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BIOBASED FUTURE: GREEN BIOPROCESSING FOR INNOVATIVE BIOACTIVE PRODUCTS

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CONFERENCE AGENDA

MONDAY, JUNE 16, 2025

09:00-10:00 Registration
10:00-10:20 Opening

Session: Interaction between human microbiota and health
Chairs: Patrick Jansen and Marija Ćorović

10:20-11:00 John Penders (Maastricht University, the Netherlands)
Plenary lecture - Human milk oligosaccharides (HMOs) and other prebiotics: Supporting microbiome resilience in early life and beyond

11:00-11:30 Mirjana Rajilić-Stojanović (Faculty of Technology and Metallurgy (FTM), University of Belgrade, Serbia)
Keynote lecture - Unlocking Plant Bioactives for Gut Microbiota Modulation through Innovative Extraction Methods

Coffee break and poster session

12:30-13:00 Ellen H. van den Bogaard (RadboudUMC, Nijmegen, the Netherlands)
Keynote lecture – Modeling the crosstalk between the microbiome and human skin

13:00-13:15 Ana Bačić (FTM, University of Belgrade, Serbia)
Exploring the Prebiotic Potential of Yarrow (Achillea millefolium) on Gut Microbiota in the TIM-2 Model

13:15-13:30 Noor van Hout (RadboudUMC, Nijmegen, the Netherlands)
Expanding the possibilities of the stratum corneum model for bacterial growth

Lunch break and poster session

Session: Prebiotics
Chairs: Milica Simović and Antonia Montilla

14:30-15:10 Dušan Veličković (Pacific Northwest National Laboratory, Richland, USA)
Plenary lecture - Analysis of carbohydrates and glycoconjugates by advanced mass spectrometry tools

15:10-15:40 Thu-Ha Nguyen (BOKU University, Wien, Austria)
Keynote lecture – Biosynthesis of novel prebiotics

Coffee break and poster session

16:10-16:40 F. Javier Moreno (CIAL, CSIC-UAM, Madrid, Spain,)
Keynote lecture – Integrated approaches to producing and designing prebiotic carbohydrates from agri-food resources

16:40-16:55 Galina Jevđenović (FTM, University of Belgrade, Serbia)
Conifer Cone as Sustainable Source of Antimicrobial and Prebiotic Compounds

16:55-17:10 Lara Denić and Teodora Zakić (Innovation Centre of the FTM, Belgrade, Serbia)
Evaluation of prebiotic potential of oilseed meals extracts

17:30 Welcome cocktail

TUESDAY, JUNE 17, 2025

09:00-10:00 Registration and poster session
10:00-10:05 Opening

Session: New and improved biocatalysts and biosensors
Chairs: Cezar Mateo and Thu-Ha Nguyen

10:05-10:45 Nenad Milosavić (Department of Medicine, Columbia University, New York, USA)
Plenary lecture - From random artificial single-stranded DNA library to real time target monitoring – through the eyes of a biochemist

10:45-11:15 Jose Miguel Palomo (ICP-CSIC, Madrid, Spain)
Keynote lecture – Innovating Biocatalyst Design: Modern Strategies for Enhanced Enzyme Functionality

11:15-11:30 Ana Vukoičić (Innovation Centre of the FTM, Belgrade, Serbia)
Synthesis of flavonoid oligomers catalyzed with artificial metalloenzymes

Coffee break and poster session

12:30-13:00 Pavle Andrić (Novonesis, Denmark)
Keynote lecture – Immobilised Enzymes: Robust Biocatalysts for Bioprocess Intensification

13:00-13:20 Anja Kostelac (BOKU University, Wien, Austria)
Novel biocatalysts for biosynthesis of specific galacto-oligosaccharide structures

Lunch break and poster session

Session: Bioprocess intensification
Chairs: Nikola Nikačević and Pavle Andrić

14:30-15:10 Dirk Holtmann (Karlsruher Institut für Technologie (KIT), Germany)
Plenary lecture - Intensification of bioprocesses

15:10-15:40 Martin Rebros (Institute of Biotechnology, Slovak University of Technology, Bratislava, Slovakia)
Keynote lecture – Recombinant enzyme production, its intensifications and applications

Coffee break and poster session

16:10-16:30 Victor Sans (Universitat Jaume I, Spain)
Advancing Biocatalysis with 3D-Printed Continuous Flow Reactors

16:30-16:50 Tamara Janković (Delft University of Technology, the Netherlands)
Advanced distillation-based purification processes for effective recovery of fermentation products

20:00 Conference dinner

WEDNESDAY, JUNE 18, 2025

09:00-10:00 Registration and poster session

Session: Bio-based products: from laboratory to industry
Chairs: Ellen van den Bogaard and Rada Pjanović

10:00-10:40 Juan Manuel Bolivar (Faculty of Chemical Sciences, Complutense University, Madrid, Spain)
Plenary lecture – Biocatalytic processes for converting waste into bio-based platform chemicals

10:40-11:05 Kristofer Cook (Carbiotix, Sweden)
Keynote lecture – Pioneering the onsite upcycling of plant-based side-streams

11:05-11:30 Alexandre Fortier (*ALGAKTIV*, Barcelona, Spain)
Keynote lecture – Upcycled Collagen-Building Peptides from Microalgae Biotechnology: A Circular Approach to Skin Nutrition with ALGAKTIV® Collage

Coffee break and poster session

12:30-12:55 Milica Perović (Faculty of Technology, University of Novi Sad, Serbia)
Keynote lecture – Enzymes as a tool for the improvement of protein isolates from underutilized plant sources: A chickpea case study

12:55-13:10 Pranesh Kannappan Karthikeyan (Loughborough University, United Kingdom)

Microbubble Plasma-Enhanced Oxidative Pretreatment for Improved Biogas Yield from Marginal Biomass

Lunch break and poster session

14:10 – 15:10 Roundtable: How to accelerate transfer of bio-based innovative ideas to industry?
Moderators: Dejan Bezbradica, Juan Manuel Bolivar, Kristofer Cook

15:10 – 15:40 Awards and closing ceremony

- BOOK OF ABSTRACTS -

Session

Interaction between human microbiota and health

Chairs: Patrick Jansen and Marija Ćorović

Human milk oligosaccharides (HMOs) and other prebiotics: Supporting microbiome resilience in early life and beyond

John Penders*

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NUTRIM School of Nutrition and Translational Research in Metabolism
Maastricht University Medical Centre

Antibiotics can induce substantial and sometimes long-lasting disruptions in gut microbiome composition and function, particularly during early life. Emerging evidence suggests that prebiotics—including human milk oligosaccharides (HMOs) naturally present in breast milk and synthetic non-digestible carbohydrates—may help mitigate these disruptions and support microbiome development and recovery. In this presentation, I will explore findings from a large birth cohort study linking breast milk HMO composition to microbial colonization patterns in infancy, as well as experimental data from ex vivo models simulating early-life antibiotic exposure.

These models reveal differential effects of HMOs and other prebiotics on microbial resilience and activity, including recovery of key taxa and restoration of short-chain fatty acid production. Translating to the human situation, I will also present results from a randomized controlled trial evaluating post-antibiotic supplementation with 2'-fucosyllactose in adults, highlighting both the potential and limitations of prebiotic support for microbial recovery and host metabolic outcomes. Lastly, I will discuss the broader implications of prebiotic strategies for reducing colonization with multidrug-resistant bacteria and for tailoring interventions to individual microbiome profiles. Together, these findings point to the promise of targeted prebiotic use in promoting microbiome resilience across the lifespan.

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Unlocking Plant Bioactives for Gut Microbiota Modulation through Innovative Extraction Methods

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Polyphenols from medicinal plants represent an underexploited reservoir of bioactive compounds with profound potential to modulate the gut microbiota. Their effects extend beyond classical antioxidant activity, encompassing support for anaerobic respiration, modulation of digestive enzyme function, and disruption of quorum sensing mechanisms that influence microbial communication and community structure. Duplibiotic activity, defined by the ability of polyphenols to simultaneously promote beneficial microbial populations and suppress undesired taxa, carries a unique potential to reshape microbial communities unmatched by any class of prebiotic or antibiotic. However, given the exceptional heterogeneity of polyphenols, their functionality is intricately linked to structural complexity, extractability, and microbial accessibility. Integration of advanced extraction technologies with biotransformation strategies has the potential to enhance the prebiotic and duplibiotic activity of polyphenol-rich extracts. Application of microwave-assisted extraction, optimized through response surface methodology, enables selective and efficient recovery of bioactive polyphenols. Incorporation of halogen elements during extraction further enhances chemical reactivity, resulting in improved antioxidant properties and antimicrobial potential. Fermentation with targeted probiotic strains complements these efforts by increasing extraction efficiency and facilitating the bioconversion of polyphenols into more bioavailable and microbiota-relevant metabolites. The integration of advanced extraction design and microbial bioprocessing enhances the functional profile of plant-based preparations while supporting principles of sustainability and precision nutrition. These insights contribute to the development of next-generation interventions for gut health and open new avenues for translational research at the intersection of ethnopharmacology, green chemistry, and microbiota science.

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Modeling the crosstalk between the microbiome and human skin

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The human skin microbiome plays a crucial role in maintaining skin barrier integrity and immune homeostasis. Dysbiosis of this microbial community is increasingly associated with dermatological conditions such as atopic dermatitis, psoriasis, and acne. The bidirectional relationship between the skin microbiota and the epidermal barrier is complex and influenced by microbial metabolites, host immune responses, and environmental factors. In this lecture current approaches to studying this interaction, ranging from clinical studies to advanced *in vitro* models will be discussed. Human cohort studies, including longitudinal and cross-sectional analyses, provide valuable insights into microbiome composition, variability, and correlation with skin barrier metrics such as transepidermal water loss and lipid profile. However, they are limited by inter-individual variability and ethical constraints. *Ex vivo* skin explants preserve the native tissue architecture and microbial interactions, yet are restricted in viability and experimental manipulation. Emerging 3D organotypic skin models — comprising keratinocytes, fibroblasts, and increasingly, immune components — allow for controlled investigation of microbial colonization and host responses. These models can be colonized with defined microbial communities or patient-derived isolates, enabling mechanistic studies on microbial modulation of barrier function. Collectively, integrating data across human and model systems provides a robust framework to decipher skin-microbiome interactions and inform therapeutic strategies targeting barrier dysfunction and microbial imbalance.

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Exploring the Prebiotic Potential of Yarrow (*Achillea millefolium*) on Gut Microbiota in the TIM-2 Model

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[2] Maastricht University, Centre for Healthy Eating & Food Innovation (HEFI), Campus Venlo, Netherlands

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Introduction: Phenolic compounds from medicinal plants have attracted growing scientific interest for their potential to influence the gut microbiota and, through it, contribute to human health. Among these plants, yarrow (*Achillea millefolium*) has a long history of use in traditional medicine, particularly for treating gastrointestinal disorders. However, despite its rich content of phytochemicals, the interaction between yarrow and the human gut microbiota has remained largely unexplored. Understanding how yarrow and its constituent compounds affect the structure and activity of gut microbial communities may reveal novel mechanisms underlying its traditional therapeutic use.
The aim of this study was to evaluate the modulatory effects of a yarrow extract and a defined mixture of its representative phenolic compounds - apigenin, caffeic acid, and chlorogenic acid, on gut microbiota composition and metabolic activity using the TIM-2 *in vitro* model of the colon.

Experimental: The dynamic, computer-controlled TIM-2 model was used to simulate the environment of the human colon under physiologically relevant conditions. The system was inoculated with pooled fecal microbiota from healthy adult donors. Following an adaptation period, the TIM-2 units were fed with Standard Ileal Efflux Medium (SIEM) with the addition of either the yarrow extract, the phenolic mixture, or a control SIEM over a 72-hour period. Samples were collected at 0, 24, 48, and 72 hours. Bacterial composition was assessed by sequencing the V_3–V_4 region of the 16S rRNA gene using Illumina MiSeq platform, followed by bioinformatics processing and taxonomic classification. To assess microbial metabolic output, short-chain fatty acids (SCFAs; acetic, propionic and butyric acids), branched-chain fatty acids (*iso*-butyric and *iso*-valeric acids), valeric, and hexanoic

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acids were quantified using gas chromatography-mass spectrometry (GC-MS).

Key findings: The yarrow extract strongly modulated the gut microbial community, promoting the growth of health-associated bacterial taxa. Notably, the growth promotion of *Lactiplantibacillus*, a genus containing probiotic species, was observed. The yarrow extract also stimulated the growth of Eggerthellaceae, Christensenellaceae, Butyricicoccaceae, and the *Eubacterium coprostanoligenes* group, microbial groups known for maintaining intestinal homeostasis and producing health-relevant metabolites. The phenolic mixture induced more selective and modest changes in microbial composition, most notably stimulating members of Eggerthellaceae and *Collinsella*. These results suggest that the whole plant extract exerts broader modulatory effects compared to phenolic compounds mixture.

Microbial activity analysis revealed that the yarrow extract significantly increased SCFAs production, particularly acetic and propionic acids, both key metabolites known to support gut barrier integrity, immune modulation, and host energy metabolism. The phenolic mixture led to a moderate rise in SCFAs levels, reinforcing the idea that synergistic effects of compounds in the full extract may be responsible for enhanced microbial activity.

These findings highlight the prebiotic potential of yarrow and its phenolics. Yarrow extract may beneficially modulate gut microbiota composition and function, promoting a community enriched in SCFA-producing and symbiotic bacteria. This study provides a foundation for future investigations into the health-promoting mechanisms of yarrow and supports its further exploration as a functional ingredient for gut health.

Expanding the possibilities of the *stratum corneum* model for bacterial growth

Noor van Hout[1], Patrick Jansen[1*], Patrick Zeeuwen[1], Ellen van den Bogaard[1]

[1] Radboud University Medical Center, Nijmegen, Netherlands

Introduction: The skin barrier, specifically the stratum corneum, plays a vital role in maintaining skin health and protecting against pathogenic microorganisms. Understanding the interactions between commensal and pathogenic bacteria on the skin's surface is important for our understanding of bacterial dysbiosis as seen in atopic dermatitis.

We focus on exploring the potential of the *stratum corneum* model, developed within our lab for pathogen testing and the evaluation of biological agents and antibiotics, to investigate microbe-microbe interactions and mimic disease specific environments. In this study, we examine the interplay between *S. aureus* and *C. acnes* in the context of atopic dermatitis.

Experimental: The stratum corneum model utilizes callus as a nutrients source for bacteria, effectively mimicking the *stratum corneum*. We investigate bacterial growth and survival on the model and explore its customizations by varying agar pH levels, utilizing callus from patients with specific skin mutations, and employing diverse bacterial inoculations combinations and densities.

Key findings: Preliminary findings reveal that the growth and survival of *C. acnes* on the model appear unaffected by the presence of *S. aureus*. Intriguingly, as the population of *C. acnes* diminishes, *S. aureus* seem to takes this opportunity to use these newfound nutrients, to further enhance its growth.

The stratum corneum model demonstrates its potential as a valuable tool for investigating microbe-microbe interactions within disease-specific environments. Our ongoing challenge is to mimic a skin disease within the model, which offers a path for future research.

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Assessment of the potential health-promoting effects of oat flour and husks on gut microbiota

Valentina Nikolić[1*], Slađana Žilić[1], Marijana Simić[1], Katarina Šavikin [2], Tatjana Stević[2], Jelena Živković[2], Beka Sarić[1], Danka Milovanović[1], Vesna Kandić Raftery[1]

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Introduction: Oats are gluten-free grains abundant in dietary fiber, *β*-glucans, phenolic acids, flavonoids, carotenoids, vitamin E, and phytosterols. Traditional medicine has used them for millennia to treat hyperacidity, severe pancreatitis, burns, and dermal irritation. Oat *β*-glucans are a type of soluble fiber that gut bacteria break down in the colon, leading to the production of short-chain fatty acids. Additionally, oat *β*-glucans act as a prebiotic that helps balance gut microbiota and supports the control of diarrhea and related issues by keeping energy levels stable, thanks to its properties such as solubility, thickening, and ability to form gels. Human digestive enzymes and stomach acid break down only small portions of oat *β*-glucan; instead, it moves to the large intestine, where gut bacteria break it down. Phenolic acids offer prospective health benefits and enhance gut health by promoting the proliferation of beneficial bacteria and inhibiting pathogens, thereby supporting overall digestive health.

Experimental: This study investigated the nutritional and phenolic content of oat flour (OF) and powdered oat husks (OHs) from different oat genotypes—white, brown, and black—as well as their antioxidant capacity. Standard laboratory, spectrophotometric and HPLC methods were applied. Antimicrobial activity evaluation was conducted on oat extracts prepared according to a recipe from traditional Serbian medicine and by conventional alkaline hydrolysis and extraction. The antimicrobial effect of oat samples was tested on six important pathogens of the gastrointestinal tract, namely *Listeria monocytogenes*, *Staphylococcus haemolyticus*, *Salmonella typhimurium*, *Enterococcus faecalis*, *Escherichia coli*, and *Shigella flexneri*. The minimal bactericidal concentration (MBC) of hydrolyzed and traditionally prepared oat extracts was determined and expressed in microliters of the preparation.

