable rheological and viscoelastic properties that enable smooth extrusion, rapid recovery, and shape fidelity. Unlike other systems, emulsion gels can prevent collapse of printed layers and allow controlled incorporation of lipids, proteins, and bioactive compounds. Printing parameters, such as nozzle diameter, layer height, infill density, and speed remain critical for defining texture, while emulsion composition, including the type and concentration of polysaccharides, proteins, and lipids, directly affects interactions and rheology [1,2]. To further optimize functionality, polysaccharide modifications (e.g., functional grafting, chemical crosslinking, and blending) can improve bioactivity, emulsifying capacity, and flowability, while protein modifications (e.g., heat, ultrasound, or microwave treatments) promote controlled denaturation, fibrillation, and interfacial stabilization, enhancing network formation [1,3]. Moreover, emulsion gels using alternative proteins (e.g., plant-based sources and microbial biomass) can enable the design of meat analogs by mimicking intramuscular fat and improving juiciness; support personalized nutrition for athletes, elderly individuals, and patients with specific dietary needs; and act as effective carriers for bioactive compounds, improving stability, bioavailability, and controlled release of antioxidants, probiotics, and functional lipids. In conclusion, 3D food printing can provide promising solutions for developing customized, sustainable, and nutritionally enriched products. Future directions should focus on designing emulsion gels with tailored physicochemical properties for different food applications, while advancing green strategies, such as extracting proteins and polysaccharides from agro- industrial by-products, to promote sustainability.

Keywords: Alternative proteins; Bioactive delivery; Meat analogs; Personalized nutrition.

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Funding: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do RS (FAPERGS).

ULTRASOUND-ASSISTED EXTRACTION OF OLIVE LEAF PROTEINS FOR EMULSION GEL DEVELOPMENT AS PLANT-BASED FAT ANALOGS

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ABSTRACT: Proteins extracted from agro-industrial residues represent sustainable alternatives for producing novel emulsions gel as fat analogs. In this study, proteins were extracted from olive leaves using an ultrasound-assisted alkaline extraction. The aqueous phase (80%, w/w) formulation consisted of 2% (w/v) protein extract dissolved in water, and 2% (w/v) sodium alginate dissolved in water. The oil phase formulation comprised soybean oil 20% (w/w). The aqueous protein solutions were heated at 95 °C for different times (10, 20, and 30 min) before emulsification. Emulsion gels were prepared by sequential homogenization with an Ultraturrax. Initially, the protein solution was emulsified with soybean oil at 15,000 rpm for 5 min, then sodium alginate solution was incorporated and homogenized at 15,000 rpm for an additional 5 min. After homogenization, the emulsions were immersed in a 1% (w/v) CaCl₂ solution for 1 h. Textural properties were assessed using Texture Profile Analysis (TPA). The 10 min sample showed moderate hardness (436 g), low adhesiveness (1.41 g s), limited springiness (0.42), and low resilience (0.17), yielding gumminess of 447 g and chewiness of 189 g, which indicated an underdeveloped network. The 20 min sample presented balanced performance, with hardness (408 g), adhesiveness (1.88 g s), higher springiness (0.70), and resilience (0.58), resulting in gumminess of 473 g and chewiness of 330 g. This profile reflected a more elastic structure capable of recovering after compression. This result could be related to the controlled unfolding and increased surface hydrophobicity, enhancing interfacial adsorption of proteins onto oil droplets. On the other hand, the 30 min sample exhibited increased hardness (1672 g) and adhesiveness (7.69 g s), low springiness (0.25), and reduced resilience (0.13), accompanied by gumminess of 1741 g and chewiness of 429 g, suggesting network densification, which could be explained by covalent interactions between residual phenolics compounds and amino acids. These results demonstrate that heating time can influence the functional property of olive leaf proteins in emulsion gel systems. Controlled denaturation at intermediate heating (20 min) yielded the most promising profile for fat analog applications.

Keywords: Sodium alginate; Denaturation; Texture Profile Analysis.

Funding: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do RS (FAPERGS).

