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Abstracts

β-LACTOGLOBULIN A NEW WHEY: PRODUCTION AND SCREENING OF NOVEL β-LACTOGLOBULIN VARIANTS THROUGH PRECISION FERMENTATION

1. Engineering microbial cell factories

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Abstract text: Protein engineering and precision fermentation provide an opportunity to increase the value of food proteins by improving their functionality or nutritional quality. Here, we use β-lactoglobulin (βLG) as a model protein to investigate whether state-of-the-art computational protein design approaches can be applied to food proteins. Novel βLG variants were designed using either the deep learning-based protein design tool ProteinMPNN to increase thermal stability and solubility or the physics-based modeling platform PyRosetta to increase branched-chain amino acid (BCAA) content. A total of 27 novel βLG variants with 84–99% sequence identity to wild-type βLG-B were generated, cloned into pET-28a expression vectors, and expressed in Escherichia coli SHuffle T7. The selected variants (n=8) were characterized by secondary structure, oligomeric state (HPLC-SEC), thermal stability, interfacial properties, retinol binding, and in vitro digestibility (INFOGEST). Several variants exhibited increased thermal stability and elevated BCAA content compared with wild-type βLG. This work provides a proof of concept that both deep learning-based and physics-based protein design approaches can be applied to food proteins and demonstrates how improvements in thermal stability or nutritional composition influence other food-relevant properties.

RAMAN SPECTROSCOPY AS A REAL-TIME PROCESS CONTROL TOOL IN PRECISION FERMENTATION

2. Scalable fermentation

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Abstract text: Optimal conditions during precision fermentation require development of process analytical techniques to give timely feedback for process control. Raman spectroscopy is a rapid and non-destructive technique suitable for in-line measurements with potential to analyze growth media composition in cellular agriculture due to its high chemical specificity, satisfactory sensitivity and insensitivity to water. In this research, we employed *Komagataella phaffii* (*Pichia pastoris*) designed to produce goose ovalbumin, using either glucose or glycerol as carbon source. Raman spectra were collected every minute during the 30+ hour fermentations with a MarqMetrix All-In-One instrument equipped with an immersion probe (Thermo Fisher Scientific Inc., Waltham, MA, USA), resulting in approx. 2000 spectra from each run. Eleven samples for HPLC reference analysis were extracted throughout each run. Glucose and glycerol concentrations started at approx. 43 g/L and ended at 0 g/L. Partial least squares regression models for glucose and glycerol concentrations were developed, where the glucose model achieved a coefficient of determination (r^2) of 0.99 with a root mean square error of cross validation (RMSECV) of 1.9 g/L, while the glycerol model achieved an r^2 of 0.99 with an RMSECV of 2.3 g/L. These results clearly demonstrate that Raman spectroscopy is a suitable tool for monitoring nutrient consumption in the growth medium, thereby enabling improved decisions on batch termination or implementation of continuous feeding strategies to optimize production in real-time. Spectra contains information about many molecules in the media, thus there is a potential to follow other critical parameters, such as nitrogen consumption or product formation.

UTILIZATION OF WHOLE LIPID-CONTAINING MICROBIAL BIOMASS IN HIGH-MOISTURE EXTRUSION PROCESSING FOR THE PRODUCTION OF PEA PROTEIN-BASED MEAT ANALOGUES

3. Pretreatment and fermenting biomass

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Abstract text: Growing demand for sustainable food systems highlights the need for alternative lipid sources that reduce reliance on land-intensive plant- and animal-based lipids. While cellular agriculture-based microbial fermentation offers a promising route, high downstream processing costs, particularly solvent-based lipid extraction, limit commercial feasibility, This study investigated the direct utilization of whole *Rhodotorula toruloides 4444* as a functional, lipid-containing ingredient in combination with pea protein isolate (PPI) to obtain high-moisture meat alternatives (HMMA), eliminating the extraction step while retaining the native intracellular lipid matrix. For the economic viability and sustainability perspectives, molasses was used as a carbon source.

R.toruloides 4444 were cultivated in two-stage (growth and lipid accumulation) fermentation at 5 L and 40 L scales, achieving dry cell weights of 21.2 g/L (8.55 log CFU/mL) and 28.8 g/L (8.88 log CFU/mL), respectively. In both scales, oleic acid was the predominant fatty acid (~53% of total lipids), followed by palmitic acid, demonstrating consistent lipid profiles across scales.