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Key findings: OF samples exhibited, on average, high protein content (15.83%), fat content (6.27%), and *β*-glucan content (4.69%), whereas OH samples were abundant in dietary fiber. OHs exhibited total insoluble-bound phenolic compound levels that were 13 to 25 times higher than in the OFs. These bioactive compounds may enhance the pronounced antioxidant and antibacterial activity detected in the extracts. Ferulic acid was the most prevalent in all samples, succeeded by *p*-coumaric, isoferulic, vanillic, and syringic acid. The samples obtained by hydrolysis showed a higher bactericidal activity, which can be associated with a higher content of polyphenols. The traditionally prepared OH extracts had the highest bactericidal effect on *Listeria monocytogenes, Escherichia coli*, and *Staphylococcus haemolyticus*, while *Salmonella typhimurium* was the least affected by the bactericidal effects of all the samples tested. These findings underscore the potential of both OF and powdered OHs for application as functional food ingredients and nutraceuticals due to their beneficial nutritional composition and potential health effects, which could offer numerous health benefits, such as better gut health, less oxidative stress, and stronger immunity. More studies are needed to fully understand how these benefits work and to improve the ways we extract and create oat-based products.

Acknowledgment: This study was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (Grant No. 451-03-136/2025-03/200040).

Long-Term Gut Microbiota Signatures in Post-COVID

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Introduction: Gut microbiota play a critical role in health, whereas its disruptions may be associated with diseases, as documented in long-term effects of COVID-19 (post-COVID). This study aimed to identify microbial and biochemical signatures in individuals after an extended period following the acute SARS-CoV-2 infection. Also, we sought potential correlations between gut bacteria members and levels of biochemical blood parameters.

Experimental: In this cross-sectional observational cohort study, a total of 90 subjects were recruited, divided into three equal groups: (I) individuals with post-COVID, (II) individuals with a history of COVID-19, but without persistent symptoms, and (III) healthy controls. Fecal and blood samples were collected from participants at varying time points post-infection, with the average of 14 months.

Fecal microbiota from 81 participants was analyzed using 16S rRNA gene sequencing (V3-V4 regions), and bioinformatic analysis was conducted using Quantitative Insights Into Microbial Ecology 2 (*QIIME*-2) and R software. Microbial diversities were calculated, with α-diversity expressed by Shannon and Observed species indices, and β-diversity calculated based on Bray-Curtis distances. Differences in microbial composition among groups were tested using the Permutational Multivariate Analysis of Variance (PERMANOVA), while differentially abundant taxa were identified via Linear discriminant analysis Effect Size (LEfSE).

Key findings: Statistical analysis showed that blood parameters and α-diversity were not significantly different among the three groups ($p > 0.05$), possibly reflecting normalization over the prolonged period following SARS-CoV-2 infection. β-diversity assessment of microbiota demonstrated a

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tight clustering of control samples, while post-COVID samples exhibited the greatest dispersion, indicating a notable variation of microbiota composition within this group. Overall microbial composition among the tested groups did not significantly differ according to PERMANOVA analysis ($p > 0.05$), but LEfSE analysis showed that the abundance of 16 bacterial taxa was significantly different, primarily representing less abundant groups from Actinomycetota and Bacillota phyla. Individuals previously infected with SARS-CoV-2 had significantly lower relative abundance of SCFA producers, such as *Phascolarctobacterium*, *Slackia*, *Enterorhabdus*, and *Senegalimassilia* ($p < 0.05$, LDA > 2) compared to healthy controls. In participants with post-COVID symptoms, *Eggerthella* was significantly enriched, with *Holdemania*, *Gemella*, and two uncultured SCFA producing *Lachnospiraceae* showed a trend toward higher abundance. Notably, *Barnesiella* was significantly enriched in individuals who fully recovered from COVID-19, suggesting that the abundance of this group could be seen as a potential microbial signature of recovery. Several microbial taxa, mostly from Clostridia class, exhibited positive correlations with HDL levels, while showing negative associations with non-HDL and triglyceride lipid parameters and CRP, highlighting their potential role in general health.

Probiotics as a Novel Therapeutic Strategy for Infection Control in Normo- and Hyperglycaemic Conditions

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Introduction: Diabetic foot ulcers (DFUs) represent one of the most severe complications of diabetes mellitus, a chronic metabolic disorder characterized by persistent hyperglycemia. Local hyperglycemia within DFUs creates an environment conducive to pathogenic microbial colonization and infection, significantly impairing wound healing and often resulting in lower limb amputation. One of the major challenges in DFU management is the simultaneous control of infection and underlying metabolic dysregulation. Conventional treatments, including systemic antibiotics and topical antimicrobials, are increasingly limited by the emergence of multidrug-resistant (MDR) pathogens [1]. Consequently, there is a pressing need for innovative, multi-targeted therapeutic strategies. In this context, probiotics have emerged as promising candidates due to their potential antimicrobial, anti-inflammatory, and immunomodulatory properties [2, 3].

Experimental: Several strains of probiotic bacteria (*Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and *Lactobacillus gasseri*) from the frozen stock were subsequently incubated under anaerobic conditions in de Man, Rogosa, and Sharpe broth at 37 °C for 18 h, twice in succession. Following incubation, supernatants and bacterial cells were separated. Cells were then washed three times with normal saline solution (0.9 % w/v NaCl), centrifuged and re-suspended in simulated wound fluid (SWF). Both, supernatants and bacterial cells were evaluated for their antimicrobial activity against resistant strain of Klebsiella pneumonia, one of the most frequently isolated bacteria from wounds. To replicate chronic wound environments, all experiments were performed under microaerophilic conditions using SWF, with testing conducted under both normoglycaemic and hyperglycaemic conditions over a 24-hour period.

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Results: The antimicrobial activity varied among probiotic strains. Among the tested supernatants, *L. plantarum* demonstrated the strongest inhibitory effect on *K. pneumoniae*, whereas *L. gasseri* exhibited the weakest activity. As expected, no significant differences were observed between normoglycaemic and hyperglycaemic conditions when using cell-free supernatants. In contrast, the activity of live probiotic cells was notably influenced by glucose levels. Under hyperglycaemic conditions, *L. plantarum* and *L. rhamnosus* significantly suppressed the growth of *K. pneumoniae*, reducing bacterial counts by approximately 5-fold and 4-fold, respectively. However, under normoglycaemic conditions, their effects were reduced, achieving only 2-fold and 1-fold reductions, respectively. Other probiotic strains showed minimal antimicrobial effects regardless of glucose conditions. These preliminary findings indicate that certain probiotic strains, particularly *L. plantarum* and *L. rhamnosus*, exhibit promising antimicrobial activity against resistant strain of *K. pneumoniae*, especially under hyperglycaemic conditions.

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Engineered live biotherapeutic products with beneficial effects on UV light-irradiated keratinocytes

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Introduction: Current treatments for chronic skin conditions, such as atopic dermatitis (AD), primarily manage symptoms, often causing side effects and economic burdens on healthcare systems. As part of the European SKINDEV project, we aim to leverage the skin microbiome to develop next-generation probiotics, or engineered live biotherapeutic products (eLBPs). Using synthetic biology, we engineer strains to detect skin signals, such as immune responses and environmental factors, and produce therapeutic agents on demand.

This study evaluates the potential of a skin-commensal eLBP producing superoxide dismutase (SOD) to mitigate UV light-induced oxidative stress.

Experimental: Western blot analysis determined whether the SOD produced by the eLBP was present in the supernatant. SOD activity in the same supernatant was assessed via specific activity assays. Additionally, a method to measure reactive oxygen species (ROS) as H_2O_2 was optimized for future application in complex models.

Key findings: Western blot revealed a protein band in the supernatant of *C. acnes* (pEND274), confirming successful SOD production and secretion. The SOD activity assay showed higher enzymatic activity in the supernatant of pEND274. Optimization of the H_2O_2 assay identified 10 minutes post-UV exposure as the optimal sampling time, yielding clear differences between UV dosages.

These findings establish a foundation for developing the eLBP as a proof-of-concept therapeutic for skin health. Building on promising ROS-reducing results in keratinocyte monolayers, this work enables future evaluation in human epidermal models.

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Session

Prebiotics

Chairs: Milica Simović and Antonia Montilla

Analysis of carbohydrates and glycoconjugates by advanced mass spectrometry tools

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This presentation will explore the forefront of mass spectrometry (MS) technologies, focusing on precise carbohydrate structure analysis, developed both in my laboratory and by other researchers. The complexity of carbohydrates is accentuated by isomeric and chiral variations, which can significantly influence their biological functions. This makes their full structural analysis both challenging and essential. The talk will highlight cutting-edge developments in matrix-assisted laser desorption ionization (MALDI), Fourier transform ion cyclotron resonance (FTICR), and innovative fragmentation techniques. It will also delve into ion mobility platforms, featuring the Structures for Lossless Ion Manipulation (SLIM) method, alongside complementary strategies such as chemical derivatization and enzymatic approaches for precise carbohydrate analysis. By leveraging these advanced tools, we aim to overcome analytical obstacles and deepen our understanding of the vital roles carbohydrates and glycoconjugates play in biological processes, creating a toolbox readily applicable to analyzing food systems, probiotics, and other carbohydrate-based products.

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Biosynthesis of novel prebiotics

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The concept of prebiotics has attracted an increasing amount of attention and stimulated both scientific and industrial interests. Prebiotic oligosaccharides can serve as fermentable substrates for certain members of the gut microbiota, and have been found to selectively stimulate beneficial gut flora. Food-grade prebiotic oligosaccharides are produced using enzymatic processes by controlled hydrolysis of polysaccharides or biosynthesis from simple sugars using glycosidases, glycosynthases or glycosyl transferases.

This talk focuses on the enzymatic production of galacto-oligosaccharides (GOS), one of the major groups of prebiotic oligosaccharides, and human milk oligosaccharide (HMO) structures using the glycosidases from probiotic microorganisms. It is anticipated that these novel prebiotics will have high selectivity for growth and metabolic activity of desired intestinal bacteria, hence it could be of interest for the food industry in the production of functional food ingredients and beneficial components for infant formulas.

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Integrated approaches to producing and designing prebiotic carbohydrates from agri-food resources

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The growing interest in gut-targeted nutrition has driven the development of both established and novel non-digestible carbohydrates with prebiotic functionality. In this talk, we will survey current manufacturing platforms for prebiotic oligo- and polysaccharides, including: (i) isolation and enrichment from underexploited plant- and by-product streams; (ii) controlled depolymerization *via* chemical (acid, alkaline) and enzymatic hydrolysis; and (iii) *de novo* assembly through enzyme engineering and microbial fermentation. Emphasis will be placed on valorizing agri-food residues, such as fruit peels and pomaces, cereal brans and vegetable wastes, as sustainable feedstocks for next-generation prebiotics.

Beyond conventional production, we will introduce a "directed-design" paradigm for tailor-made carbohydrate structures. This approach integrates experimentally determined or *in silico*-predicted 3-D structures of enzymes and protein receptors with molecular docking and dynamic simulations, guiding the engineering of glycosidases to yield tailored, non-digestible carbohydrates. Until recently, rational design efforts were hampered by the limited availability of high-resolution protein structures. Advances in computational structure-prediction (*e.g,*. AlphaFold-derived models) and subsequent molecular modelling now overcome this bottleneck, enabling the identification of active-site mutations that enhance specificity toward novel sugar acceptors.

Case studies will illustrate how coupling agri-food substrate diversity with enzyme-based approaches can generate prebiotic candidates that selectively stimulate previously untargeted beneficial microbes.

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Conifer Cone Extracts as Natural Antimicrobial and Prebiotic Agents: A Sustainable Biotechnological Solution

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Introduction: The increasing demand for alternatives to synthetic antimicrobial agents has prompted extensive research into natural bioactive compounds with antimicrobial potential. These naturally derived agents present a promising solution to antibiotic resistance by offering a safer, more sustainable approach to microbial control, with broad applicability across various industries. Notably, selected rare natural compounds demonstrate both antimicrobial and prebiotic properties, facilitating the targeted suppression of pathogenic bacteria while simultaneously fostering the growth of beneficial microbial communities. This selective modulation of microbiota composition may contribute to improved gastrointestinal function, immune homeostasis, and overall systemic health.

Experimental: Microwave-assisted extraction (MAE) was employed to isolate bioactive compounds from mature *Pinus nigra* and *Thuja orientalis* cones. The resulting extracts were evaluated at various concentrations to assess their antimicrobial and prebiotic activities. The prebiotic potential was assessed using three probiotic strains – *Saccharomyces boulardii*, *Lactobacillus rhamnosus GG*, and *Lactobacillus plantarum* 299v, while antimicrobial activity was tested against pathogenic strains *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. Following incubation, colony-forming units (CFU) were quantified on both extract-free control and extract-containing agar plates to determine the influence of the extracts on microbial viability.

Results: The study demonstrated a positive correlation between extract concentration and antimicrobial efficacy, while prebiotic activity followed a concentration-dependent biphasic trend, peaking at an optimal threshold before declining. The opportunistic pathogen *S. aureus* was completely inhibited (100.00%) at nearly all tested concentrations of both extracts,

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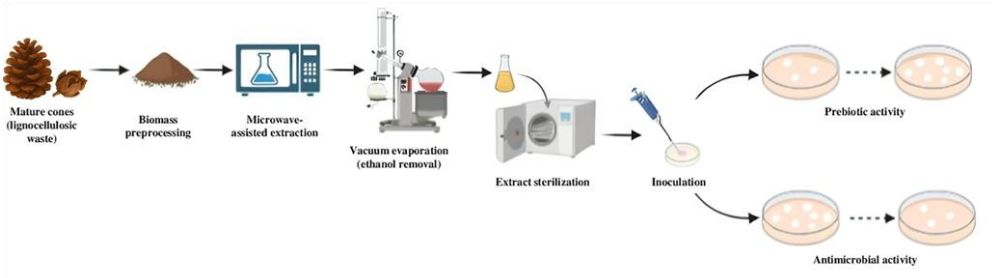
while *T. orientalis* extracts exhibited stronger antimicrobial effects against *C. albicans* (99.66%) and *E. coli* (52.50%) compared to *P. nigra* extracts. Lower concentrations of *T. orientalis* extracts promoted prebiotic activity, whereas higher concentrations resulted in a sharp decline. The highest activation of *L. rhamnosus* GG (67.88%) and *S. boulardii* (19.73%) was achieved with the use of lower concentrations of this extract, whereas *L. plantarum* 299v (32.14%) responded favourably to both lower *T. orientalis* and higher *P. nigra* extract concentrations. These findings highlight the significant potential of mature conifer cones as a lignocellulosic waste resource and open new avenues in the maintenance of beneficial microbiota and antimicrobial formulations for use in food preservation, medicine, pharmaceuticals, and cosmetics. Utilizing these bioactivities from an overlooked waste material in accordance with green chemistry principles presents an innovative and sustainable biotechnological approach with broad applicability.

Acknowledgment: This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-136/2025-03/200135).



Agro-Waste Valorization: Extraction and Characterization of a Polyphenol-Rich Fraction from Sunflower Meal

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Introduction: The global demand for sunflower oil has driven extensive production, resulting in large quantities of a by-product known as sunflower meal (SFM). Traditionally, SFM has been utilized mainly as a protein source in ruminant diets, as it contains approximately 30 % protein in its dry matter (DM) and about 40 % lignocellulosic fiber. However, its nutritional value is compromised by the presence of phenolic compounds (PCs). Yet PCs alone have many health-benefiting properties, including antioxidant, anti-inflammatory, bacteriostatic, and antitumor effects. Consequently, extracting PCs from SFM could enhance its quality and facilitate the development of a new product rich in phenolic compounds.

Experimental: The sunflower meal (SFM) extract, obtained through a three-stage ethanol extraction process, was subjected to chemical and microbiological analyses. To assess the SFM extract's composition, HPLC was employed. Additionally, spectrophotometric assays, including DPPH, FRAP, CUPRAC, and ABTS, were conducted to evaluate the antioxidant capacity of the SFM extract. Additionally, microbiological tests on various representatives of intestinal microbiota (*Escherichia Coli* ATCC 25922, *Lacticaseibacillus rhamnosus* GG, *Saccharomyces boulardii* CBS 5926, *Lactiplantibacillus plantarum* 299v) were performed to determine the prebiotic potential of SFM extract.