DEVELOPMENT OF VEGAN CHEESE WITH BARU NUT (*Dipteryx alata*)

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ABSTRACT: The search for plant-based food alternatives has driven the development of products that combine health, sustainability, and innovation. In this context, the baru nut (*Dipteryx alata*), a fruit typical of the Brazilian Cerrado, is a promising raw material for the formulation of vegan cheeses. This study aimed to develop and characterize a vegan cheese made from baru, evaluating physicochemical and microbiological parameters to verify its technological and nutritional viability. The baru nuts were selected and oven-dried for approximately 7 hours, followed by controlled roasting until reaching 100°C. After cooling, they were weighed and homogenized in a blender with purified water, cassava starch, sunflower oil, nutritional yeast, lemon juice, salt, and cornstarch until a dense paste was obtained. The mixture was heated to approximately 100°C for 10 minutes to achieve firmness, then molded, pressed, and refrigerated for 7 hours, resulting in the vegan cheese. All steps followed Good Manufacturing Practices (GMP) protocols. Physicochemical analyses revealed average moisture (54.70%), lipids (4.47%), proteins (11.23%), and ash (2.01%), indicating an adequate nutritional profile. Notably, the cheese showed lower fat content compared with conventional cheeses, combined with relatively high protein levels. Microbiological analyses included thermotolerant coliforms, *Staphylococcus aureus*, *Salmonella* spp., fungi, and yeasts, confirming that all samples complied with the legal limits established by Normative Instruction No. 161/2022, ensuring product safety. The vegan baru cheese stood out for its technological and nutritional viability, meeting the demands of vegan and flexitarian consumers while promoting a regional cerrado ingredient. It can be concluded that baru nut cheese represents an innovative and sustainable alternative in the plant-based dairy market, with potential for diversification of the food industry and greater use of national raw materials.

Keywords: food industry; Good Manufacturing Practices; physicochemical analysis; microbiology; plant-based proteins.

COMPARATIVE SENSORY ACCEPTANCE STUDY: A2 COALHO CHEESE, VEGAN BARU CHEESE (*Dipteryx Alata*) AND HYBRID COALHO CHEESE

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ABSTRACT: The increasing demand for innovative and sustainable food products has encouraged the development of plant-based dairy alternatives. This study evaluated the sensory acceptance of three formulations: A2 coalho cheese, vegan cheese made from baru nuts, and hybrid coalho cheese (A2 + baru). All processing steps were conducted in accordance with Good Manufacturing Practices (GMP), and the sensory evaluation was approved by the Research Ethics Committee of the Federal University of Mato Grosso (Opinion No. 6,402,125/2025). The A2 cheese was produced from pasteurized A2 milk, with calcium chloride, coalho, salt, and subsequent pressing to obtain a firm and fresh texture. The vegan cheese was prepared with roasted baru nuts, water, starches, vegetable oil, nutritional yeast, lemon juice, and salt, molded, and refrigerated after heating. The hybrid cheese combined A2 curd with a roasted baru nut base, forming a mixed matrix. Prior to sensory evaluation, microbiological control (thermotolerant coliforms, *Staphylococcus aureus*, *Salmonella* spp., fungi, and yeasts) confirmed compliance with Normative Instruction No. 161/2022, ensuring safety. All samples were free of *Salmonella* spp., with no detectable thermotolerant coliforms or *S. aureus*, and 10² CFU/mL of fungi and yeasts in the hybrid and vegan cheeses. Sensory analysis was performed with 50 untrained tasters using a 9-point hedonic scale (flavor, color, aroma, texture, appearance, overall impression) and a 5-point purchase intention test. ANOVA and Tukey's test revealed significant differences ($p < 0.05$). The A2 coalho cheese showed the best performance in flavor and texture, with an acceptability index (AI) of 88.6%. The hybrid cheese achieved an AI of 82.3%, standing out for its innovative character and higher purchase intention (76%). The vegan baru cheese achieved an AI of 79.4%, with good acceptance, particularly in flavor (7.6 ± 0.8) and aroma (7.4 ± 1.0). In conclusion, all formulations showed satisfactory acceptance and microbiological safety. The A2 cheese maintained the highest sensory preference, the hybrid cheese stimulated greater purchase interest, and the vegan baru cheese demonstrated viability as an inclusive and sustainable alternative.

Keywords: food preservation; sensory analysis; microbiology.

PROTECTIVE EFFECT OF INULIN AND PEA PROTEIN ON THE VIABILITY OF *Lactococcus garvieae* (SC004) and *Streptococcus thermophilus* (RS254)