Dried yeast biomass (13.3% w/w) and PPI (86.7% w/w) were combined and extruded to obtain HMMA. Processing parameters included barrel temperatures of 130–150 °C, screw speeds of 150–450 rpm, and a water content of 60% (w/w). Texture profile analysis showed that biomass inclusion significantly reduced hardness, gumminess, and chewiness relative to control extrudates while maintaining structural integrity. These results indicate that whole-cell *R.toruloides 4444* could potentially contribute to desirable tenderness in HMMA, providing a cost-effective route for incorporating microbial lipids in food applications.

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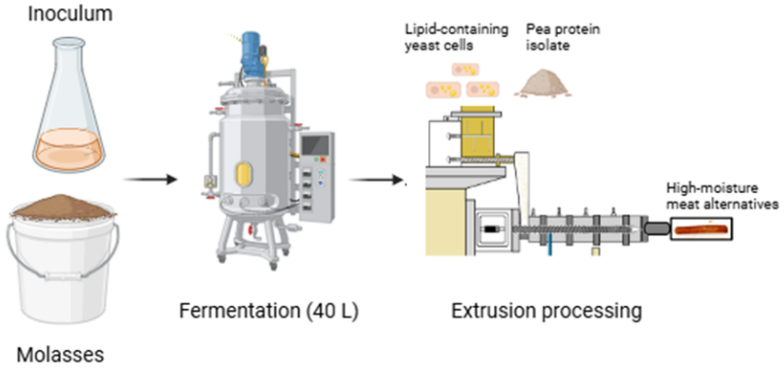


Figure 1. Process overview of the production and incorporation of lipid-containing yeast biomass in high-moisture meat alternatives.

Upcycling Potato Side Streams via Fusarium venenatum Solid State Fermentation: An Approach to Sustainable Mycoprotein Production

4. Solid-state and low-energy pathways

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Abstract text: The valorization of industrial side streams represents a key opportunity for improving the sustainability and circularity of modern food systems. Potato processing generates substantial volumes of nutrient-rich side streams that remain underexploited despite their potential as substrates for microbial biomass production. In collaboration with an industrial partner seeking to reduce waste and increase resource efficiency, this study investigates the solid-state fermentation (SSF) of potato side streams using *Fusarium venenatum*, a filamentous fungus widely recognized for its capacity to produce high-quality mycoprotein. The objective is to characterize the biochemical, structural, and molecular transformations occurring during SSF and to evaluate the nutritional and functional properties of the resulting mycoprotein.

Preliminary results indicate that *F. venenatum* effectively colonizes potato substrates under SSF at pH 5.8 and 28°C, leading to measurable shifts in macromolecular composition and the formation of a protein-rich biomass, including an increase of 3% (dry weight) of lysine, with potential food application. The integration of analytical and physicochemical approaches is expected to provide insights into structure–function relationships relevant to texture formation, digestibility, and techno-functional behavior. Carbohydrate profiling can also elucidate substrate utilization patterns and their influence on fungal growth and metabolite production.

By combining SSF with high-resolution analytical workflows, this work aims to establish a framework for upgrading potato side streams into value-added, sustainable protein ingredients that can be included in the production line of the industrial partner. The findings will contribute to the broader understanding of fungal bioconversion processes and support future efforts toward process optimization and industrial upscaling.

Beyond Orchestration: How Anchor Organizations Shape Research-to-Impact Pathways in Sustainable Protein Innovation Ecosystems Under Uncertainty

5. From reactor to reality

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Abstract text: The development of sustainable protein innovation ecosystems (SPIE) has significantly benefited from the orchestrations provided by anchor organizations, such as innovation hubs, and collaborative platforms, which play important roles in the research-to-impact pathways. However, increasing technological, regulatory, market, and geopolitical uncertainties are creating new challenges for those organizations in terms of adaptability and resilience beyond orchestration.

Orchestration has been defined as a set of actions undertaken by anchor organizations to coordinate networks and resources to create value from the innovation ecosystem (IE). Existing research has largely examined orchestration roles of anchor organizations (e.g., universities, firms) isolated within relatively stable contexts and predefined boundaries of developed IE, while limited attention has been paid to how anchor organizations such as innovation hubs and collaborative platforms navigate uncertainty and shape IE under changing conditions. To address this gap, rather than assuming orchestration as the primary function, the study examines whether additional roles emerge in practice by exploring the roles of anchor organizations in SPIE under uncertainty. In doing so, a comparative and explorative case study is conducted between the Bezos Centre for Sustainable Protein at Imperial College London in UK, and Biotech Heights in Sweden.