Results: By employing a three-stage ethanol extraction of PCs from SFM, an optimal balance was achieved between material extraction efficiency and resource input, resulting in a dry matter yield of 16.8 ± 1.06%. The analysis revealed that carbohydrates and phenolic compounds (PCs) constitute approximately 73% of the SFM extract's DM. Specifically, the chlorogenic acid was identified as the dominant PC, with a concentration of 103 mg/g

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DM. Regarding carbohydrates, disaccharides (301 mg/g DM), trisaccharides (179 mg/g DM), and tetrasaccharides (61.7 mg/g DM) were detected. The extract's highest antioxidant activity was observed using the CUPRAC assay, measuring 2.14 ± 0.0554 mmol Trolox equivalents/g DM. Other antioxidant assessments also confirmed that the SFM extract exhibits strong antioxidant properties compared to other phenolic-rich by-products [1]. Overall, these findings suggest that the extract has potential as a natural source of antioxidants. Moreover, the addition of an adequate amount of the extract to the nutrition medium led to notable stimulation of probiotic microorganisms (*S. boulardii, L. plantarum, L. rhamnosus*), while suppressing the growth of the pathogen bacteria *E. coli*. For more refined observation of such a complex microbial community, the prebiotic activity score was calculated, and achieved values were promising (as high as 8.65). Creating new strategies that would focus on harnessing the antioxidant and prebiotic potential of the extract can result in the development of a new product, with the benefit of increasing the industrial value of SFM.

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Reference

[1] Petrov Ivanković A., Ćorović M., Milivojević A., Simović M., Banjanac K., Veljković M., Bezbradica D., **2024,** *Int. J. Fruit Sci.*, 24, 85–101.

Production of polyphenol-rich fraction from rapeseed meal as a potential source of antioxidants and prebiotics

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Introduction: Rapeseed is widely cultivated globally for oil production, with the resulting by-product, rapeseed meal (RSM), ranking second only to soy meal in volume. RSM is notable for its high protein content (35-40 %) and, unlike other oilseed meals, is rich in sulfur-containing amino acids such as methionine and cysteine. It also provides essential minerals and vitamins. However, its inadequate to be used in animal or human diet due to the presence of lignocellulosic fibers and antinutritional compounds, including glucosinolates, phytic acid, and phenolic compounds (PCs). Nevertheless, recent research highlights that many PCs extracted from plant based byproducts provide health benefits and have antioxidative properties, and therefore, it seems worth considering their application as potential prebiotics.

Experimental: To assess the significance of the PC-rich extract, a three-stage ethanol extraction of PCs from RSM was applied, and the RSM extract's composition was characterized through spectrophotometric methods, HPLC, and MS. Additionally, to evaluate the antioxidant potential of the obtained RSM extract, various assays utilizing different substrates to measure antioxidant activity were applied. Additionally, the prebiotic activity of the extract was analysed with *in vitro* tests by adding different concentrations of PC extract to a depleted microbiological medium using probiotic strains like *Saccharomyces boulardii* CBS 5926, *Lactiplantibacillus plantarum* 299v, *Lacticaseibacillus rhamnosus* GG, and an indicator strain of the pathogenic species *Escherichia coli* ATCC 25922.

Results: The RSM fraction obtained through a three-stage extraction process yielded a dry matter (DM) of 17.7 ± 0.7 %. This fraction was mainly composed of simple sugars (649 mg glucose/g DM) and PCs (89.5 mg galic acid/g DM), with a relatively low protein content (19.8 mg BSA/g DM). The

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presence of sinapine in the extract was detected through HPLC and MS analysis, indicating that sinapine is a prominent bioactive compound within the obtained RSM extract. The antioxidant activity of the RSM extract was measured as 730 ± 32 μmol trolox equivalents (TE)/g DM (CUPRAC), 324 ± 3 μmol TE/g DM (FRAP method), 392 ± 5 μmol TE/g DM (ABTS), and 20.0 ± 0.7 μmol TE/g DM (DPPH), confirming its potential as an antioxidant for food industry applications. The results of the prebiotic study showed that all tested concentrations of the extract have a pronounced ability to stimulate the growth of beneficial gut microorganisms, as well as to inhibit the growth of the *E. coli*. Accordingly, high values of prebiotic activity scores were obtained. Overall, these findings highlight the antioxidant and prebiotic potential of the RSM extract, suggesting its potential as a novel and valuable product derived from an oil production waste. Emphasizing the multifunctionality of RSM extract could open new avenues for its application in food and pharmaceutical industries, reinforcing the importance of innovative valorization strategies for industrial byproducts.

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Circular economy principles integration in probiotic production – whey utilization in cultivation of *Bacillus amyloliquefaciens* BioSol021

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Introduction: The raising problem of antimicrobial resistance calls for an action to develop novel solutions to prevent and minimize the existing risks for human/animal health. Probiotics application as dietary supplements, food ingredients or medicines has raised in recent years due to increased awareness of their health-beneficial effects exerted *via* contribution to microbiome balance, imunomodulation or production of host-beneficial or antimicrobial compounds with antagonistic effects to human/animal pathogens. Next-generation probiotics should enlarge the spectre of the conventionally used strains, simultaneously developing novel technologies for their large-scale production, thus contributing to minimization of the production footprint and cost while maximizing product quality and quantity. The aim of this study was to investigate probiotic traits of the strain *Bacillus amyloliqefaciens* BioSol021, but also to make initial steps in development of technology for probiotic production in accordance with the circular economy principles, using whey as a by-product from dairy industry and a circular substrate for microbial growth.

Experimental: Probiotic traits investigation has included numerous methods investigating survival of *Bacillus amyloliqefaciens* BioSol021 in experimental setup simulating gastrointestinal (GIT) conditions, such as low pH values, presence of bile salts and tolerance to simulated gastric and intestinal fluids containing GIT enzymes. *In vitro* tests have been performed to determine sensitivity towards antibiotics, but also antimicrobial potential against *Lysteria monocytogenes* ATCC 19115 and *Staphylococcus aureus* ATCC 29213. Lab-scale cultivation of *Bacillus amyloliqefaciens* BioSol021 (30 °C, 150 rpm, spontaneous aeration) has investigated the potential of whey as a dairy industry effluent to be used as the substrate for probiotic production, without the medium adjustment before cultivation.

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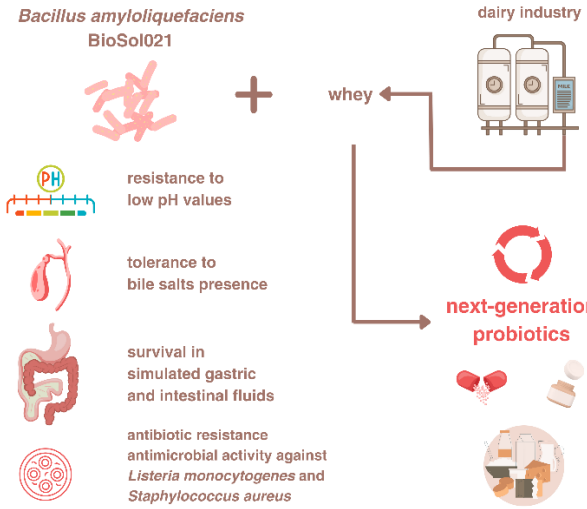

Results: *Bacillus amyloliqefaciens* BioSol021 has shown a satisfactory survival in simulated GIT conditions, with 30% reduction in viable cell number after 4 h-exposure to pH value 2, as well as 26% reduction after exposure to 2% bile salts solution in the similar timeframe, while less than 5% reduction in viability was observed in artifical gastric and intestinal fluid after 2 h-exposure. *Bacillus amyloliqefaciens* BioSol021 has shown to be moderately sensitive to streptomycin (10 µg) and sensitive to amoxycycline (25 µg) and tetracycline (30 µg). The antimicrobial potential against *Lysteria monocytogenes* and *Staphylococcus aureus* was indicated by the inhibition zone diameters of 33.50±2.50 mm and 15.50±0.50 mm, respectively. The lab-scale cultivation course using whey as a substrate has indicated maximal viable cell number after 72 h (9.5 log CFU/mL), while longer cultivation period resulted in cell death after 96 h, suggesting possible time, labor and economic savings by adopting the shorther cultivation batches. The obtained results provide a basis for further development of techno-economically viable upstream bioprocess solutions integrating the circular economy principles in the next-generation probiotics production, with necessity to invest additional efforts in development of downstream processing procedures and formulation protocols preserving the probiotic viability and defining the future application routes in medicinal products, food/feed or cosmetic industry.

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Glycosidases from *Lactiplantibacillus plantarum* WCFS1: studying their potential use for the synthesis of prebiotic oligosaccharides

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Introduction: *Lactiplantibacillus plantarum* is a species of lactic acid bacteria found in a wide variety of niches, which also forms part of the microbiota of the human gastrointestinal tract. *L. plantarum* strain WCFS1 is considered as a model strain of this species. In this strain, 2.9% of the genes encode enzymes that act on carbohydrates, including numerous proteins involved in carbohydrate transport and utilization. These proteins allow it to grow on many different carbon sources. *L. plantarum* WCFS1 has 55 proteins, classified into 18 families, annotated in the Carbohydrate-Active enZymes (CAZy) database (http://www.cazy.org/b125.html) as potential glycosyl hydrolases (GH), also known as glycosidases or glycoside hydrolases. Over the last few years, members of the Bacterial Biotechnology Research Group at ICTAN and those of the Bioactivity and Food Analysis Group at CIAL, in collaboration with researchers from the IQM, IQFR and ICP institutes of the CSIC, have developed a line of research focused on the glycosyl hydrolases of *L. plantarum* WCFS1. This line of research is based on the fact that the presence of a wide range of enzymes involved in the hydrolysis and synthesis of carbohydrates makes it a versatile model for the

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identification of new enzymatic activities useful for the design of new compounds with beneficial properties for human health such as prebiotics. In this context, the exhaustive structural and functional characterization of both, the glycosyl hydrolases of *L. plantarum* WCFS1 and the high added value products synthesized by them, allows the design of new strategies for the efficient production of bioactive compounds of interest in the food and/or nutraceutical field.

Experimental: Protein production and purification: GH genes from *L. platarum* WCFS1 were PCR-amplified and inserted into the pURI3-Cter vector using a restriction enzyme- and ligation-free cloning strategy in which the gene is used as primers and the vector is used as a template. This vector produces recombinant proteins in *E. coli* having a six-histidine affinity tag in their C-termini. Recombinant proteins were purified by affinity chromatography using imidazole. *L. plantarum* WCFS1 GH enzymatic assays: i) GH hydrolytic activity was carried out by using a library of 24 *p*-nitrophenyl glycoside derivatives ii) Transglycosidase activity was evaluated on natural substrates by GC-FID iii) purification of novel oligosacharides was performed by HILIC with a RID and their structural elucidation by NMR.

Results: The research groups involved have produced and characterized 20 glycosyl hydrolases belonging to 6 different families (GH1, GH13, GH32, GH36, GH42 and GH78). Using natural carbohydrates and synthetic carbohydrates derived from p-nitrophenol, eleven different glycosyl hydrolase activities have been identified (*α*-D-maltosidase, *α*-D-glucosidase, 6-P-*β*-D-glucosidase, α-D-maltopentaosidase, β-D-galactosidase, β-D-fucosidase, α-L-arabinosidase, *α*-L-rhamnosidase, *α*-D-galactosidase, 6-P-*β*-D-galactosidase and 6-P-*β*-D-thioglucosidase). In addition to the hydrolytic capacity of these enzymes, their ability to synthesise oligosaccharides was studied. Thus, it was observed that Lp3469 (LacA) was able to synthesise galactosyl-polyols whereas Lp_3485 (MelA) synthesised heteroligosaccharides and *α*-galacto oligosaccharides.

Medicinal herb extracts as promising natural inhibitors of *α*-amylase

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Introduction: Carbohydrate-rich foods influence blood sugar levels, and their effect is measured using the glycemic index (GI), which ranks foods based on how quickly they raise blood glucose. Consuming high-GI foods can cause rapid spikes in blood sugar, which may contribute to health concerns like insulin resistance and diabetes. Managing diabetes requires a multifaceted approach, including medication, diet, and lifestyle modifications. In recent years, the role of gut health in diabetes management has gained increasing attention, particularly concerning prebiotics—non-digestible dietary fibers that promote the growth of beneficial gut bacteria. Prebiotics help regulate blood sugar levels, improve insulin sensitivity, and reduce inflammation by enhancing gut microbiota composition. Their role in diabetes management is an emerging area of research, offering a natural and dietary-based strategy to support metabolic health. Medicinal herb extracts contain a high concentration of polyphenols, prebiotics that can enhance gut microbiota and inhibit the activity of α-amylase and glucoamylase, enzymes responsible for breaking down starch molecules into glucose monomers, thereby increasing blood glucose levels. By inhibiting amylolytic enzymes, digestible starch is converted into resistant starch, which serves as a substrate for gut microbiota fermentation, which can lead to bloating, but also can lead to the production of health-beneficial metabolites such as butyrate.

Experimental: This study focused on the preparation and analysis of extracts derived from various plant materials, including green coffee beans (*Coffea arabica*), roasted coffee beans (*Coffea arabica*), common nettle leaves (*Urtica dioica*), blueberry leaves (*Vaccinium sect. Cyanococcus*), and common wormwood leaves (*Artemisia absinthium*). The primary objective was to assess their ability to inhibit α-amylase, an enzyme involved in carbohydrate digestion. In the obtained extracts the total polyphenol content was determined. The Folin-Ciocalteu method was applied, a widely used spectrophotometric technique for measuring phenolic compounds. The inhibition of α-amylase was analyzed by measuring the amount of reducing sugars released during enzymatic hydrolysis, using the DNS method. These

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analyses aimed to explore the potential of these plant extracts in modulating carbohydrate metabolism, which may have implications for managing blood sugar levels and supporting overall metabolic health. A correlation test was done to determine whether polyphenols are the carriers of the inhibition activity.

Results: Several of the tested plant extracts demonstrated strong α-amylase inhibitory potential, with some exhibiting inhibition rates exceeding 99%. Blueberry leaf extracts were among the most effective. Similarly strong inhibition rate was observed for green coffee bean and roasted coffee bean extracts. In contrast, extracts prepared in glycerol showed noticeably lower inhibitory activity across all plant types. Regarding total polyphenol content, the highest concentrations exceeding 2000 mg GAE/l were found in roasted coffee bean and blueberry leaf extracts, particularly those obtained using ethanol or hot water. Green coffee bean extracts also exhibited high polyphenol levels, while nettle and wormwood leaf extracts showed more moderate values. Glycerol extracts consistently yielded the lowest polyphenol content. The correlation test suggests a moderate positive correlation, indicating that a higher polyphenol content in the extract corresponds to greater inhibition of α-amylase activity. These findings highlight the potential of medicinal plant extracts as both natural prebiotics and α-amylase inhibitors, providing a promising dietary approach for managing blood sugar levels and preventing diabetes mellitus.

Polyphenol-rich strawberry extract as potential modulator of skin microbiota

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Introduction: Enzyme-assisted extraction (EAE) is an efficient method for obtaining phytochemicals, plant-derived compounds known for their antioxidant properties. By using enzymes to break down plant cell walls, EAE enhances the release of these bioactives. Among various sources, strawberries are particularly rich in polyphenols such as anthocyanins, flavonoids, and ellagic acid, which are known for their potent antioxidant activities. Recently, interest has grown in using such extracts to support skin health, particularly by influencing the skin microbiota - the diverse community of microorganisms that live on the human skin. Maintaining a balanced skin microbiome is essential for healthy skin, and phytochemicals extracted from fruit via EAE offer promising natural solutions to help regulate and support this delicate ecosystem. Therefore, the aim of this research is to obtain polyphenol-rich extract from strawberry using EAE and to evaluate their potential as skin prebiotics.

Experimental: Phytochemicals from strawberries were extracted using a mixture of enzyme preparations Rohapect® MC and Viscozyme® L in final concentration of 0.1 mL/g. Abundance of different polyphenol subclasses and antioxidant activity were determined spectrophotometrically. The prebiotic effect of the extract (25-100 µg GAE/mL) was evaluated on the common skin-associated bacteria, including commensal *Staphylococcus epidermidis* and pathogen *Staphylococcus aureus*. Monoculture growth was monitored by measuring optical density at 600 nm, while co-culture growth was determined using mannitol salt agar plates and colony counting method.

Results: The strawberry extract contained 20.8 mg GAE/g of total polyphenols, with the highest share attributed to water-soluble tannins (5.8 mg GAE/g), followed by phenolic acids (4.3 mg CAE/g) and flavonoids (2.1 mg QE/g), demonstrating an antioxidant activity of 156 µmol TE/g as

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measured by the ABTS assay. When applied at a concentration of 25 µg GAE/mL, the extract stimulated the growth of *S. epidermidis* by 15%, while higher concentrations resulted in growth inhibition. In contrast, all tested concentrations of the extract inhibited the growth of *S. aureus*, with inhibition levels reaching up to 60%. The maximum prebiotic capacity observed in monoculture was 0.245. In co-culture with *S. aureus*, the prebiotic capacity increased to 5.7, suggesting that *S. epidermidis* exhibits superior probiotic properties. This effect likely results from both the selective stimulation of *S. epidermidis* and the concurrent inhibition of *S. aureus*. Such findings point to a potential synbiotic interaction — the combination of the polyphenol-rich strawberry extract and the commensal skin bacterium *S. epidermidis* may work synergistically to suppress pathogenic bacteria through natural competitive and inhibitory mechanisms. It is hypothesised that similar effects may occur *in vivo*, providing a promising strategy for modulating the skin microbiota which should be investigated in the future.