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ABSTRACT: The application of Lactic Acid Bacteria (LAB) in food requires strategies to ensure their stability during processing and storage. Freeze-drying is widely used for microorganism preservation, but it can reduce cell viability due to the osmotic stress caused by freezing and sublimation. Therefore, the use of protective matrices is essential. Pea protein stands out as a plant-based alternative due to its functional properties, such as film formation and thermal stability, while inulin acts as a prebiotic and osmotic protective agent. This study aimed to evaluate the effectiveness of combining pea protein and inulin in protecting *Lactococcus garvieae* (SC004) and *Streptococcus thermophilus* (RS254) during freeze-drying, and to assess their shelf-life at 4 °C and 25±2 °C over 35 days. The RS254 isolate showed greater sensitivity to both freeze-drying and storage, with cell counts decreasing from 7.14 log CFU·g⁻¹ on day 1 to 5.70 log CFU·g⁻¹ after 35 days at 4 °C. Samples stored at 25±2 °C became non-viable (below the detection limit <1.00 log CFU·g⁻¹) after 28 days. In contrast, the SC004 isolate exhibited better stability, maintaining viable counts between 6.56 log CFU·g⁻¹ on day 1 and 5.48 log CFU·g⁻¹ after 35 days at 4 °C. Viability at 25±2 °C fell below the detection limit after 21 days for SC004. These results may be associated not only with the physicochemical properties of the freeze-dried powders but also with the intrinsic physiological tolerance of each isolate to oxidative stress and dehydration. The powders had moisture contents and aw of 0.19 and 0.14 for RS254, and 0.22 and 0.16 for SC004, respectively. Although low aw is important for limiting degradation, excessively low levels can intensify cellular damage. The superior performance of SC004 suggests that both a balance of residual moisture and aw and higher intrinsic tolerance to oxidative stress favor microbial stability. Furthermore, storage at 4 °C proved to be a critical factor for cellular survival, reinforcing the importance of matrix composition and storage conditions in LAB preservation.

Keywords: Lactic cultures; Lyophilization; Viability.

Funding: The authors are thankful to Financiadora de Estudos e Projetos (FINEP 2892/22), Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC 2022TR002005), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 402338/2023-5 303672/2024-2) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/Proex) for the financial support.

CASHEW NUT PROTEIN CONCENTRATE AS A POTENTIAL INGREDIENT FOR THE EMERGING ALTERNATIVE PROTEIN INDUSTRY

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ABSTRACT: We evaluated cashew kernel protein concentrates (PCs) produced after three defatting routes—mechanical pressing (PC-P), hexane extraction (PC-H), and aqueous extraction (PC-A). The PCs were obtained by alkaline extraction followed by isoelectric precipitation and characterized for proximate composition, amino-acid profile, in-vitro digestibility, solubility, interfacial properties (interfacial tension and ζ -potential), Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), water-holding capacity (WHC), oil-holding capacity (OHC), least gelation concentration (LGC), emulsifying activity index (EAI), emulsion stability index (ESI), and flow-behavior (Powder Flow Tester). Protein contents were 59.34% (PC-P), 85.45% (PC-H), and 69.02% (PC-A). All samples showed high in-vitro digestibility (>90%). Solubility was minimal near pH 4.0 and increased under alkaline conditions; at ~neutral pH, PC-H was most soluble (63.83%), followed by PC-A (51.60%) and PC-P (39.70%). Functionally, PC-A delivered the highest OHC (1.09 g/g) and EAI (35.45 m²/g), supporting rapid droplet formation. PC-P achieved the highest ESI (61.00 min), consistent with its more negative ζ -potential (-52.67 ± 2.53 mV) and micrographs evidencing pronounced interfacial coverage; PC-A exhibited similarly high $|\zeta|$ (-51.23 ± 1.22 mV). In contrast, PC-H showed the smallest $|\zeta|$ magnitude (-44.10 ± 2.81 mV) and the lowest ESI (21.19 ± 1.76 min). Microscopy revealed polydisperse droplets with partial coalescence in PC-A; thicker interfacial domains compatible with viscoelastic films in PC-P; and a more uniform field in PC-H, which did not translate into long-term stability. Thus, PC-A and PC-P are considered post-cohesive ($2.0 < FI < 4.0$), and PC-H is considered easy-flowing ($4.0 < FI < 10.0$). Together, the results delineate a practical structure–function framework for PCs. Together, these results delineate a practical framework linking interfacial composition/film quality and ζ -potential magnitude to emulsification outcomes. Aqueous extraction emerges as an eco-friendlier route to concentrates with strong emulsification and oil binding, suiting dressings, spreads, and sauces where rapid droplet formation is prioritized. Hexane extraction maximizes protein purity and solubility, favoring high-protein or clearer systems. Mechanical pressing yields concentrates with robust interfacial stabilization and sustained emulsion stability. Overall, cashew protein concentrates are versatile ingredients for plant-based foods; choosing the defatting route enables fit-for-purpose formulation by balancing EAI and ESI while aligning with performance and sustainability.

Keywords: plant-based; alternative protein; green chemistry; rheology; food processing.