This study extends the understanding of traditional orchestration perspectives by emphasizing collective learning across interconnected actors in terms of adaptability and resilience of SPIE. By drawing insights from the national contexts between the UK and Sweden, practical implications are offered for universities, innovation hubs, firms, funding agencies, and policymakers seeking to strengthen the capability of SPIE under uncertainty.

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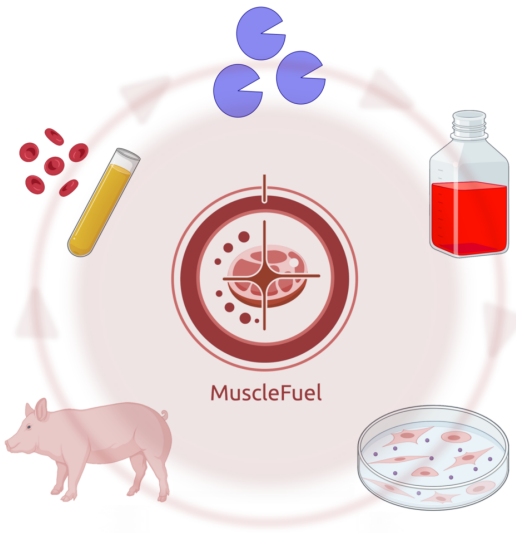
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REPLACEMENT OF BASAL MEDIA AMINO ACIDS THROUGH TARGETED HYDROLYSIS OF PORCINE BLOOD FROM SLAUGHTERHOUSE BY-PRODUCTS

6. Cultivation of eukaryotic cells

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Abstract text: Media formulation is one of the main challenges when producing cultivated meat. In recent years, advances have led to the transition towards the use of serum-free media, tailored for specific development stages such as proliferation or differentiation. However, to improve the circular bioeconomy of the food sector, there is a potential in using side streams and byproducts from the food industry to produce basal-media macronutrients. In the present study, we explore the use of slaughterhouse blood as a potential source of amino acids to produce cultivated meat. We hydrolyzed porcine plasma using 10 different proteases and tested the hydrolysates as a replacement of basal media, targeting the amino acids. Moreover, we further analyzed the amino acid composition of each hydrolysate. Our results show that, using pepsin and trypsin hydrolysates can support cell proliferation by replacing DMEM/F12 from the media formula. Further research is needed to verify and improve the efficiency of using byproducts from the food industry as replacers of basal media.



ADAPTIVE LABORATORY EVOLUTION AND METABOLIC NICHE PARTITIONING ENABLE EFFICIENT VALORIZATION OF WHEAT STRAW HEMICELLULOSIC HYDROLYSATE

1. Engineering microbial cell factories

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Abstract text: The increasing demand for sustainable alternatives to fossil-based products has brought attention to the need for efficient microbial cell factories capable of valorizing lignocellulosic hydrolysates. Hemicellulosic hydrolysate (HCH), derived from agricultural residues such as wheat straw, represents a promising renewable feedstock for the production of value-added chemicals. However, microbial growth and bioconversion of HCH are hindered by toxic compounds and the complexity of utilizing multiple carbon sources simultaneously.

In this study, *Escherichia coli* strains were engineered to valorize the main carbon sources present in wheat straw HCH. The strains were designed as acetic acid auxotrophs and engineered either to co-utilize pentoses and hexoses in a single strain or to utilize specific substrates through niche partitioning in a microbial consortium. To improve growth and tolerance to inhibitory compounds present in HCH, the strains were subjected to adaptive laboratory evolution (ALE).

The evolved strains were evaluated for growth, substrate utilization, and production of isobutyric acid, a precursor for various platform chemicals. Several evolved variants exhibited improved growth and metabolic performance in HCH and demonstrated efficient conversion of hemicellulose-derived sugars into isobutyric acid. During fed-batch fermentations, the optimized consortium outperformed the co-valorizing strain, achieving a final titer of 21 g L^{-1} and a carbon yield of 0.40 Cmol $Cmol^{-1}$, corresponding to 65% of the theoretical maximum.

These results demonstrate that ALE combined with acetic acid auxotrophy and consortium-based approaches can significantly improve the valorization of complex hemicellulosic hydrolysates.

Machine learning assisted optimization of essential omega-3 production in yeast Apiotrichum brassicae

1. Engineering microbial cell factories

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Abstract text: Microbial lipids represent a sustainable route to produce tailored fatty acid profiles, with potential to improve both nutritional value and flavour development in plant-based foods. Polyunsaturated fatty acids such as linoleic acid and α-linolenic acid (ALA) contribute to health benefits and serve as precursors for meaty flavour. In this work, we applied a dual-strategy approach involving optimisation of cultivation parameters using machine learning, specifically Bayesian modelling, and genetic engineering to increase omega fatty acid content in the oleaginous yeast *Apiotrichum brassicae*.