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Separation of xylo-oligosaccharides using ultra- and nanofiltration membranes and investigation of their prebiotic effect

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Introduction: Xylo-oligosaccharides (XOS) are potential prebiotics composed of xylose units linked by β-(1-4)-xylosidic bonds. In addition to health benefits, their favourable physicochemical properties and moderate sweetness make them suitable nutritional ingredients for food and feed products. Unlike other prebiotics, XOS can be produced from agricultural and wood residues (corn cobs/stalks, bagasse, rice hulls, birch wood, etc.) by enzymatic hydrolysis of xylan, the main hemicellulosic part of the mentioned sources. This strategy, which includes using renewable plant sources as a source of xylan and enzymes, represents an environmentally friendly process compared to conventional methods and results in a mixture of XOS of various degrees of polymerization (DP), unreacted xylan, and monosaccharide units (xylose). Given that the prebiotic potential of XOS depends on the DP and purity, this research is focused on applying membrane separation techniques to obtain a mixture of higher purity and defined molecular weight with optimal prebiotic activity.

Experimental: Using commercial xylan from beech wood and an enzyme preparation Rohalase® SEP-Visco as a source of endo-xylanases, a mixture of XOS with different DP was obtained. The mixture was purified using a thin film composite polyamide membrane for ultrafiltration (XT3-1812TM (MWCO 1000 Da)) and nanofiltration (NFW (MWCO 300-500 Da)). The processes were characterized by rejection coefficients, while the purification course was monitored by analysing the samples on the HPLC system. The obtained fractions were then tested for the growth of individual gut microbes to investigate the prebiotic potential.

Results: By performing the hydrolysis of xylan with endo-xylanases under selected optimal conditions, a mixture with 28.53% XOS DP (1-6) was obtained, while the rest was made up of XOS with a higher DP and

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unreacted xylan. Purification of the obtained mixture under defined conditions (temperature 35 °C, flow rate 22 mL/min, and substrate concentration of 1%) using an ultrafiltration membrane showed that XOS with DP (1-6) pass through the membrane, while the application of a nanofiltration membrane showed limited passage of trisaccharides and complete retention of tetra, penta and hexa-oligosaccharides. In this regard, at the end of the purification process, fractions of different compositions were obtained. Permeate after ultrafiltration contained 84.2%, retentate 1.2%, and retentate after nanofiltration 32.5% XOS DP (1-6). The microbiological test results showed the highest percentage of stimulation of probiotic culture *Lactiplantibacillus plantarum* 299v and *Saccharomyces boulardii* CBS 5926 in the presence of permeate after ultrafiltration. By calculating the prebiotic effect, which illustrates the collective effect of the fractions on the growth of the probiotic and pathogenic microbes, it was found that the retentate after nanofiltration has the greatest effect.

Acknowledgements: This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-136/2025-03/200135 and 451-03-136/2025-03/200287) and has received funding from Science Fund of the Republic of Serbia, programme IDEAS, No. 7750109 (PrIntPrEnzy) and the Horizon Europe 2021-2027 research and innovation programme under grant agreement ID 101060130 (TwinPrebioEnz). Rohalase® SEP-Visco was kindly donation from AB Enzymes GmbH (Germany).

Holistic and sustainable utilization of broccoli by-products

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Introduction: Broccoli (*Brassica oleracea var.* Italica) is a highly appreciated vegetable, especially popular for its nutritional and sensory properties. Its production has experienced an impressive growth in the last years. By 2022, Spain was the fifth global broccoli producer, with a total production of 0.677 million of tons of broccoli. Their by-products are especially rich in compounds of interest such as phytochemicals, fiber polysaccharides and oligosaccharides. However, studies on the utilization of these residues are practically limited to obtaining extracts enriched in phytochemicals, and to a lesser extent in obtaining pectin. Of special relevance is the fact that, despite all the advances, there is no protocol that allows the integral utilization of broccoli residues, as there is in other vegetable matrices.

Experimental: The present work focused on the sequential obtention of different high value-added extracts from broccoli by-products, giving priority to the utilization of sustainable methodologies. Firstly, freeze-dried broccoli by-products were subjected to a phytochemical extraction with 70% ethanol (50 ºC, 30 min) and plate stirring. Afterwards, citric acid pectic polysaccharide extraction from the alcohol-insoluble residue was optimized through ANN, using a CCD model with parameters varied in defined ranges: X1, temperature (52.7-84.2 ºC); X2, % of citric acid (1.6-5.4%); X3, time (45-135 min). Outcomes were: Y1, yield; Y2, % of galacturonic acid; Y3, % of neutral sugars; Y4, % of glucose and mannose. Finally, solid depectinised residue was subjected to enzymatic hydrolysis with Rapidase® FIBER (50 ºC, 6 h, concentration of solids 12.5 mg/mL and of enzyme 100 μL/g DW, pH 5.5), in order to obtain an oligosaccharide-enriched extract, whose low molecular weight carbohydrate (LMWC) content and monomeric composition were determined by GC-FID and molecular weight (M_w) distribution by HPSEC-ELSD.

Results and conclusion: Phytochemical extract was obtained with a yield of 32.4%, a high total polyphenol content (16.3 mg GAE/g DW), an antioxidant

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activity (EC_{50} of 12.2 mg/mL, DPPH method; EC_{50} of 5.4 mg/mL, ABTS method), as well as a content of total soluble carbohydrates of 447 mg/g DW. The optimal conditions to extract pectic polysaccharide were 85 ^{o}C, 135.6 min, 6.1% citric acid, obtaining a yield of 7.8%. The polysaccharide showed a galacturonic acid content of 54.8%, a neutral sugar content of 39.3%, and a degree of methyl-esterification of 48%. Finally, the oligosaccharide-enriched extract showed a LMWC content (mainly monosaccharides) of 267.7 mg/g DW and a content of carbohydrates with M_w > 0.25 kDa of 280 mg/g DW (HPSEC-ELSD). Regarding monomeric composition, major monosaccharide quantified were glucose (46.6%), followed by galacturonic acid (34.3%). Additionally, the possibility of incorporating the final residual fractions into the production chain through bioethanol production with *Saccharomyces. cerevisiae* and *Scheffersomyces stipitis* is under investigation. In conclusion, a holistic protocol that allows the sequential obtainment of phytochemicals, polysaccharides and pectic oligosaccharides has been designed. The integral and sustainability-oriented approach could allow its incorporation to the industry in the standards required by the current problems of food waste and the use of polluting methodologies.

Integrated extraction and enzymatic hydrolysis for emerging prebiotic production from agro-industrial waste

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Introduction: Due to its availability and abundance, lignocellulosic biomass (LCB) has become a feedstock of interest for obtaining a variety of products, such as biofuel and food additives [1]. Xylo-oligosaccharides (XOS) and cello-oligosaccharides (COS) are considered emerging prebiotics - a class of food additives which exhibit beneficial effects on human health [1]. These products are traditionally derived from LCB through chemical processes at harsh conditions, which generate toxic by-products and can have a negative environmental impact. Moreover, the majority of these processes rely on multiple pretreatment steps, which only aim to reduce LCB recalcitrance, without generating any products [2]. This work presents a novel biocatalytic process for simultaneous XOS and COS production based on the integrated liquid-solid extraction and enzymatic hydrolysis, using sunflower residues as raw material. In line with the biorefinery concept, raw material was obtained after the pretreatment of sunflower meal designed to obtain value-added products, namely phenolics-rich extract and protein hydrolysate. Compared to the traditional methods, this process represents a green and sustainable solution with improved product quality, which could become industrially relevant.

Experimental: A two-step pretreatment method included ethanol extraction and enzymatic protein hydrolysis [3]. Prebiotic oligosaccharides were obtained from the deproteinized sunflower meal (DP-SFM) using the enzymatic cocktail Cellic CTec3 HS. Firstly, temperature (30 – 60 °C) and pH (3.8 – 6.3) were optimized, after which the reactions with varied DP-SFM (5 – 10 wt%) and enzyme concentrations (1 – 10%) were conducted for 12 h. The modified DNS method and HPLC were used for sample analysis.

Results: The starting point of this study investigated the temperature and pH influence on DP-SFM hydrolysis using a commercial enzymatic cocktail, which comprises both cellulase and hemicellulase activity. Highest relative

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activity and reducing sugar concentrations were observed at 40 °C and pH 4.9, which have been selected as optimal conditions. The subsequent experiments were performed at different DP-SFM and enzyme concentration for 12 h. DP-SFM concentration of 10 wt% resulted in maximum concentrations of all hydrolysis products. However, the economically preferable medium enzyme concentration (5%) proved favourable for limiting the production of monomers. The selection of reaction times up to 300 min secured lower xylose formation, while longer reaction times expressed limited influence on the oligosaccharide concentration (Figure 1a). During the first 30 min, some compositional changes of the obtained XOS and COS species are detected. However, this compositional difference becomes less pronounced at higher reaction times (Figure 1b), with XOS2 and XOS5 as the dominant oligosaccharide products.

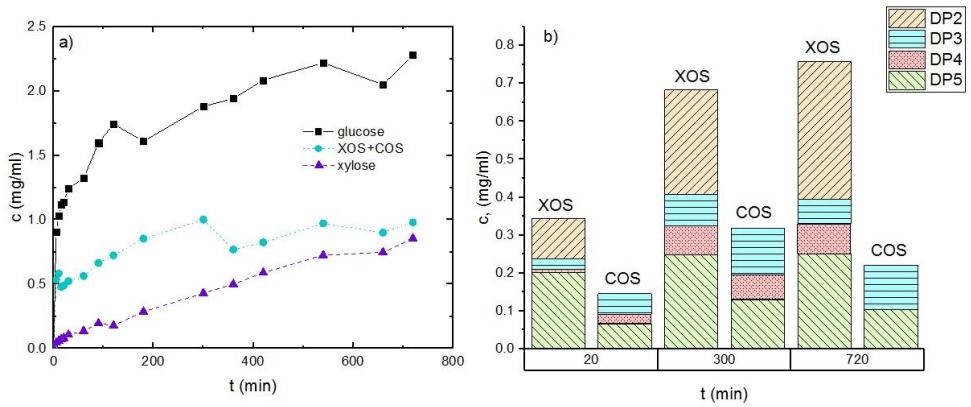

Figure 1: Product profiles (a) and oligosaccharide composition (b) (10 wt% DP-SFM, 5% enzyme)

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Influence of avocado seed extract obtained by ultrasonic extraction on rebalancing skin staphylococci

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Introduction: The skin microbiota, a complex and diverse community of microorganisms residing on the skin's surface, has become an area of increasing interest in the cosmetic industry. Disruptions to microbial balance can lead to a decline in diversity and have been associated with several skin disorders, including acne, atopic dermatitis, and psoriasis. Therefore, maintaining a well-balanced skin microbiome is vital for preserving the skin's barrier function and overall health. In this study, we investigated the effect of avocado seeds extract (ASE) obtained by ultrasonic extraction on skin microorganisms, the pathogenic *Staphylococcus aureus* and the beneficial *Staphylococcus epidermidis*, aiming to assess its potential in restoring a healthy skin microbiome balance.

Experimental: Avocado seeds extract was prepared using ultrasonic extraction. The total phenolic content was determined using the Folin–Ciocalteu colorimetric method. The influence of different ASE concentrations on the growth of two bacterial strains, opportunistic pathogen, coagulase-positive *S. aureus* ATCC 25923, and commensal, coagulase-negative *S. epidermidis* DSM 20044, was evaluated in liquid cultures using a 96-well microtiter plate. Bacterial growth was monitored by measuring optical density at 600 nm over 24 hours using an automated plate reader. Callus-based *in vitro stratum corneum* model was used for testing the effect of selected ASE concentration on the bacterial growth which was monitored by counting the number of colonies. By comparing the growth of each bacterium in the presence of extract and in control samples without extract, prebiotic capacity (PC) was calculated.

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Results: Ultrasonic extraction yielded 12.11% of extract, with a total phenolic content of 6.31 mg GAE/g. The effect of the ASE on bacterial growth was assessed over a concentration range of 0.015 to 0.97 mg GAE/ml of medium. The results from microwell plate experiments indicated the concentration-dependent response. An intermediate concentration of 0.06 mg GAE/ml demonstrated the highest prebiotic potential, promoting the growth of the beneficial *S. epidermidis* (by 42.04%) while inhibiting the growth of the pathogenic *S. aureus* (by 28.81%). This concentration was tested in more complex environment, the callus-based *stratum corneum* model, which mimics the upper layer of the human skin. Strong inhibitory effect on the growth of pathogen *S. aureus* was shown, since no growth after 24h was observed. At the same time, commensal *S. epidermidis* was stimulated (4-fold CFU increase) and resulting PC reached the value of 4.4. These findings suggest that avocado seeds extract may possess skin microbiome-modulating properties beneficial for skin health. Further studies using more advanced models, such as *in vitro* 3D skin equivalents or *in vivo* clinical trials would provide a more accurate representation of the skin's barrier environment and allow for a deeper understanding of the avocado seed extract's effects on the skin microbiota.

Acknowledgment: This work was supported by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia (Contract No.: 451-03-136/2025-03/200287 and 451-03-136/2025-03/200135) and bilateral project: Republic of Serbia - Republic of Slovenia (no. 337-00-110/2023-05/35 and no. BI-RS/23-25-034) and has received funding from the Horizon Europe 2021–2027 research and innovation program, TwinPrebioEnz (grant agreement ID 101060130).

Optimized production of oligosaccharides using arabinogalactan-rich fraction isolated from blackcurrant pomace as raw material

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Introduction: Growing awareness of gut microbiome importance is leading to an increased interest in novel compounds able to selectively stimulate growth of intestinal microorganisms conferring a health benefit. Beside established prebiotics, such as galacto-oligosaccharides (GOS, derived from lactose), and fructo-oligosaccharides (FOS, from inulin-rich sources or derived from sucrose), other oligosaccharides classes (*e.g.,* xylo-oligosaccharides (XOS), pectic-oligosaccharides (POS)) produced from various plant-based substrates, including agro-industrial residues, are being intensively investigated as potential prebiotics. At the same time, harnessing bioactive potential of food waste has stood out as promising way for achieving sustainable development goals and circularity. Particularly rich sources of different valuable compounds are berry-fruit pomaces obtained during juice production as by-products, making about 20-30% of the fresh fruit weight [1]. Blackcurrant is polyphenol- and polysaccharide-rich berry-fruit which annual production reaches 764 499 tons globally and most of the fruit is being processed to juices leaving large amounts of its pomace unused [2]. Here, we examined possibility of using blackcurrant pomace as a raw material for the production of prebiotic oligosaccharides.

Experimental: The monosaccharide composition of the polysaccharide fraction extracted with citric acid was characterized by GC-FID, and its molecular weight (Mw) distribution was determined by HPSEC-ELSD. Three different commercial enzyme preparations were tested and their efficiency was monitored by the amount of oligosaccharides (molecular weight 0.3-5 kDa) produced. For the selected preparation, statistically

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designed experimental plan was employed for maximizing oligosaccharide yield and response surface methodology was used for determining optimum values of key experimental factors (reaction time, temperature, and enzyme concentration).

Results: Mw distribution analysis revealed two dominant polysaccharide fractions: one high-Mw fraction (247.3 kDa, 60.6%) and one low-Mw (12.3 kDa, 39.4%). Structural characterization of isolated polysaccharides demonstrated that dominant monosaccharide was galactose (49.1%), followed by arabinose (31.6%) and galacturonic acid (9.4%). Low amounts of rhamnose, xylose, glucose, and mannose were also detected (all below 5%). Such monosaccharide composition suggests that isolated polysaccharides are composed of arabinogalactan chains with certain amounts of pectic polysaccharides. Using the Rapidase® Fiber (DSM-Firmenich) enzyme preparation under optimal conditions (46 °C, 76.7 µL of enzyme per g substrate, and a 29.1 h reaction time) gave a maximum oligosaccharide yield of 61%. Our results indicate that blackcurrant pomace is a suitable raw material for extracting an arabinogalactan-rich fraction and producing oligosaccharides with potential prebiotic activity. Further investigations should be directed towards purification and *in vitro* assessment of these oligosaccharides' influence on intestinal microbiome.

Acknowledgements: This work was supported by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia [Contract No. 451-03-136/2025-03/200135 and 451-03-136/2025-03/200287] and has received funding from Science Fund of the Republic of Serbia, programme IDEAS, PrIntPrEnzy [grant number 7750109] and Horizon Europe 2021–2027 research and innovation program, TwinPrebioEnz [grant agreement ID 101060130].

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Blackcurrant Extract as a Botanical Deodorant Alternative via Microbiome Modulation

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Introduction: Unpleasant armpit odor is caused by specific bacteria, mainly by *Corynebacterium* species, that convert sweat secretions into volatile and malodorous compounds. In contrast, another mayor habitant of the axillary microbioom, *Staphylococcus epidermidis*, does not have this property. Therefore, the balance between these bacterial strains can affect the production of unpleasant odor. Since the use of conventional antiperspirants leads to an enrichment of odor-causing species, this does not solve the problem but actually makes it worse. The use of natural extracts such as blackcurrant extract (BCE) offers an interesting alternative to deodorants, helping to maintain microbial balance.