CASHEW APPLE FIBER AS A SUSTAINABLE ALTERNATIVE FOR PLANT-BASED PRODUCTS

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ABSTRACT: Cashew apple fiber (CAF), a byproduct of the cashew juice industry, is often discarded as waste but holds potential to be valorized as an ingredient in plant-based products. In order to improve its microbiological, chemical, technical and sensorial characteristics, the aim of this work was to evaluate the effect of thermal treatment, number of sequential pressing cycles and drying methods on the characteristics of CAF. To address this, cashew apple bagasse was processed at a fiber-to-water ratio of 1:2 (w/w) through one and two sequential pressing cycles. Thermal treatments were applied at room temperature (25 °C), 80 °C and 90 °C, followed by two drying strategies: freeze-drying and oven-drying. The resulting fibers were characterized through chemical, microbiological, technological, and sensory analyses. Thermal treatments at 80 °C and 90 °C effectively ensured microbiological safety (aerobic mesophiles, molds and yeasts, *E. coli*, *B. cereus*, and *Salmonella* spp.), independent of the drying method or number of pressing cycles. Physicochemical and technological analyses indicated satisfactory properties for all treatments, with fibers showing low moisture content and water activity. Color varied according to the drying method: oven-dried samples appeared darker compared to freeze-dried fibers. NMR analysis combined with multivariate evaluation revealed the hierarchical impact of processing variables on the chemical profile of CAF, in the order: sequential pressing cycles → drying method → thermal treatment. UPLC-QDA analysis identified 14 compounds, with freeze-dried samples presenting higher levels of the flavonoid's quercetin and myricetin (and their derivatives), regardless of pressing cycles. In contrast, oven-dried samples showed higher concentrations of anacardic acids, kaempferol, and luteolin. Additionally, 23 volatile metabolites were detected in samples, with composition varying according to processing conditions. Sensory evaluation showed that the number of pressing cycles did not affect any descriptors. Conversely, the drying method influenced fiber characteristics: oven-dried fibers were larger, darker, exhibited more dark spots, and were chewier compared to freeze-dried fibers. The results show fibers washed once or twice are suitable ingredients for plant-based products, and supporting sustainable valorization of cashew-processing byproducts for clean-label formulations, enhancing functionality, sensory acceptance, scalability, and circular-economy alignment in industry.

Keywords: *Anacardium occidentale* L.; clean-label; thermal treatment; ingredient; sensory.

Funding: The Good Food Institute - GFI.

COWPEA FLOUR AND PROTEIN ISOLATE AS INGREDIENTS FOR 3D FOOD PRINTING APPLICATIONS

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ABSTRACT: Cowpea flour (CF) is a commercially available product in Brazil. This study focuses on comparing the water retention, rheological properties, and printing performance of CF-based ink formulations with those prepared using isolated protein (IP) obtained from cowpea flour. On a dry matter basis, CF contained 41.17% protein, 4.54% lipids, 5.31% ash, and 48.96% carbohydrates, while IP contained 83.48% protein, 6.24% lipids, 6.94% ash, and 3.35% carbohydrates. Hydrogels containing the same amount of total solids were tested as inks for 3D printing. For ink preparation, CF or IP was dispersed overnight in distilled water, heated at 90 °C for 30 min, and stirred for 2 min before being loaded into a syringe. Rheological tests showed that the inks exhibited elastic behavior ($G' > G''$) within the linear viscoelastic region (LVER). The intersection point of G' and G'' in a shear stress sweep test occurred at approximately 400 Pa for CF ink and around 1000 Pa for IP ink. The G' and G'' values from the three-interval thixotropy test (3ITT) confirmed that the inks behaved as solids under low shear stress (stabilization), as liquids under high shear stress (extrusion), and recovered their solid structure during the third step (regeneration). However, due to its higher yield stress, the protein-rich IP ink required greater stress to initiate flow. In contrast, CF ink exhibited better flowability, and its lower shear stress requirement is generally preferred for smooth extrusion, facilitating the transitions that occur during the 3D printing process. Printing tests using a 1.2 mm nozzle demonstrated better printability and stability for CF ink, whereas IP ink showed superior water-holding capacity despite the brittleness of its printed structures. Overall, this study suggests that protein flours can serve as cost-effective alternatives to isolated proteins in the production of edible inks, potentially reducing the cost of 3D-printed foods.

Keywords: Rheology; beans; plant-based ink.

Funding: CNPq and FAPESP

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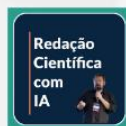
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ISBN
978-85-89436-02-1