Bayesian modelling identified temperature and carbon-to-nitrogen ratio as key factors influencing polyunsaturated fatty acid content. Optimising cultivation parameters for the wild-type strain increased ALA levels from 0.4% to 6.6% of total lipids. To further increase ALA levels, a genetically engineered strain overexpressing a bifunctional Δ12/Δ15 desaturase was constructed. When cultivated under model-derived conditions, this strain exhibited a substantial increase in ALA accumulation, reaching above 20% of total lipids.

To evaluate scalability of the model-derived cultivation conditions, 1 L bioreactor cultivations were performed using the wild-type strain. Comparable or higher ALA titres were obtained relative to shake flask experiments, despite a lower ALA proportion in the lipid fraction.

Overall, this work demonstrates the potential of *Apiotrichum brassicae* as a flexible platform for producing tailored lipids and highlights the importance of predictive modelling and targeted genetic engineering for systematic optimisation.

PRODUCTION OF AROMATIC AMINO ACIDS AND DERIVATIVES FROM BEECH WOOD CELLULOSIC HYDROLYSATES

1. Engineering microbial cell factories

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Abstract text: The three aromatic amino acids L-tyrosine, L-phenylalanine, and L-tryptophan form the basis for production of a variety of products ranging from medicine over materials, to feed and food additives. Being derived from the common central shikimate pathway, they provide a biological alternative to otherwise chemically synthesised building blocks. If produced via fermentation of renewable resources this cannot only lead to the production of complex compounds but can also improve the ecological footprint of otherwise petrol-derived compounds.

In this project, we designed and engineered three strains based on *Escherichia coli* BL21 for the efficient production of L-tyrosine, L-phenylalanine or L-tryptophan. To speed up the process of generating the numerous deletions and integrations needed to obtain efficient production strains, we first developed a new integrative deletion and marker removal system applicable to all *E. coli* strains. We subsequently used this system to introduce 12-16 deletions and 11-19 overexpressed genes in different genomic locations under the control of an inducible promoter to increase the metabolic flux towards the aromatic amino acids. By using a tight, inducible, and titratable promoter, we further enabled concomitant tuning of the flux towards both the aromatic amino acids, as well as downstream pathways controlled by alternative inducible promoters for more efficient production of downstream products.

Shake-flask cultivations demonstrated that the strains show strong production performance from cellulosic beech wood hydrolysate compared to classical refined glucose. Overall, this work provides a robust foundation for sustainable biomanufacturing from lignocellulosic feedstocks and expands the toolbox for microbial production of aromatic compounds.

BIOREACTOR PRODUCTION OF RECOMBINANT AVIAN OVALBUMINS IN KOMAGATAELLA PHAFFII

2. Scalable fermentation

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Abstract text: Precision fermentation is being explored as a route for animal-free production of food proteins, and ovalbumin is a relevant target because it is the major protein in egg white. This study evaluated controlled bioreactor production and downstream characterisation of recombinant ovalbumin from chicken (*Gallus gallus*) and goose (*Anseranas semipalmata*) in engineered *Komagataella phaffii*. Two codon-optimised chicken ovalbumin constructs encoding the same protein were first compared using shake-flask cultivation , and the higher-producing construct was selected for subsequent recombinant chicken ovalbumin experiments. A codon-optimised goose ovalbumin homolog was also expressed and secreted, extending the system beyond a single avian protein. In controlled batch bioreactor cultivation, both recombinant chicken and goose ovalbumin strains reached high cell density and showed similar overall process trajectories, with harvest titers approaching 50 mg L^{-1}. Electrophoretic analysis and Endoglycosidase H treatment showed that both recombinant products displayed glycosylation-related heterogeneity. Ammonium sulphate precipitation enabled effective enrichment of recombinant ovalbumin, while anion-exchange chromatography revealed broad dominant peaks containing multiple ovalbumin variants consistent with glycosylation differences. Together, the results demonstrate bioreactor production of recombinant avian ovalbumins in engineered *K. phaffii* and highlight heterogeneity in the secreted products, with potential implications for ongoing characterisation and future application.

REPLACING FRESH WATER IN SUBMERGED FUNGAL PROTEIN PRODUCTION BY PAECILOMYCES VARIOTII

2. Scalable fermentation

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Abstract text: Fresh water scarcity is an increasing risk for food systems, including submerged microbial fermentation as a developing approach to sustainable protein production. Replacing pure water in cultivation media could enhance the sustainability of production by reducing dependence on fresh water.