Here, we evaluated whether and how BCE has an effect on the main microbial components (represented by *S. epidermidis* and *C. striatum*) of the armpit microbiome.

Experimental: A home-made callus model was used as a model for *stratum corneum* on which monocultures of bacteria were grown in the absence or presence of various BCE concentrations. Subsequently, co-cultures of the two most common bacteria of the axillary microbiome were tested at various inoculum ratios. Finally, the effect of BCE was evaluated in a therapeutic setting.

Key findings: BCE has growth promoting properties for *S. epidermidis*, while the growth of *C. striatum* is not affected by BCE compared to cultures without BCE. This was found in both the mono- and co-cultures with various inoculum ratios as well as in the therapeutic setting.

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These results indicate that BCE has potential use as a prebiotic to change the axillary microbiome towards a microbiome that produce less volatile and malodorous compounds. These findings needs to be validated with a broader range of staphylococci and corynebacteria, before moving to *in vivo* experiments.

Session

New and improved biocatalysts and biosensors

Chairs: Cezar Mateo and Thu-Ha Nguyen

From random artificial single-stranded DNA library to real time target monitoring from the perspective of a biochemist

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This presentation will cover the process from synthetic random DNA library to point of care devices (POC) based on electrochemical aptasensors. The term "aptamer" is derived from the Latin word "*aptus*" which means "to fit," and the Greek word "*meros*" meaning "part". This term, invented by Nobel laureates Szostak and Ellington, reflects the way aptamers can bind specifically to their target molecules. Aptamers are short, synthetic single-stranded oligonucleotides with high precision in detection of the target molecules, like antibodies but with several advantages. They are synthesized chemically, making them more stable, less expensive to produce, and easier to modify. Compared to antibodies, aptamers have a much more diverse range of targets, starting from ions, small molecules and biomolecules, and even cells themselves, with nearly limitless applications.

The utilization of antibodies as a bio-recognition element in POC devices is currently limited due to obstacles associated with cost and production. This significantly hinders the widespread use and adoptions of antibodies in wearable devices. In the rapidly evolving landscape of nanomedicine, aptamers have emerged as powerful molecular tools, demonstrating huge potential in targeted therapeutics, diagnostics, and drug delivery systems. Additionally, small molecule size, simplicity of the chemical modification process, nonimmunogenic characteristics of aptamers, and their reproducibility within a short generation time are some of the main advantages of aptamers in developing sensitive and portable POC systems.

Aptamers represent one of the most exciting and promising developments in molecular recognition technology, offering a bridge between laboratory diagnostics and POI devices.

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Innovating Biocatalyst Design: Modern Strategies for Enhanced Enzyme Functionality

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The development of efficient and robust biocatalysts is a key enabler for innovation in biotechnology, green chemistry, and industrial bioprocesses. This lecture explores a range of modern strategies used to enhance enzyme functionality, focusing on approaches that improve activity, selectivity, stability, and adaptability to non-natural environments. Central to this discussion are rational design, as well as the increasing role of computational modeling, structure-guided mutagenesis, and chemical modification in enzyme engineering. We will critically evaluate the advantages and limitations of each strategy, highlighting how their integration can lead to more efficient and targeted improvements in biocatalyst performance. Particular attention will be given to the importance of combining enzymes and metals in order to create biocatalysts that exhibit activities that do not occur naturally. Case studies from diverse application areas —including pharmaceutical synthesis, fine chemical production, and environmental remediation—will illustrate how these strategies are being successfully applied in both academic and industrial settings. The session aims to provide a comprehensive overview of the state of the art in biocatalyst design, offering insight into how innovative strategies are reshaping enzyme functionality and expanding the frontiers of biocatalysis in the 21st century.

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Immobilised Enzymes: Robust Biocatalysts for Bioprocess Intensification

Pavle Andrić*

Novonesis, Denmark

Introduction

Enzymes are biologically derived catalysts – biocatalysts – capable of radically enhancing rate of numerous classes of industrially relevant reactions. Novonesis (legacy Novozymes A/S) is a world-leader in producing commercial enzymes. Our products today enable applications, ranging from consumer products to industrial processes, within detergents (laundry, automatic and hand-dish washing), animal feed, human and animal health, baking, fruit, juice, beer and wine processing, starch conversion, biofuels (bioethanol and biodiesel), oils and fats processing, textiles, pulp, leather processing, and recently CO_2 capture, pharma and therapeutics.

As biocatalysts, enzymes are perceived as versatile, effective, and precise, providing high regio- and chemical selectivity, while working at mild conditions (pH, T, pressure) and producing virtually no waste streams. Herewith, enzymes are clearly distinguished from many commercial (chemical-based) catalysts, and enzymes and enzyme formulations are perfect candidates for use in green and sustainable processes. However, enzymes are still perceived (in some applications) as more sensitive, less stable and more cost intensive, than traditional catalysts.

Enzyme immobilization for creating robust biocatalysts

Immobilization is a technique that makes enzymes reusable and hence cost-effective (by reducing enzyme Cost-in-Use) and enables their use in processes where enzyme molecules presence in final products is not allowed. Due to the demand for enzyme reuse and fixation, immobilization is inherently stabilizing enzymes against loss of activity during storage and under the challenging industrial conditions, in application processes, and hence turning enzymes into much more robust (bio)catalysts.

Immobilized enzymes are designed as reusable cores, particles, beads, encapsulates, pellets, structures integrated with e.g. membranes, etc., that are insoluble, easy to separate, with high porosity and high specific surface. All of the above allows for a variety of reactor configurations and process designs that can potentially be utilized, leading to the process intensification

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– as e.g. integrated reaction and separation, in continuous mode – which normally would not be possible with enzymes in soluble format.

Figure 1. *Enzyme immobilization concept and 3 basic types of immobilized products*

Immobilized enzymes for process intensification - success cases from industry

We will discuss successful cases involving immobilized enzymes that have been upscaled and commercialized. We will show how this valuable technology can provide robust catalysts and enhance process intensification for current and future, green and sustainable processes and eventually allow broader use of enzymes. We will also show the most important classes of enzyme action as well as briefly introduce to the main applications of enzymes.

Novel biocatalysts for biosynthesis of specific galacto-oligosaccharide structures

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Introduction: *Lactobacillus*, which is the largest genus of lactic acid bacteria, comprises around 200 species. High number of reports reflects the importance of this 1.8-billion-year-old genus, focusing mainly on the production of probiotics and the health benefits they confer to the host [1]. Among many potential genetic components that are important for the metabolism and life cycle of lactobacilli, the enzyme *β*-galactosidase is of our great interest. *β*-Galactosidase (EC 3.2.1.23; lactase), a member of the glycoside hydrolases (GH), has two functional activities – hydrolytic and transgalactosylating [2]. The hydrolytic activity has been explored since 1950s and widely exploited in the food industry. Besides, transgalactosylation activity of *β*-galactosidases has attracted significant interest for the production of added-value lactose derivatives, such as galacto-oligosaccharides (GOS), which are known as prebiotics and beneficially affect the composition and function of the gut microbiota [3].

Experimental: We have recently solved the crystal structures of a *β*-galactosidase from *Lactobacillus reuteri*, which is the LacLM-type *β*-galactosidase. We use rational design based on molecular docking to improve the yields of GOS formation catalyzed by the enzyme. There are three different enzyme engineering strategies to improve GOS yield: (i) limit hydrolysis and alter transgalactosylation rate, (ii) improve substrate binding within the active site, and (iii) optimize transition state of the enzyme-substrate-product complex.

Key findings: In this research, we present the first crystal structure of the dominant LacLM-type *β*-galactosidases from *Lactobacillus* spp. Looking at the structures of LacLM from *L. reuteri* and LacZ from *Escherichia coli*, the overall fold of the catalytic domains and the active center of the LacLM show similarities to the LacZ from *E. coli*. Based on the crystal structure of LacLM from *L. reuteri* and the docking results of AutoDock Vina, we design engineering strategies to biosynthesize several specific structures of galacto-oligosaccharides, which are reported to be highly prebiotic molecules [4].

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Additionally, we also analysed the sequence space of bacterial *β*-galactosidases to explore the differences between various insertion loops to design further strategies to obtain improved variants

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Zinc-Based Nanobiohybrids as Artificial Metalloenzymes for Efficient Photocatalytic CO_2 Conversion to Bicarbonate

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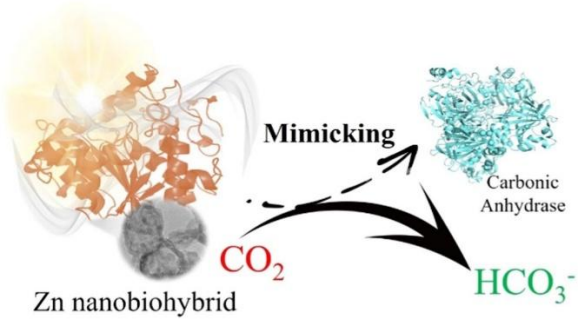
Introduction: Given the rising global carbon dioxide (CO_2) emissions and the urgent need to address climate change, the development of efficient and sustainable CO_2 conversion strategies has gained significant importance. This study introduces, for the first time, the synthesis and design of photocatalytic zinc nanobiohybrids—artificial metalloenzymes that integrate enzymes with zinc nanostructures—for CO_2 conversion under sustainable conditions (aqueous medium at room temperature). Various proteins were evaluated as scaffolds, differing in nature, behavior, and size, to identify the most suitable candidates that could replicate specific natural enzyme activities involved in CO_2 transformation.



Results: Depending on the protein used, distinct ZnO nanocloud-like structures were formed, influencing their catalytic performance. These structures were tested in a model reaction involving the selective hydrolysis of *p*-nitrophenyl propionate (*p*NPP) to *p*-nitrophenol (*p*NP), where Zn coordination with the protein conferred esterase-like activity. Furthermore, the synthesized Zn-protein hybrids displayed activity in hydrolyzing CO_2 to bicarbonate, mimicking carbonic anhydrase activity. The Zn nanobiohybrids also exhibited photocatalytic properties, with conversion efficiencies varying according to the light source. Among them, Zn-CALB demonstrated the highest photocatalytic performance: under ultraviolet (UV) light, bicarbonate conversion was doubled (61 ppm/h) compared to natural light (33 ppm/h), and with Xe light, the rate increased by 2.5 times (83 ppm/h).

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Metal Nanoparticles-Enzyme Complexes as Multi-Functional Heterogeneous Catalysts

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Introduction: One-pot multi-step transformations present a significant chemical challenge today. Advancing this field requires the development of new catalytic systems capable of efficiently performing bio- and chemical transformations in a sustainable manner. To address this, we have designed and synthesized enzyme-metal hybrid nanomaterials that show great potential as multiactive heterogeneous catalysts. Various nanostructures were developed by inducing the *in situ* formation of metal nanoparticles (MeNPs) on a preformed *Candida antarctica* lipase-graphene (G@CALB) complex. In these systems, metal nanoparticles were synthesized exclusively under enzyme induction and uniformly dispersed across the enzyme scaffold.

Results: This biosynthesis, carried out in aqueous media under mild conditions, enables the creation of bifunctional systems where enzymatic activity is preserved while the metallic activity is enhanced, resulting in synergistic effects. We evaluated different metals, including palladium, copper or silver, testing their performance in various one-pot cascade reactions of synthetic interest. Furthermore, we explored the generation of a bimetallic-enzyme system, leading to the development of a trifunctional catalyst with significantly enhanced metallic activity due to synergistic interactions.

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Multicatalytic systems based on immobilized enzymes for glucose production from cellulose in ionic liquids

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Introduction: Glucose is a compound of great interest across various fields due to its broad applicability as a building block for the synthesis of other chemical compounds, as well as a renewable energy source, since it can be converted into various biofuels through chemical processes or fermentation, such as in the production of ethanol. In this context, the production of glucose from cellulose is proposed using an enzymatic cocktail composed of immobilized enzymes, including cellulases, β-glucosidases, and esterases, which act synergistically to hydrolyze the polysaccharide. Since cellulose is insoluble in conventional aqueous media, the use of ionic liquids capable of efficiently dissolving cellulose is proposed, thereby facilitating its transformation into glucose during the hydrolysis process. Subsequently, the implementation of a selective extraction system is explored to recover the glucose from the ionic liquid medium allowing the obtention of the compound.

Key findings: By immobilizing the enzymes in the form of CLEAs (Cross-Linked Enzyme Aggregates) and immobilizing them into magnetic particles, highly active and stable multicatalytic biocatalysts have been developed, enabling high glucose yields to be achieved.

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Ochratoxin A detoxification by microbial enzymes

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Introduction: Ochratoxin A (OTA) is among the most prevalent and toxic mycotoxins. Following good practices during both pre- and post-harvest can effectively reduce its presence in food. However, once OTA is present in the product, its complete elimination presents difficulties. In this context, different chemical, physical and biological methods have been described to eliminate the presence of this toxin. Biological methods, including enzymatic methods, are considered the most promising strategy due to their higher specificity, their ability to preserve food quality, their possible application in mild conditions and their environmental friendliness. In this study, microbial enzymes capable of degrading OTA by hydrolyzing its amide bond, producing the non-toxic products ochratoxin α and L-β-phenylalanine, were identified. A common strategy for discovering new proteins with similar functions is the identification of homologous sequences, typically through sequence alignment-based methods. Here, the fungal ochratoxinase from *Aspergillus niger* (M38 peptidase family) and the bacterial N-acyl-L-amino acid amidohydrolase from *Alcaligenes faecalis* (M20D peptidase family) were selected as reference enzymes, as they are efficient OTA-degrading enzymes.

Experimental: A homology search was performed using BLAST and Clustal Omega to identify proteins with potential similarity to known ochratoxinases. Genes encoding proteins that were predicted to exhibit ochratoxinase activity were cloned, hyperproduced recombinantly in *E. coli*, and purified. PCR amplification was used to amplify and clone the selected genes into the pURI3-Cter vector through a restriction enzyme- and ligation-free strategy. This vector facilitates the expression of recombinant proteins with a C-terminal six-histidine affinity tag in *Escherichia coli*. Purification was carried out by affinity chromatography using imidazole.

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The enzymatic transformation of OTA (5 μM) was analyzed by HPLC. Proteins displaying confirmed ochratoxinase activity were further characterized in terms of optimal temperature, pH, and thermal stability. Characterization assays were conducted using the OTA analog N-(4-methoxyphenylazoformyl)-phenylalanine (4MF) as a substrate. Enzymatic activity was assessed by measuring the decrease in absorbance at 350 nm, which resulted from the hydrolysis of 4MF.

Results: A total of nine proteins similar to previously described ochratoxinases from *A. niger* and *A. faecalis* were identified, seven of which exhibited OTA-degrading activity. These active enzymes were further characterized based on their optimal pH and temperature, thermostability, the influence of various additives on their activity, and substrate specificity. Overall, the enzymes demonstrated peak activity at neutral pH (6.5–7) and elevated temperatures (45–55 °C). In terms of substrate specificity, despite sharing similarities in their catalytic centers, the enzymes exhibited diverse hydrolytic profiles.

Converting a Prolidase from *Cupriavidus necator* N-1 into an OTA-Degrading Enzyme: AlphaFold2-Guided Active Site Engineering

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Introduction: As part of our ongoing efforts to identify new Ochratoxin A (OTA)-degrading enzymes belonging to the subtype I amidohydrolase family, we discovered a promising candidate (*Cn*PRO) encoded in the genome of the Gram-negative bacterium *Cupriavidus necator* N-1. This protein, annotated as a metal-dependent hydrolase (WP_013953161.1), should be homologous to the highly efficient OTA-degrading ochratoxinase from *Aspergillus niger* (*An*OTA) and ADH3 from *Stenotrophomonas acidaminiphila*. As expected, AlphaFold2-based 3D structure prediction of *Cn*PRO revealed a protein fold nearly identical to that of *An*OTA and ADH3, confirming its classification as an amidohydrolase. However, analysis of the hydrolytic activity of a recombinant *Cn*PRO variant expressed in *E. coli* showed that this enzyme exhibits only aminoacylase activity, primarily against N-acyl-Proline, and lacks amidohydrolase activity against OTA.

Experimental: A homology search was performed using BLAST and Clustal Omega to identify proteins with potential similarity to ochratoxinase from *Aspergillus niger* (*An*OTA) and ADH3 from *Stenotrophomonas acidaminiphila*. The 3D structures were predicted using AlphaFold2. Pairwise 3D alignments were performed with Fatcat Server. Genes were PCR-amplified and inserted into the pLATE31 vector using a restriction enzyme- and ligation-free cloning strategy, which involved generating protruding ends in both the vector and the gene using T4 DNA polymerase. A mutated prolidase gene was constructed via site-directed mutagenesis PCR using mutagenic primers. The pLATE31 vector produces recombinant proteins with a six-histidine affinity tag at the C-terminus. Proteins were purified by affinity chromatography using imidazole. The enzymatic transformation of OTA (5 μM) was analyzed by HPLC. The substrate

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specificity of prolidase and mutated prolidase was assessed using synthetic carboxypeptidase substrates containing various C-terminal amino acid residues. Based on their chemical composition, the tested substrates were classified into three families: family I consists of carbobenzyloxy-*β*-alanyl-L-alanine, family II of potential carbobenzyloxy-derived substrates and family III of hippuryl-derived compounds.