In this study, alternative liquids were evaluated for submerged cultivation of the edible filamentous fungus *Paecilomyces variotii.* YPD (yeast extract-peptone-dextrose) media were prepared using Milli-Q water, artificial sea water, artificial brackish water, artificial urine, Baltic Sea water, or Atlantic Ocean water. Shake flask trials were used to screen growth and crude protein content. Following screening, Baltic Sea and Atlantic Ocean waters were further studied in 10 L bioreactors to quantify biomass yield, protein content, and amino acid composition. A sensory evaluation compared biomass produced with Baltic Sea water to the Milli-Q control.

In shake flask screening, *P. variotii* showed robust growth in YPD prepared with each alternative liquid, with no reduction in biomass formation compared to the control. In bioreactor cultivations, Ocean water significantly increased biomass yield to 19.9 g/l compared to 11.2 g/l in Milli-Q, whereas Baltic Sea water resulted in similar growth (10.6 g/l). Amino acid profiles remained similar, but total protein content increased when using Baltic Sea (29.5 % DM) and Ocean water (31.6 % DM) compared to Milli-Q (26.0 % DM). Sensory evaluation detected no statistically significant differences between biomass produced with Baltic Sea water and that produced with Milli-Q.

Findings indicate that alternative water sources can maintain fungal protein production, supporting more resilient food systems.

Sustainable production of biodegradable plastics from bioethanol

2. Scalable fermentation

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Abstract text: Polyhydroxyalkanoates (PHAs) production from biomass still faces challenges, including high costs, low yields, and poor scalability. Bioethanol, derived from biomass, offers an attractive alternative feedstock thanks to its availability and established industrial infrastructure.

In this project, commercial ethanol at 60 g/L was efficiently oxidized into acetic acid using resting cells of *Gluconobacter oxydans*, achieving a 74% yield with 100% selectivity. Ethanol (45%) from Lantmännen was also tested with *G. oxydans* DSM 50049 in a lab-scale bioreactor. At a higher concentration of 41 g/L ethanol, cell activity dropped, resulting in only a 62% yield despite full selectivity. The reduced yield was linked to ethanol evaporation caused by the higher aeration requirements and extended reaction time. This limitation was overcome by lowering the ethanol concentration to 27.5 g/L, applying 40% aeration, condensing vapors at +4 °C, and shortening the reaction time. Hence, the final acetic acid yield was improved to reach 100% after 31h of the reaction.

For PHA production, sodium acetate was used as a carbon source by *Cupriavidus necator* and *Cobetia marina* in a lab-scale bioreactor. The results showed that *C. marina* displayed much higher tolerance, growing and accumulating PHA at 20 g/L acetate, compared with *C. necator*, which managed only 10 g/L. Under controlled conditions, *C. marina* further demonstrated the ability to consume 35 g/L acetate in fed-batch mode, reaching an OD of 15.

Together, these findings show a renewable pathway to PHA production that reduces dependence on fossil fuels while supporting the global push for sustainable plastics.

THE BIOTECHNOLOGY FERMENTATION FACTORY (BFF): FROM CONCEPT TO MARKET!

2. Scalable fermentation

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Abstract text: As the biomanufacturing sector expands across industries—from healthcare and agriculture to food and consumer products—integrated solutions that bridge upstream and downstream processes are critical to meet growing and diverse product demands. The Biotechnology fermentation factory (BFF) is a state-of-the-art technical facility uniquely equipped to support the full bioprocessing spectrum, offering advanced infrastructure and deep expertise in process development, scale-up, and the path for regulatory approval. The BFF enables seamless transition from lab to pilot to manufacturing scale, supporting recovery, purification, and formulation of a wide array of biologically-derived products, as well as preparing material for clinical trials, speeding up the path from concept to market. With a strong focus on holistic process integration, product validation and cost optimization, the facility empowers innovators to navigate complex development challenges while enhancing scalability, productivity, and sustainability across biomanufacturing workflows. The BFF is enabled by NIZO, offering the largest foodgrade pilot plant in Europe, analytics and EFSA support.

An example will be shared regarding precision fermentation with methanol induction, an automation program was developed to both maximize productivity and keep operating within safe conditions.

EVALUATING THE PREBIOTIC POTENTIAL OF GREEN MACROALGAE

3. Pretreatment and fermenting biomass

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Abstract text: Keywords: marine macroalgae, green macroalgae, ulvan polysaccharide, ulvan oligosaccharides, gut microbiota

Macroalgae are fast-growing, photosynthetic organisms found globally and play an important role in the marine environment and their high abundance makes them an attractive source for food, pharmaceuticals and biotechnological applications [1]. Green macroalgae are rich in highly sulphated polysaccharides, predominantly ulvan, with a rare monosaccharide composition which is vastly different from other terrestrial polysaccharides [2]. Given its complex structure, ulvan remains non-digestible in the upper gastrointestinal tract which highlights its potential as a dietary fiber. This allows it to reach the colon intact, where it can act as a selective carbon sourse for health-promoting gut bacteria [3]. This study aims to evaluate the prebiotic potential of *Ulva* sp. as well as ulvan polysaccharide and depolymerized ulvan oligosaccharides generated by an in-house characterized ulvan lyase. To investigate their metabolic fate, the substrates will be processed through anaerobic fermentation using an *in vitro* anaerobic gut model with human fecal sample. Gas production and pH will be monitored throughout the fermentation. Additionally, changes in the algal carbohydrate composition, the accumulation of microbial metabolites including short-chain fatty acids and the taxonomic shift in the microbiota composition will be analyzed.

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PROCESS MINIMISATION OF COLLAGEN-GELATIN EXTRACTION FROM HERRING BONE RAW BIOMASS

3. Pretreatment and fermenting biomass

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Abstract text: The growing accumulation of marine processing waste presents a significant environmental challenge and an economic loss for both the global and regional fishing industries. Fish bones accounting for a substantial portion of fish filleting by-products, represent an underutilized yet rich source of proteins. Traditional methods often overlook the high-value potential of these proteins, which can be upcycled into premium bio-ingredients for various food and biotechnology applications. Extracting collagen and gelatine fractions from raw fish bone biomass offers a sustainable and commercially viable alternative to conventional mammalian sources. Baltic herring (*Clupea harengus membras* L.) bone biomass is one of the remaining unexploited sources of collagen and gelatine. Well-established methods for protein extraction from fish bones could be relatively easily implemented for extracting collagen and gelatine fractions from this type of biomass, however, the process requires fine-tuning, particularly to develop a food-grade, scalable extraction pipeline. Wet-frozen herring bones collected after filleting (Skillinge Fisk-Impex AB) were used for extraction. The initial food-grade extraction was based on extensive biomass washing with high-ionic-strength salt solutions and acid-temperature extraction of the protein fraction, followed by liquid extract desalting and moisture removal via lyophilization. This extraction process ensured a yield of high-quality collagen reaching approximately 20 g/kg of bone biomass. Amino acid composition analysis confirmed the expected ratios typical for collagens. Process optimization was performed to reduce time and cost by improving the moisture-removal and extraction steps, as well as simplifying the process to ensure direct gelatine extraction.

PROCESS DEVELOPMENT AND TECHNO-ECONOMIC ASSESSMENT OF ENZYME PRODUCTION

5. From reactor to reality

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Abstract text: Keywords: sustainability, biorefinery, industry, technoeconomic analysis

Sustainability has become an increasingly important focus over the last decade, driving the development of innovative strategies to address environmental challenges and resource limitations. At the same time, the global food and consumer markets continue to expand, increasing the demand for raw materials while placing pressure on carbon emissions and non-renewable resources. Therefore, more sustainable approaches for valorizing industrial side-streams are urgently needed. One promising strategy is the use of recombinant bacterial strains to produce enzymes for the targeted hydrolysis and conversion of biomass feedstocks, enabling the development of new food products and biopolymers for high-value material applications. In addition, carbohydrate-rich side-streams generated during the processing steps can be valorized as feedstocks for microbial fermentation, thereby supporting the development of a circular bioeconomy approach. Enzymatic hydrolysis is a mild treatment that enables specific bond cleavage that preserves product quality under moderate temperatures and near-neutral pH. When lead enzyme candidates with specific activities have been identified and characterized at laboratory scale, the data can then be used to assess large-scale industrial feasibility using process simulation software, such as SuperPro Designer and Aspen Plus. Here, we report on the concept development and industrial application of enzymes produced by recombinant bacteria, highlighting its potential as a sustainable alternative for future large-scale biomass valorization.