Results: A comparison of the predicted 3D structure of *Cn*PRO with those of *An*OTA and *Sa*OTA revealed that all three proteins share a common fold characteristic of subtype I amidohydrolases. However, while *An*OTA and *Sa*OTA hydrolyze the amide bond of OTA, *Cn*PRO lacks this hydrolytic activity. A detailed analysis of their active sites showed that *An*OTA and *Sa*OTA have strictly conserved amino acid residues, whereas *Cn*PRO exhibits notable differences. This insight allowed us to design a *Cn*PRO mutant (*Cn*OTA) with an active site potentially optimized for OTA binding. Subsequent substrate profiling confirmed that *Cn*OTA efficiently hydrolyzes OTA.

Amino-lignin microspheres loaded with Fe_3O_4 nanoparticles as support for xylanase immobilization: application in prebiotics synthesis

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Introduction: In recent years, hybrid nanomaterials engineering has emerged as an exciting research area in materials design. One promising strategy concerning enzyme immobilization is to use hybrid polymer nanomaterials consisting of inorganic nanoparticles coated with natural polymers to create more efficient industrial enzymes, particularly for producing green and sustainable energy or biomass-derived bioactive compounds. Specifically, a hybrid system integrating lignin and magnetite oxide nanoparticles shows promise due to lignin's abundance as a low-cost, renewable resource for producing eco-friendly adsorbents and coatings. Additionally, superparamagnetic magnetite (Fe_3O_4) particles, known for their biocompatibility and low toxicity, have diverse applications in biocatalysis, drug delivery, and bioimaging. This combination of lignin and Fe_3O_4 nanoparticles could yield a hybrid material with enhanced stability, chemical resistance, magnetic functionality, and a strong affinity for enzyme molecules.

Experimental: The spherical amino-modified lignin-based microspheres were prepared by inverse copolymerization of lignin (kraft) from suspension with poly(ethylene imine) and amino-functionalized magnetite nanoparticles (A-LMS_Fe_3O_4). The optimal parameters for immobilization of commercial preparation ROHALASE® SEP-VISCO xylanase on hybrid nanosystem A-LMS-Fe_3O_4 were determined by measuring glucose production from Birchwood xylan using the DNS method. Furthermore, the application of the A-LMS_ Fe_3O_4-xylanase was tested in the production of potential prebiotic xylo-oligosaccharides (XOS). The XOS synthesis reaction progress was monitored using HPLC. Sunflower meal (SFM), a by-product of sunflower

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oil production, represents a valuable agricultural residue rich in xylan; thus, it was used as a substrate for XOS production. By utilizing SFM for xylan extraction and subsequent production of XOS, a sustainable and eco-friendly bioprocess was developed.

Results: The A-LMS_Fe_3O_4 nanoparticles used for ROHALASE® SEP-VISCO xylanase immobilization possess specific characteristics: a surface area of 3.38 m^2/g, 64% porosity, and a particle diameter of 10.05 to 20.25 nm. To optimize the immobilization process, the initial enzyme concentration was varied in the range of 200 to 950 mg/g of support. The optimal protein loading of 389 mg/g was achieved with a protein immobilization efficiency of 40% after 30 min. The highest hydrolytic activity recorded was 1537 IU/g of support at the highest initial enzyme concentration after 2 hours, with the immobilized enzyme retaining approximately 57% of the initial activity. The specific activity of 7.02 IU/mg of protein was detected, indicating that nearly all immobilized enzyme molecules were active. Consequently, the immobilized preparation obtained after 2 h at 950 mg/g of support and pH 6.0 was selected for the production of XOS from SFM xylan. This research highlights the potential of the A-LMS- Fe_3O_4 hybrid nanosystem as an eco-friendly approach to generate prebiotics from agro-food waste, achieving an XOS productivity yield of 25% based on the total xylan content from SFM after 3 hours.

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Evaluation of amino-modified nanocellulose carrier for immobilization of xylanase and application in xylo-oligosaccharide synthesis

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Introduction: Biocatalysis is increasingly recognized as a viable alternative for synthesizing complex molecules that are significant in industrial applications. This is achievable through the use of enzymes immobilized on biopolymers, which serve as environmentally friendly carriers with specific attributes. In particular, the immobilization of enzymes on nanocellulose (CNC), which features a well-organized crystalline structure, favorable morphology, and properties such as non-toxicity and biocompatibility, represents a promising approach for enhancing the performance of crucial industrial enzymes. With this in mind, the main objective of this study is to develop an efficient preparation of immobilized xylanase for the production of potentially prebiotic xylo-oligosaccharides (XOS) using xylan extracted from sunflower meal (SFM) as a substrate. Specifically, two strategies for immobilizing xylanase on amino-modified nanocellulose (Amino-CNC) will be employed: adsorption and covalent binding via glutaraldehyde crosslinking.

Experimental: Silanization reaction using 3-aminopropylethoxysilane to introduce amino groups to the surface of the CNC was applied. The resulting Amino-CNC was characterized as nanorods ranging from 100 to 800 nm in length and 40 to 60 nm in width. To enable covalent immobilization of xylanase, the amino-CNC was activated with the crosslinking agent glutaraldehyde (GA-amino-CNC). Under optimized conditions (2 hours, 60 ºC, and pH 6.0), the xylanase immobilized on both Amino-CNC and GA-amino-CNC, which exhibited the best characteristics, was evaluated in the conversion of SFM xylan into XOS.

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Results: At the highest achieved protein loading (250 mg/g of support), xylanase attached via adsorption on Amino-CNC displayed an activity of 416 IU/g of support, yielding a specific activity of 1.67 IU/mg of protein. However, high loading corresponded to a low activity immobilization yield of only 17%. In contrast, when protein loading was lower, 150 mg/g, the activity yield improved significantly to 50%, with a specific activity of 1.5 IU/mg of protein, while the expressed activity of the immobilized preparation dropped 2-fold. Xylanase immobilization on GA-amino-CNC via covalent attachment exhibited similar trends. In the case of the protein loading of 190 mg/g of support, the activity reached 240 IU/g of support with a 12% activity yield, while a 30% lower protein loading increased the activity yield up to 40%. The specific activity of GA-amino-CNC-xylanase reached a similar value (1.45 IU/mg proteins) to Amino-CNC-xylanase at the lower protein loading. These trade-offs highlight the importance of customizing immobilization strategies to meet specific process requirements. In the context of using these immobilized preparations for XOS synthesis, covalently immobilized xylanase generated 28% XOS based on the total xylan content from SFM after 3 h, whereas absorbed xylanase required 5 h to yield the same amount of XOS. These findings suggest that formed covalent bonds enable the attached enzyme molecules to adopt a more favorable conformation, facilitating easier substrate access to the active site, thereby enhancing XOS synthesis.

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Session

Bioprocess intensification

Chairs: Nikola Nikačević and Pavle Andrić

Intensification of bioprocesses

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Strategies to reduce cost and emission profiles are becoming increasingly important for the development of affordable and sustainable biobased production. The overall goal of process intensification in various industries is to achieve significant benefits in terms of cost, product concentration and quality, while eliminating waste and improving process safety. Bioprocess intensification could be a valuable tool to increase efficiency and reduce resource consumption in bioproduction. In general, bioprocess intensification is defined as an increase in bioproduct output relative to cell concentration, time, reactor volume or cost. Key strategies in this area include the combination of reaction and downstream, enzymatic and microbial electrosynthesis, the use of CO_2 as a carbon source, the conversion of waste materials into valuable products, and the use of unconventional reaction media. This review provides a definition of process intensification in biotechnology, presents some general and specific examples, and addresses some of the current challenges.

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Recombinant enzyme production, its intensifications and applications

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The application of recombinant enzymes in different areas of industry including pharma, food, feed, chemical and biorefinery sectors is highly demanded. Its effective production plays a key role in their further applications. Our team is principally focused on the production of recombinant enzymes, intensification of fermentation process and its further applications. Among the wide portfolio of proteins and enzymes we are currently focusing on are enzymes applied in biocatalysis, biorefineries and pharma industry.

For the purposes of effective recombinant enzymes production we are applying both main producers: *Escherichia coli* and *Pichia pastoris*. In the presentation different strategies of recombinant enzyme production even on waste substrates and its further applications will be presented.

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Advancing Biocatalysis with 3D-Printed Continuous Flow Reactors

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Introduction: In recent years, 3D printing (3DP) has evolved into a well-established manufacturing technology, extending its applications far beyond rapid prototyping. The development of advanced materials has broadened its impact across various fields, offering cost-effective solutions for next-generation devices and engineering challenges. However, achieving both high-resolution fabrication and customized functional properties in printable materials remains a significant challenge.

Key findings: Our approach leverages 3DP's flexibility for reactor design while incorporating surface modifications to develop stable biocatalytic systems. Additive manufacturing enables the creation of complex geometries using a wide range of materials, which can be tailored for process intensification. The ultimate goal is to integrate continuous-flow reactor technology with biocatalysis, utilizing immobilized enzymes that are both stable and reusable, thereby enhancing the efficiency of biotransformations. Moreover, 3DP facilitates the rapid fabrication of structured reactor architectures, significantly improving process optimization capabilities.

This work presents our latest advancements in printable materials with tunable chemical functionalities, emphasizing their role in enhancing biocatalytic reactions.

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Advanced distillation-based purification processes for effective recovery of fermentation products

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Introduction: Although biorefineries present a promising alternative to traditional petrochemical production, improving fermentation and downstream processing is often essential to enhance the competitiveness of large-scale bioprocesses. A key challenge in the downstream processing is low product concentration in the fermentation broth (often <10 wt%) due to the end-product inhibition phenomenon. Additional challenges that complicate recovery processes include by-product formation, thermodynamic constraints (e.g. azeotrope formation), and microbial presence. Consequently, downstream processing accounts for 20-40% of the total production costs in bulk biochemical production. Advanced separation and purification technologies are vital for improving bioprocess efficiency. Hence, this research aims to enhance the competitiveness of industrial fermentation by developing advanced downstream processes, based on process intensification principles, for the effective recovery of various fermentation products. Aspen Plus software was used as a computer-aided process engineering tool for process design.

Key findings: Continuous product removal from the fermentation broth is a commonly employed technique for mitigating end-product toxicity. When products are sufficiently volatile, a distillation-based loop around the bioreactor can be used for initial separation. This process should be conducted under reduced pressure to lower operational temperatures and enable the reuse of most of the broth and microorganisms, improving fermentation efficiency by reducing biomass loss, enhancing substrate-to-product yield, and conserving water. For products with insufficient volatility, distillation must be coupled with additional techniques such as biomass removal via centrifugation or alternative *in-situ* methods like liquid-liquid extraction. Advanced downstream processes have been designed for the recovery of various low-boiling (e.g., ethanol, isopropanol, acetone, isobutanol, hexanol) and high-boiling biochemicals (e.g., 1,3-propanediol, butanediol isomers). These processes were evaluated based on their economic and environmental impact. The integration of process

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intensification and energy recovery techniques has been shown to reduce costs by 40-80% and energy consumption by 60-80% compared to conventional methods. However, the performance of recovery processes depends significantly on the product concentration in the fermentation broth, which is mainly determined by the product hydrophobicity. Thereby, lower product concentrations result in more energy-intensive and expensive recovery. Nonetheless, the costs of the developed advanced recovery processes make up only 8-16% of current market prices, indicating their potential for improving the economic viability of biorefineries.

Therefore, advanced purification processes based on process intensification are essential for enhancing bioprocess efficiency. To remain competitive and ensure the long-term viability of industrial biotechnology, it is critical to simultaneously develop both upstream fermentation processes and downstream processing technologies. Finally, this study emphasizes the crucial role of process design and simulation in developing sustainable (bio)chemical processes, enabling the application of a process systems engineering (PSE) approach in biotechnology.

Enhancement of the Biological Potential of Herbal Extracts through Fermentation, Microwave-Assisted Extraction, and Iodine Catalysis

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Introduction: The biological potential of herbal extracts can be significantly enhanced through fermentation, followed by microwave-assisted extraction (MAE). The selection of microorganisms capable of producing enzymes such as hemicellulase, cellulase, tannase, and glucosidase facilitates the release of polyphenols from the plant matrix, the depolymerization of tannins, and the conversion of glycosides into aglycones. This enzymatic activity results in fermented extracts with superior biological properties compared to non-fermented counterparts. Moreover, the addition of iodine during MAE, as a catalyst, enables the production of extracts with enhanced biological activity. The catalytic properties of iodine arise from its ability to form intermolecular bonds with electron-rich atoms within various functional groups, thereby facilitating nucleophilic reactions and enabling the synthesis of new compounds.

Experimental: In this study, *Achillea millefolium* (yarrow) was fermented using two microorganisms: *Lacticaseibacillus rhamnosus* A71 and *Saccharomyces boulardii*. After fermentation, MAE was performed. A non-fermented control sample was prepared under the same conditions without the addition of microorganisms. For the iodine treatment, a second set of samples was prepared by adding iodine at a concentration of 1% (w/w) during the MAE process, while a control sample was extracted without iodine under identical conditions. The extracts were analyzed for polyphenol content, antioxidant activity (measured by ferric reducing antioxidant power and DPPH radical scavenging assay), antimicrobial properties (minimum inhibitory concentration, MIC, against pathogenic microorganisms), and acetylcholinesterase (AChE) inhibition.

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Results: Fermentation with *L. rhamnosus* A71 and *S. boulardii* led to a significant increase in polyphenol content in extracts, from 231 mg GAE/g to 332 mg GAE/g and 289 mg GAE/g, respectively. Antioxidant activity, expressed as ferric reducing antioxidant power, increased from 2.00 mmol Fe^{2+}/g to 2.85 mmol Fe^{2+}/g and 2.16 mmol Fe^{2+}/g, while the IC_{50} values for DPPH radical scavenging decreased from 6.76 μg/mL to 6.23 μg/mL and 6.52 μg/mL, following fermentation with *L. rhamnosus* A71 and *S. boulardii*, respectively, indicating enhanced antioxidant potential. Antimicrobial activity was notably improved, particularly against *Staphylococcus aureus*, where the MIC decreased 16-fold (from 5 mg/mL to 0.32 mg/mL) for both fermented extracts, and against *Pseudomonas aeruginosa*, with an 8-fold reduction in MIC (from 2.5 mg/mL to 0.32 mg/mL) for the *S. boulardii* fermented extract. AChE inhibition increased from 31.7% to 42.6% and 43.1%, following fermentation with *L. rhamnosus* A71 and *S. boulardii*, respectively.

When iodine was incorporated during MAE, the MIC against *S. aureus* decreased 2-fold compared to the extract without iodine. Antioxidant activity further improved, with ferric reducing antioxidant power increasing from 5.1 mmol Fe^{2+}/g to 5.4 mmol Fe^{2+}/g and the IC_{50} value for DPPH radicals decreasing from 6.96 μg/mL to 3.97 μg/mL.

These findings demonstrate that fermentation with selected microorganisms followed by MAE and the addition of iodine during MAE enhance the bioactivity of herbal extracts, offering a promising strategy for developing functional plant-based products.

"Dark-Photosynthesis" in *Rhodospirillum rubrum*: Biohydrogen and Carotenoid Synthesis from Agroindustrial Waste Under Semiaerobic Dark Conditions

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Introduction: Biohydrogen production is crucial for a sustainable energy transition, while lycopene and carotenoid production have great pharmaceutical values. *Rhodospirillum rubrum* as a facultative photosynthetic purple non-sulfur bacterium, produces these resources completely independent of light at semi-aerobic dark conditions ("Dark Photosynthesis"), but the efficiency depends on optimal substrates. The "Dark Photosynthesis" growth regime thus opens a new door to the biotechnological potential of photosynthetic purple bacteria using common bioreactor technology at industrial scales.

Experimental: This study investigates the use of grape pomace juice (GPJ) and hydrolyzed molasse as renewable and economic carbon sources in 10-30 L bioreactors with the wild-type strain S1 and the genetically modified lycopene-producing strain SLYC18. Semi-aerobic conditions were controlled *via* pH-DO coupling, focusing on the fructose content (80 g/L) of GPJ.

Results: At a dilution of 1:10, GPJ enabled high growth and production of 373 ml H_2/L with a yield of 6.57 mol H_2/mol fructose. The strain SLYC18 achieved a high yield of lycopene (~50 mg/L) without compromising growth. Although transcriptomic analyses revealed expression of *nif* genes, encoding nitrogenase components, the majority of the observed biohydrogen likely originated from the fermentative enzyme formate-hydrogen lyase.

In summary, GPJ as well as hydrolyzed molasse are effective alternatives for the production of biohydrogen and carotenoids with *R. rubrum*, eliminating the need for light. This approach paves the way for sustainable purple bacteria biorefineries based on agricultural waste.

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Natural deep eutectic solvents for efficient mild pretreatment of crop residues

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Introduction: Crop residues consist predominantly of lignin, cellulose, and hemicellulose, organized together as lignocellulose. With approximately 200 billion tons produced per year, lignocellulosic biomass is the most abundant source of plant-based raw materials available in nature [1]. Burning crop residues worldwide emits around 70,000 kt of CO_2eq in total [2] and this can be prevented by implementing these residues in biorefinery. The prerequisite step to utilize these residues is to pretreat them and break down the complex lignocellulose structure. Natural deep eutectic solvents (NADES) are emerging as a green, biocompatible, biodegradable, and cheaper alternative to ionic liquids that are widely used as a highly efficient pretreatment. Their physicochemical properties can be fine-tuned by altering the number, type, and molar ratio of NADES constituents. Acidic NADES composed of choline chloride and carboxylic acids proved to be suitable for lignocellulosic biomass delignification. However, to increase the pretreatment's efficiency, elevated temperatures and prolonged pretreatment duration are needed. This study aimed to test the possibility of subjecting corn stalks to NADES pretreatment at room temperature and in short time intervals to improve the sustainability of the pretreatment.

Experimental: Taguchi orthogonal array design (L9) was used to select the best pretreatment conditions for achieving the highest delignification rate. Key parameters of the process, namely choline chloride to lactic acid ratio in NADES, water addition, and pretreatment duration, were optimized through nine experimental runs. Biomass was pretreated at room temperature while being stirred constantly. After pretreatment, biomass was recovered, analyzed for residual lignin content, and subsequently subjected to enzymatic hydrolysis using commercial Cellic CTec2 mixture to estimate the pretreatment's efficiency in terms of improving both biomass delignification and digestibility.

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Results: The optimal conditions for achieving the highest delignification rate were defined based on the results obtained, as follows: choline chloride-to-lactic acid ratio 1:5, addition of 20% water, and 10-minute-long pretreatment performed at room temperature. Analysis of variance confirmed that the composition of NADES contributed mostly to the pretreatment's efficiency. Sugar yields obtained after 48-hour-long enzymatic hydrolysis were mostly correlated to the biomass delignification. This suggests that corn stalks could be successfully delignified and further used through various fermentation processes with reduced energy demands. However, different types of NADES could be tested to make this pretreatment energy-efficient and cost-effective on a larger scale. Moreover, the reusability of NADES and the potential valorization of extracted lignin should also be examined to shift this pretreatment toward a zero-waste approach.

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Intensified expression of metal-dependent enzymes

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Introduction: Metalloenzymes are a ranged group of enzymes whose activity and structural integrity rely on the presence of certain metal ions as cofactors such as Zn^{2+}, Mn^{2+}, Fe^{2+}, or Mg^{2+}. Metal ions are frequently coordinated into the active site to engage in chemical transformations such as redox reactions or hydrolysis, which often stabilize transition states or facilitate electron transfer. Metalloenzymes play an essential role in various biological processes, including respiration, oxidative phosphorylation, DNA or RNA synthesis, and antioxidant defense [1].

Alcohol dehydrogenases are zinc-dependent enzymes, which are widely used in the pharmaceutical and biofuel industries for stereoselective synthesis, environmental remediation, and ethanol production [2]. Magnesium-dependent DNA and RNA polymerases are fundamental tools in molecular biology, including PCR, sequencing, and diagnostics. In recent years, RNA-dependent RNA polymerase (RdRp) has gained popularity. The availability of purified recombinant RdRp will allow for not only biochemical analysis of the role of the enzyme´s role in virus replication, but also the study of its structure and function, as well as the search for its potential inhibitors, all of which are required to combat viral diseases such as COVID-19, gastroenteritis, hepatitis C, ebola, and many others [3]. Understanding their expression and metal incorporation is crucial for optimizing enzyme performance for expanding their industrial use, expanding their biotechnological application, or advancing drug design.

This study focuses on the intensified expression and functional characterization of selected metalloenzymes in bacterial host. *Escherichia coli* provides a platform for the rapid and cost-effective synthesis of recombinant proteins. However, the creation of inclusion bodies caused by the overproduction of heterologous proteins is a significant barrier to large-scale production. Proper and efficient protein folding might require specific cofactors in the growth media, such as metal ions. The addition of these crucial components to the culture media may significantly boost the yield and folding rate of the soluble proteins [2].

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Experimental: This study focuses on the heterologous expression and functional characterization of selected metalloenzymes in bacterial host E. coli. The intensification of the recombinant enzyme was carried out according to optimized protocol in DASbox® laboratory fermenters. The obtained cells were mechanically disrupted, and the enzyme was purified using affinity chromatography. The activity and substrate specificity of the enzyme was determined.

Key findings: We optimized expression conditions to ensure proper metal incorporation, using various metal supplementation strategies and co-expression of metallochaperones. Our results highlight the importance of metal homeostasis in achieving functional enzyme expression. These findings contribute to the development of efficient production systems for industrial and biomedical applications of metalloenzymes.

Acknowledgment: This work was supported by the HE Project 101159534 — WIDEnzymes.

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Computational Fluid Dynamics Analysis of Two-Phase Bioreactors with Smooth Periodic Constrictions

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Introduction: In biochemical production, one of the key aspects of process intensification is the transition from batch to continuous processing. In multiphase reactor systems, for optimal performance, it is essential to ensure proper fluidization and uniform distribution of solid particles. This is particularly challenging as biochemical reaction rates are inherently slow, requiring long residence times and efficient mixing. Achieving these conditions simultaneously remains a key obstacle, limiting the widespread adoption of continuous processes in the industry. To address these limitations, innovative reactor designs with enhanced mixing at low flow rates are currently researched, including oscillatory flow reactors. The application of advanced computational tools, particularly Computational Fluid Dynamics (CFD), has proven to be a strong asset in optimizing reactor designs and process parameters.

Experimental: In this study, Simcenter STAR-CCM+ is employed to analyze and compare the hydrodynamics and fluidization characteristics of reactor geometries with semi-continuous operation, where particles are contained within the reactor, while the liquid phase flows continuously. The study investigates the use of both constant and oscillating flow conditions of the continuous liquid phase. Depending on the flow rate, the fluid was modeled using either the laminar or the k-ε turbulence model. To simulate the behavior of solid particles, a Lagrangian multiphase model is developed, including the drag force (Schiller-Naumann model), shear lift force (Sommerfeld model), pressure gradient force, gravity and virtual mass. The initial simulations assume particles are spherical and uniform with a 400 μm diameter. As the volume fraction of solids does not exceed 5 vol.%, it is assumed that their presence does not influence the liquid flow and two-way coupling was not considered.

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Key findings: Two tubular reactor designs, both with smooth periodic constrictions (SPC), with a 30 mm diameter, were considered based on their ability to promote changes in velocity and the creation of vortices, while avoiding sharp edges that could lead to particle accumulation. To replicate experimental conditions, particles are introduced into the reactor before initiating the liquid flow. This is done via part injectors distributed within the entire reactor volume. The analysis of simulation results focuses on identifying the optimal liquid flow conditions, which ensure both extended residence time and effective mixing of solids for the selected reactor geometry. This can be assessed by calculating the particle volume fraction across equidistant reactor cross-sections and quantified via standard deviation from the ideally mixed solid phase. Figure 1 shows the distribution of the solid phase for normal flow conditions with Re = 212. It shows that even quite low flowrate can keep the particles fluidized, but most of them stay concentrated closer to the axis of the reactor. Thus, the entire volume of the reactor is not fully utilized. This could be improved by using oscillatory flow, and simulations to analyze this option will be further analyzed.

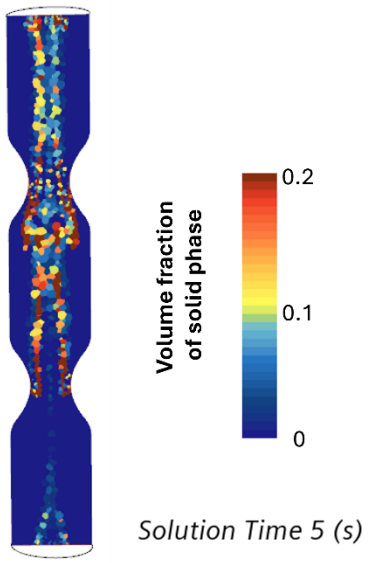

Figure 1. CFD simulation results for normal flow with Re = 212

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Modeling and Optimization of Membrane Reactors for Production of Prebiotics

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Introduction: Fructooligosaharides (FOS) are an established type of prebiotics used in the supplement industry to improve the composition of human gut microbiota. They can be produced using enzymatic transfructosylation reaction of sucrose, which is conventionally done in batch reactors. A major drawback of these conventional reactors is that FOS yields and purity are low. This study analyses the improvement in FOS production by using a membrane reactor under optimized process conditions.

Experimental: The experimental setup included a stirred tank and a spiral wound nanofilter membrane. The stirred tank was initially filled with 5 wt% sucrose solution and a commercial enzyme Pectinex® Ultra SP-L (Novozymes, Denmark) with 1 vol.% concentration. The experiments included two operation modes: the reaction without separation (for 60 and 120 minutes) and the reaction with separation (at constant volume). Separation is mainly done to remove unwanted byproducts (i.e., fructose and glucose).

The mathematical model of the membrane reactor was derived as a set of material balance equations for the stirred tank and the membrane module, including all the reaction components (sucrose, enzyme, glucose, fructose, and FOS). The gPROMS ModelBuilder software (Siemens PSE) was used to solve the model equations and later to perform numerical optimization. The dynamic model optimization included an objective function defined to maximize FOS production, while considering enzyme consumption and time required. It was used to determine optimal values of main process parameters: initial sucrose and enzyme concentrations, all relevant flowrates in the system, as well as durations of different modes of operation.

Results: The results of experiments performed in the batch and membrane reactors showed that the maximum mass fraction of FOS were 47 and 92%,

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respectively. Thus, the membrane reactor showed a significant increase in FOS purity. This is due to the selective removal of glucose and fructose in the case of the membrane reactor.

Since the membrane reactor model showed a good qualitative match to experimental data, its numerical optimization was used to analyze further improvements that can be made in terms of FOS production. Our optimizations showed that FOS yield above 75% can be achieved while maintaining purity at 90% or more. This would be a considerable improvement to the conventional batch reactor operation, where both yield and purity are typically around 50%. The results showed this can be achieved by increasing the number of operation periods with optimized flowrates and durations of each period. Further work is being done on exploring the transition to fully continuous FOS production using membrane reactors.

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Polyphenol Extraction from *Teucrium montanum* Using Fluidized Bed

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Introduction: Fluidized beds are systems that ensure efficient contact between the solid phase and the fluid, making them suitable for extraction processes. In this study, a fluidized bed was used for the extraction of polyphenols from plant material. Polyphenols are micronutrients and natural antioxidants found in fruits, vegetables, plants, and nuts, obtained through extraction from plant materials. Extracting polyphenols from medicinal plants is particularly important, as these compounds can be utilized in medicine, cosmetics, and the food industry. The quality of the obtained plant extracts depends on the choice of solvent and the applied extraction method.

Experimental: The objective of this study is to optimize the extraction of polyphenols from *Teucrium montanum* by evaluating three different methods: (1) a fluidized bed of plant material, (2) a three-phase fluidized bed with inert particles, and (3) conventional maceration. All extractions were performed at room temperature to preserve the stability of thermolabile polyphenols. Water and a fifty percent aqueous ethanol solution were used as solvents.

Key findings: text The extraction kinetics of total polyphenols were monitored to evaluate the efficiency of all three extraction methods. The results showed that both fluidization techniques yielded a higher polyphenol extraction rate compared to conventional maceration. The study also showed that a higher polyphenol yield is obtained when a 50% aqueous ethanol solution is used as the extraction solvent.

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Session

Bio-based products: from laboratory to industry

Chairs: Ellen van den Bogaard and Rada Pjanović

Biocatalytic processes for converting waste into bio-based platform chemicals

Celia Alvarez-Gonzalez, Jorge Garcia-Montalvo, Alvaro Lorente-Arevalo, Victoria E. Santos, Miguel Ladero, Juan M. Bolivar*

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Bioprocess engineering plays a central role in advancing sustainable development and enabling a circular bioeconomy. The valorization of raw materials derived from food industry by-products and agricultural residues offers significant potential, but also presents distinct processing challenges. The development of intensified bioprocesses—from biomass to platform chemicals—requires coordinated upstream and midstream operations, where hydrolytic and oxidative biotransformations are particularly important. In Spain, abundant feedstocks such as rice straw and winery residues exemplify this opportunity. Rice straw is a valuable glucose source, while grape pomace and vine prunings are rich in phenolic compounds and lignin. Mild pretreatment strategies are essential for improving cellulose accessibility in rice straw, facilitating subsequent enzymatic saccharification. Likewise, solid-liquid extraction, acid hydrolysis, and organosolv treatments are key for the effective fractionation of winery residues.

Efficient conversion of glucose, its derivatives, and phenolic compounds through biocatalytic oxidation represents a promising strategy for biomass upgrading. Achieving this requires an integrated approach to enzyme, catalyst, and reactor design, addressing challenges such as the gap between enzyme engineering and process implementation, complex reaction kinetics and mass transfer, and long-term operational stability. This presentation will also explore catalyst formulation and reaction engineering strategies to support the development of robust biotransformations.

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Pioneering the onsite upcycling of plant-based side-streams

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Unfortunately, our current food production system discards many of the nutrient rich parts of plants we should be utilising. One example of this is the dietary fibre present in hemicellulose side-stream products typically sent to animal feed. This dietary fiber deficiency in processed foods is one contributing factor leading to roughly 90% of adults lacking over 50% of the soluble fibre or prebiotics they should be consuming in their diets on daily basis and the related health implications. Carbiotix decided in 2024 to address this problem, building on over 10 years of experience working with the extraction of soluble fibers, proteins and other bio-active extracts from plant-based materials, and launched its NutraCycle service. Over the past 12 months and up to the end of April 2025, the company commenced 47 development projects on 58 different plant-based side-stream materials, including fruits, vegetables, cereals, legumes, nuts, berries, grasses, seeds or other plant-based materials. The NutraCycle service is a very good example of efficiently designing a customised process at laboratory scale to hydrolyse a side-stream that is translatable to an industrial scale. With a focus on product complexity as a driver of efficacy rather than purity, Carbiotix is pioneering the cost-effective upcycling of F&B plant-based side-streams, focusing on the fortification of onsite products and creating business opportunities with Value Added Manufactures. This approach provides an opportunity to expedite the use of extracts, redefining the ingredient supply chain, and allows for processed F&B products to be converted into functional products high in soluble fiber, protein and other bio-active extracts at a low cost.

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Upcycled Collagen-Building Peptides from Microalgae Biotechnology: A Circular Approach to Skin Nutrition with ALGAKTIV® Collage

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The rising demand for effective, sustainable, and functional cosmetic ingredients has catalyzed innovation in biotechnology, particularly in the valorization of industrial by-products. ALGAKTIV® Collage exemplifies this shift through the upcycling of *Haematococcus pluvialis* biomass from Iceland—traditionally discarded after astaxanthin extraction—into a zero-waste cosmetic active rich in vegan collagen-building peptides and bioavailable amino acids.

This novel active contains glycine, proline, and hydroxyproline—key structural amino acids that constitute native human collagen. With a molecular weight between 75–130 Da, these peptides are optimized for topical absorption, enabling direct nourishment of skin fibroblasts and stimulation of collagen synthesis. The formulation also features Spermidine, a cellular rejuvenation booster that promotes dermal regeneration by activating autophagy and upregulating collagen synthesis genes.
ALGAKTIV® Collage's clinical data demonstrate instant and long-term effects: a 15% reduction in wrinkle depth within 10 minutes, a significant increase in hydration and plumping after single use, and enhanced dermal density and radiance over time. *In vitro* studies confirm its capacity to restore aquaporins AQP3 and AQP5 and to stimulate native collagen production by over 40% at 1% concentration.

By integrating circular economy principles, advanced peptide science, and proven efficacy, ALGAKTIV® Collage bridges the gap between environmental responsibility and high-performance skincare. It represents a new frontier in biocosmetics—where discarded microalgal biomass is reimagined as a physiologically active, dermonutrient solution from lab to market.

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Enzymes as a tool for the improvement of functional properties, nutritional quality and *in vitro* digestibility of protein isolates from underutilized plant sources: A chickpea case study

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Introduction: The use of enzymes in the treatment of plant raw materials generally allows lower energy consumption, higher efficiency and mild processing conditions. Another advantage of enzyme-assisted extraction is that the obtained compounds could be of superior and preserved quality, making them highly suitable for human nutrition. Nevertheless, usage of enzymes for the improvement of protein extraction has not been fully exploited yet.