EFFECT OF MICROBIAL EXTRACTS ON MAMMARY EPITHELIAL CELLS VIABILITY AND REAL-TIME GROWTH FOR CELLULAR AGRICULTURE APPLICATIONS

6. Cultivation of eukaryotic cells

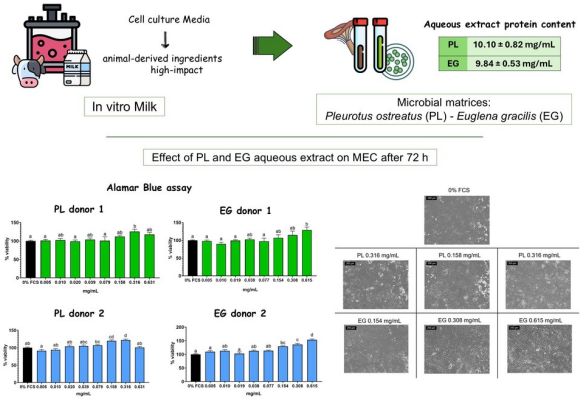
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Abstract text: The identification of innovative low-impact, non-animal-derived cell culture ingredients is essential for the sustainable advancement of cellular agriculture. Microbial matrices may provide bioactive compounds with potential beneficial effects on cell growth and differentiation. This study evaluated aqueous extracts from *Pleurotus ostreatus* (PL) and *Euglena gracilis* (EG) on primary bovine mammary epithelial cells (MEC) isolated from two donors. Extracts were prepared from 0.5 g of lyophilized microbial matrix in 10 mL ddH_2O. MEC were exposed for up to 72 hours to serial dilutions of each extract, corresponding to protein concentrations of 0.631–0.005 mg/mL for PL and 0.615–0.005 mg/mL for EG. Cell viability and morphology were assessed using AlamarBlue assay and inverted light microscopy. Since preliminary results showed no difference between 0% and 10% fetal calf serum (FCS), serum-free conditions were used as control. Three most effective concentrations were selected for real-time monitoring of MEC proliferation using the xCELLigence® system. Initial stock protein concentrations, determined by BCA assay and expressed as BSA equivalents, were 10.10±0.82 and 9.84±0.53 mg/mL for PL and EG, respectively. PL increased MEC viability at 0.316, 0.158 and 0.079 mg/mL, EG was effective at 0.615, 0.308 and 0.154 mg/mL. PL at 0.316 mg/mL and EG at 0.615 mg/mL significantly increased viability compared with 0% FCS. At these concentrations, cells appeared more compact, with reduced intercellular spacing. xCELLigence-derived parameters did not reveal a consistent proliferative advantage. Overall, PL and EG extracts may transiently enhance MEC viability and growth, although further optimization is needed to determine their sustainability as functional components.

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https://doi.org/10.1016/j.fufo.2024.100340

Effect of microbial extracts on Mammary epithelial cell viability and real-time growth for cellular agriculture applications

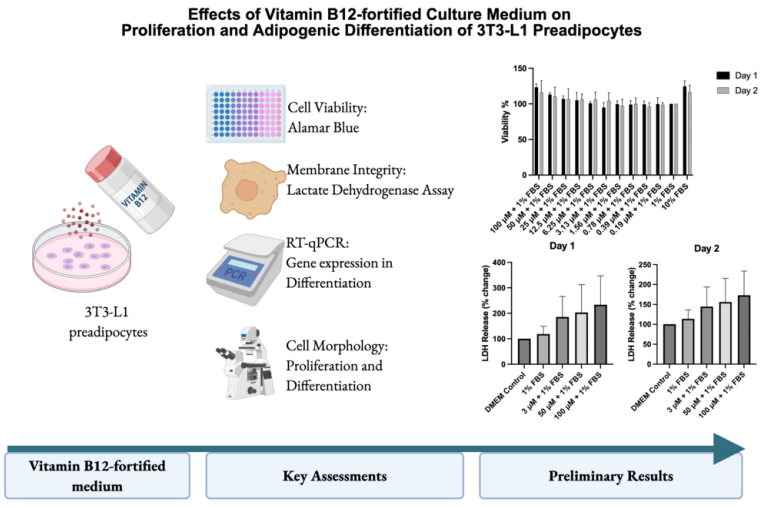


Effects of Vitamin B12-fortified Culture Medium on Proliferation and Adipogenic Differentiation of 3T3-L1 Preadipocytes

6. Cultivation of eukaryotic cells

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Abstract text: Although cultivated meat (CM) is emerging as a potential alternative protein source, evidence suggests that it may lack certain micronutrients that are abundant in conventional meat products, especially vitamin B12. This study investigated the effect of vitamin B12-fortified proliferation medium on 3T3-L1 preadipocytes by evaluating cell viability (Alamar Blue assay), membrane integrity (lactate dehydrogenase; LDH), and cell morphology during proliferation (Days 1 and 2) and differentiation (Day 10). Furthermore, four key genes associated with adipogenic differentiation (CEBPa, CEBPb, FASN, PLIN) were assessed after complete differentiation on Day 10 for selected concentrations. During proliferation, preadipocytes were cultured in medium containing (i) vitamin B12 (0.1–100 µM) with 1% fetal bovine serum (FBS), (ii) 10% FBS (positive control) or (iii) 1% FBS (negative control). Subsequently, cells were switched to adipogenic differentiation medium (10% FBS, 0.5 mM IBMX, 1 µM dexamethasone, 10 µg/mL insulin) until Day 10. Our preliminary results showed that these concentrations (0.1–100 µM) did not significantly alter viability or membrane integrity ($p > 0.05$). Based on the absence of significant cytotoxicity across the tested range, three representative concentrations, 100, 50, and 3 µM with 1% FBS, were selected for further gene expression analysis. Our preliminary RT-qPCR analysis showed that increasing concentrations of vitamin B12 were associated with decreased expression of differentiation-related genes. Further investigation of vitamin B12 accumulation in the cells is needed.



STIMULATION OF MUSCLE STEM CELLS USING AGRI-FOOD SIDE STREAMS FROM THE FOOD PROCESSING SECTOR

6. Cultivation of eukaryotic cells

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Abstract text: One of the key challenges for cultured meat (CM) is achieving economically and environmentally sustainable large-scale production. A major cost driver is the cell culture medium required for nutrient supplementation and efficient growth of muscle stem cells (MuSCs). The medium must be affordable, available in large quantities at industrial scale, and free of animal-derived ingredients.

Plant-based ingredients are increasingly recognized as a nutrient-rich, sustainable and ethical alternative to animal-derived inputs. Despite their limited proliferative capacity, the utility of MuSCs as a cell source for CM production can be enhanced by enriching the culture medium with stimulatory compounds such as bioactive phytochemicals. These compounds have been shown to promote muscle cell proliferation, reduce protein degradation, and improve muscle metabolic function.

Agri-food side streams, such as pomace and surplus plant materials from the frozen vegetables industry, are rich in bioactive phytochemicals and remain a largely underutilized resource. However, the discovery of bioactive compounds from these natural sources is often challenged by the complexity of crude extracts.

To address this, we utilize a bioassay-guided fractionation (BGF) approach where crude plant extracts undergo multiple rounds of fractionation and biological testing until pure bioactive or anti-proliferative compounds are isolated and identified. BGF is achieved by preparative high-performance liquid chromatography (prepHPLC) and characterized using liquid chromatography-mass spectrometry (LC-MS).

In this study, we demonstrate that fractions from phytochemical-rich cauliflower extracts have potential as stimulators of MuSCs proliferation.

VALORISATION OF FRUIT AND VEGETABLE PROCESSING SIDE STREAMS IN PLANT CELL PROPAGATION– NOVEL PLANT CELL CULTURE INGREDIENTS FOR FOOD AND COSMETIC USE

6. Cultivation of eukaryotic cells

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Abstract text: Agri-food side streams offer an underutilised resource for improving sustainability and cost-efficiency of cellular agriculture. We studied ten fruit- and vegetable-derived side streams from BonneJuomat, a Finnish producer of plant-based juices and purees, and a potato processing side stream from Finnamyl, as culture medium components. Rinsing waters, spillover juices or purees from bilberry, cherry, apricot, carrot and mango were tested as carbon sources and water replacement, while the potato side stream was assessed as macronutrients. Several side streams supported the growth of wild raspberry, cloudberry, arctic bramble, bilberry and licorice cell lines. Based on screening, selected side streams were scaled upto 5–100L cultivations for biomass and ingredient evaluation. In 5L cultivations, cherry and bilberry side streams yielded 0.6–1.6kg fresh weight biomass of wild raspberry, cloudberry, bilberry and licorice, while 100L cultivation produced up to 22.7kg wild raspberry biomass. Biomass grown in side stream-based media contained 19–27% protein of dry weight, and total phenolic content was maintained at level of conventional medium, with increases observed in some cases. Exploratory sensory evaluation indicated that differences between biomass produced in side stream-based and conventional media were smaller than those between plant cell biomass and conventional berry reference materials. In smoothies, samples were broadly similar in odour and taste, with the main differences in texture-related attributes. Promising results were also obtained in cosmetic testing. The results demonstrate feasibility of valorising food industry side streams as functional culture medium components for circular and resilient production of plant cell-based food and cosmetic ingredients.