Among different peas, chickpea emerged as promising but underutilized source of protein. Although it is widely consumed in some countries in Asia and Africa, its implementation in diet in European countries as well as in Serbia has been limited. So, our research is focused on the potential of this underutilized legume as sustainable source of proteins and possible enzymatic technology that can be employed for the enhanced protein production from this plant source. The aim of the study was to evaluate yield, amino acid composition, functional and nutritional properties and *in vitro* digestibility of protein isolates produced from chickpea seeds. The study compares protein isolates prepared with the aid of enzymes (individual α-L arabinofuranosidase or combination of xylanase and cellulase) with that from protocol without them (conventional extraction). Results revealed that usage of enzymes in extraction of proteins resulted in enhanced protein yield and in addition higher protein content in these protein isolates. On the other side, protein isolates produced with assistance of enzymes showed improved functional properties such as solubility, water and oil holding capacities and emulsifying and foaming properties. Enzymatic treatment led to changes in amino acid composition of extracted proteins. Highest Essential Amino Acid Index, biological value and nutrition index were characteristics of proteins extracted with the assistance of enzymes. In addition, enzymatically obtained

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chickpea proteins are reflected in well-balanced amino acids content as well as in high bioavailability and superior digestibility compared to the one from conventional protocol. Obtained results revealed huge potential of enzymes as a tool for the improvement of yield, functional and nutritional properties of chickpea protein isolates.

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Whey protein isolate hydrogels as a delivery vehicle for phytochemicals in biomedical applications

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Introduction: Due to the aging populations in developed countries, there is a constant and growing need for implantable biomaterials to (i) restore lost tissue function; (ii) promote wound healing, and (iii) deliver drugs, both hydrophilic and hydrophobic. Furthermore, with the increasing prevalence of microbes resistant to antibiotics, new phytochemicals with antimicrobial activity are beneficial additives to biomaterials, to prevent undesirable microbial colonisation during implantation. Whey Protein Isolate (WPI) is a cheap byproduct from the dairy industry, which is commonly used as a food supplement for bodybuilders. WPI hydrogels can be formed by heating and sterilized by autoclaving, a ubiquitous and clinically accepted sterilization technique. This work will summarise our research group's work on WPI hydrogels for biomedical applications, and its use as a carrier for phytochemicals and biotechnologically produced polymers with biological activity.

Experimental: In previous work, we have improved WPI hydrogels for biomedical applications by the addition of biotechnologically produced polymers such as Poly-gamma-glutamic acid (PGGA) [1] and inorganic materials such as Calcium Silicate [2]. Furthermore, we have developed WPI hydrogels as carriers for Cannabidiol for oral drug delivery of anticancer drugs [3] and phloroglucinol, the building block of phlorotannins, which are phlorotannins found in seaweeds which display antibacterial activity [4].

Key findings: WPI is a versatile biomaterial, which can serve as a delivery vehicle for both hydrophilic and hydrophobic substances, including phloroglucinol, which retains its bioactivity. Furthermore, it supports the adhesion, proliferation and osteogenic differentiation of bone-forming cells, highlighting its potential as a biomaterial to support tissue regeneration.

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Microbubble Plasma-Enhanced Oxidative Pretreatment for Improved Biogas Yield from Marginal Biomass

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Introduction: Lignocellulosic biomass is a renewable resource that is abundantly available for producing sustainable energy. However, its complex matrix, particularly due to the recalcitrant lignin component, limits biogas yield in anaerobic digestion (AD) processes. Despite the development of numerous pretreatment strategies, there is still a requirement for sustainable, scalable, technically feasible, and industrially applicable technologies that can effectively degrade lignin and improve the accessibility of substrates to microbes. This study investigates the integration of the Fenton process with a microbubble plasma technology as a novel oxidative pretreatment strategy to enhance lignin degradation in whole maize and improve biogas production through AD.

Experimental: The pretreatment was performed by introducing maize into a Fenton reagent (a mixture of hydrogen peroxide and iron salts) within the microbubble plasma reactor, which operated under atmospheric pressure. The Fenton solution initiates hydroxyl radical formation, while the plasma enhances the effect by producing more reactive oxygen and nitrogen species, including hydroxyl radicals, ozone, peroxynitrites, and nitric acid. This combination creates a synergistic oxidative environment, facilitating lignin breakdown. Varying Fenton solution molar ratios of hydrogen peroxide to iron salts from 1:10 to 1:75 and treatment durations of 30 min to 90 min were investigated. To evaluate structural and chemical modifications to lignocellulosic biomass, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) were used to characterise both treated and untreated samples. The National Renewable Energy Laboratory (NREL) standard protocol was implemented to ascertain the lignin content of biomass samples. The biomethane yield is evaluated through batch tests of the Biochemical Methane Potential (BMP) using the AMPTS II system. Concurrently, a continuous stirred anaerobic digestion system has been

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studied to assess the performance of the process under semi-continuous conditions.

Key findings: The FTIR results demonstrate a notable decrease in the intensity of peaks associated with lignin linkages, suggesting that the plasma-assisted Fenton oxidation effectively contributed to the disruption of lignin structure. Increased surface disruption and porosity are evident in pretreated maize as evidenced by SEM images. The pretreated samples exhibited a significant improvement in methane production, as indicated by the batch Biochemical Methane Potential test results. Furthermore, the liquid fraction collected post-treatment was analysed for solubilised lignin and hemicellulose, which may hold potential for resource recovery or biorefinery integration.

Probiotic immobilization on food industry waste

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Introduction: The production of cold-pressed oils is a process with a low resource utilization percentage, representing a fast-growing industry with a tendency for large waste accumulation. When producing 1kg of flaxseed oil, 2kg of cake remains, which is not recognized in the Serbian market, and (>90%) ends up as waste. A flaxseed cake is rich in fibers, proteins (~35%), and bioactive or potential bioactive components. Fibers and proteins make it a potential good carrier for probiotic immobilization.

Experimental: Flaxseed cake obtained from the cold-pressed oils industry was used for probiotic bacterium *Lactobacillus acidophilus* immobilization. The composition of the raw material was determined before immobilization. Flaxseed cake was ground in a ball mill and sieved through a sieve with a pore size of 0.6 mm. The flaxseed powder obtained in this way was sterilized in an autoclave at 120 °C, with a pressure of 1.5 bar for 30 minutes. After sterilization, flaxseed powder was diluted in distilled water at 10% (w/v) concentration, and a probiotic culture was inoculated. One sample was fermented and then freeze-dried, while the other was subjected to freeze-drying immediately after inoculation of the culture. Before freeze-drying, samples were stored at 4 °C for 1h and then frozen at -80 °C. After 24 hours of lyophilization, the probiotic culture's survival percentage was determined, as well as the antioxidant capacity of probiotic-flaxseed powder.

Results: The high fiber content of about 18% and protein of about 37% in flaxseed cake makes it a material with good potential for use as a probiotic carrier. The probiotic culture showed a high survival rate in both samples. The non-fermented sample showed a survival rate of *L. acidophilus* of 80 % while fermentation decreased probiotic viability during the lyophilization procedure by 13%. These results show that flaxseed cake powder is a good material for immobilizing probiotics, providing them with a high percentage of viability. Fermentation is often a good method for improving the properties of materials and products. In this research, fermentation of flaxseed significantly increased the antioxidant activity of probiotic powder. The reason for the reduced number of viable cells in the fermented compared to the non-fermented sample may be the adaptation of the culture to the

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fermentation temperature as well as the introduction of the culture into the accelerated growth phase when it is highly sensitive to external factors. This can be remedied by extending the time of storing the samples in the refrigerator before freezing and lyophilization.

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Valorization of wine industry wastewater for the production of *Bacillus*-based biocontrol agents against *Botrytis* spp.

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Introduction: Wine production, despite its reputation as an environmentally sustainable process, generates substantial quantities of waste, including winery wastewater rich in organic and inorganic compounds. The disposal of this effluent poses a significant environmental challenge. A promising approach to mitigating this issue aligns with circular economy principles through biotechnological valorization of winery waste. Cultivation of beneficial microorganisms on industry-derived effluents offers an alternative route for biocontrol agents production, which provides a more sustainable solution compared to conventional chemical pesticides that persist in the environment and accumulate in the food chain, posing a significant health risk for animals and humans due to acute/chronic exposure. Among microbial biocontrol agents, *Bacillus* species are particularly recognized for their dual role in plant growth promotion and phytopathogen suppression. Their biocontrol activity is mediated through multiple mechanisms, including the induction of systemic resistance in plants, biofilm formation, competition for nutrients and growth space, lytic enzymes production, and the secretion of antimicrobial metabolites such as lipopeptides (e.g., surfactin, iturin, and fengycin). This study investigates the potential of winery flotation wastewater as a substrate for the cultivation of *Bacillus* sp. 18B, an isolate derived from grape berries, and its application as a biocontrol agent against *Botrytis* spp., the causal agent of grey mold in grapevine.

Experimental: The strain's enzymatic activity was investigated in vitro using media containing selective substrates for enzymatic activity, along with its capabilities for production of the cyclic lipopeptide surfactin, known for its antimicrobial and surface-active properties, using the colorimetric assay. The cultivation bioprocess using the flotation wastewater as a substrate (diluted with tap water in the ratio 1:4) was performed at 28 °C under spontaneous aeration and agitation (150 rpm). The biocontrol efficacy of

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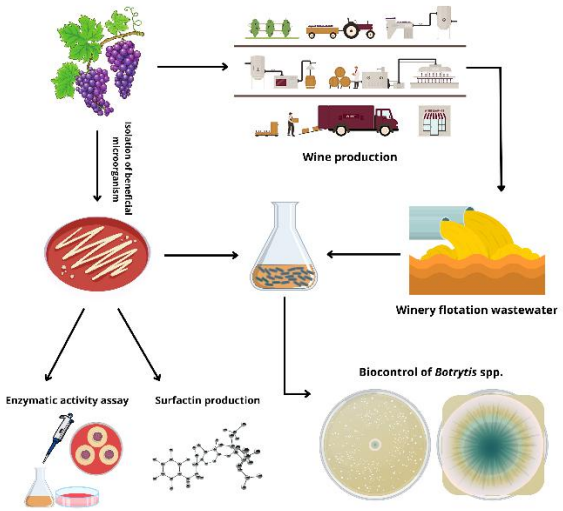
Bacillus sp. 18B cultivation broth samples was assessed through dual-culture assays against two *Botrytis* isolates from grapevine.



Results: Enzymatic activity was quantified using an enzymatic activity index, yielding values of 1.67 for cellulases, 1.00 for xylanases, 6.33 for pectinases, 3.96 for proteases, 5.67 for gelatinases, and 1.00 for lipases. Surfactin yield in cultivation broth, measured by colorimetrimetric assay, reached 64.89 mg/L. In biocontrol assays, Bacillus sp. 18B suppressed the growth of Botrytis sp. A107 by 82.35% and Botrytis sp. A89/1 by 90.59%, compared to control cultures grown on agarized winery flotation wastewater. Beyond substrate decomposition, enzymatic activity of investigated bacteria is crucial for its role in fungal cell wall degradation, serving as an additional biocontrol mechanism that enhances the suppression of fungal pathogens. The surfactin produced by this strain and its demonstrated antimicrobial activity against Botrytis isolates highlights the strain's potential as an effective biocontrol agent. This study establishes new directions for further research on the potential production of biocontrol agents using alternative waste streams, while also providing a foundation for investigating the underlying mechanisms of microbial antagonism, bioprocess optimization, and the formulation of circular bioproducts for agricultural applications.

Acknowledgement: This research was supported by the Science Fund of the Republic of Serbia, #14906, Eco-innovative circular bioprocess design for grey mold management in wine production – EcoInvent.

Thermoresponsive pNIPAAM Hydrogels: eco-friendly synthesis and antibacterial properties

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Introduction: Stimuli-responsive hydrogels have found applications in various fields due to their easily tunable properties. Among them, poly(N-isopropylacrylamide) (pNIPAAM) hydrogels are well-known for their biocompatibility and temperature-sensitive behavior. Above their lower critical solution temperature, which is close to human physiological temperature, pNIPAAM hydrogels undergo polymer network contraction, which lead to the release of encapsulated active substances. This property makes them highly suitable for controlled drug release applications. Previous studies have demonstrated that a system based on vitamin C and hydrogen peroxide can initiate polymerization of various monomers. In this study, a novel initiator system, utilizing peroxidase isolated from food waste material, hydrogen peroxide, and vitamin C, was employed for the polymerization of N-isopropylacrylamide. The use of peroxidase extracted from food waste aligns with circular economy principles by valorizing waste materials. Antibacterial properties of obtained pNIPAAM hydrogels with encapsulated gentamicin were further analysed.

Experimental: pNIPAAM hydrogels were synthesized using an initiation system composed of peroxidase isolated from food waste material, hydrogen peroxide, and vitamin C. The polymerization was conducted under ambient conditions, leading to successful hydrogel formation with reduced reaction time. The composition of synthesized pNIPAAM hydrogels were confirmed using Fourier-transform infrared spectroscopy (FTIR). The effects of peroxidase activity on hydrogel swelling in phosphate buffer pH 6.8 at different temperatures were investigated. Additionally, the antibacterial properties of these hydrogels were evaluated. Gentamicin was incorporated post-synthesis by immersing xerogels in an aqueous gentamicin solution. Two model bacteria were chosen for this analysis: *Escherichia coli K12* (*E. coli*; Gramm negative) and *Staphylococcus capitis* (*S. capitis*; Gramm positive). Fresh cultures of both bacteria were diluted to the same optical

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density (OD600=0.1) prior starting the experiment. Two mL of bacteria culture was added in falcons with samples. After 1 h, OD of all samples were measured and 5 μL of each suspension was seeded onto agar in 24 well plates. Measurement of OD and bacteria seeding was performed also after 8 h and 24 h of incubation with hydrogel samples. Control was bacterial culture starting at OD600=0.1 without any contact with tested samples during 24 h. After seeding, petri dishes were placed at 37 °C and they were imaged 24 h later using light microscope. The pNIPAAM hydrogel samples were UV sterilized prior the analysis.

Key findings: The composition of the synthesized pNIPAAM samples was confirmed by the presence of characteristic peaks of poly(N-isopropylacrylamide) in the FTIR spectra of all samples: the peak at 1524 cm^{-1} corresponding to bending vibrations of the -NH group, the peak at 1639 cm^{-1} corresponding to C=O stretching vibrations, and the peak at 3281 cm^{-1} corresponding to N-H vibrations. The equilibrium swelling ratio of the hydrogels decreased with increase in temperature, confirming the thermoresponsive nature of pNIPAAM. Higher peroxidase activity led to increased swelling ratios. The pNIPAAM hydrogels were effective against *E. coli* after incubation of 24h.

Acknowledgment: This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-136/2025-03/200287).

Synthesis and Characterization of Unsaturated Polyester Resin Using Aromatic and Aliphatic Bio-Based Monomers

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Introduction: Unsaturated polyester resins are among the most widely used polymer materials, valued for their versatile properties and broad applicability in industries such as automotive manufacturing, plastics, and biomedical engineering. However, the increasing environmental concerns associated with petroleum-based polymers have prompted research into sustainable alternatives. In this study, a bio-based unsaturated polyester resin was synthesized using renewable monomers to reduce dependency on fossil-derived materials while maintaining the desired performance characteristics.

Experimental: Key bio-based platform chemicals, identified by the U.S. Department of Energy as among the most promising for polymer applications, were selected for the resin formulation. These include itaconic acid, succinic acid, and 2,5-furandicarboxylic acid, which serve as bio-based alternatives to conventional petroleum-derived monomers such as maleic acid, fumaric acid, and terephthalic acid. Due to their structural similarity, these monomers offer a viable route for developing sustainable materials with properties comparable to their commercial counterparts. In addition to these acids, propylene glycol and dimethyl itaconate, both bio-based monomers, were used for unsaturated polyester synthesis, with dimethyl itaconate also serving as a reactive diluent. Fourier-transform infrared spectroscopy, differential scanning calorimetry, and uniaxial tensile testing were applied for the characterization of resins.

Results: The successful synthesis of the bio-based unsaturated polyester resin was confirmed via Fourier-transform infrared spectroscopy. Thermal properties were assessed using differential scanning calorimetry, providing

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insights into the resin's glass transition temperature. Mechanical properties were evaluated through uniaxial tensile testing, which determined key performance parameters such as tensile strength and elongation at break. This study demonstrates the feasibility of incorporating bio-based monomers into unsaturated polyester resin synthesis while maintaining structural integrity and functional performance. The findings contribute to the ongoing efforts to develop sustainable polymeric materials that align with circular economy principles and reduce reliance on petroleum-derived resources.

Acknowledgment: This research was supported by the Science Fund of the Republic of Serbia, GRANT No. 6793, Project title - Step2PolyGreen.

Fourier transform infrared spectroscopy as a tool for chemical analysis of nanoliposomes with aloe extract

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Introduction: Aloe or *Aloe vera* (L.) Burm. f. (Asphodelaceae family) has been known and used for centuries for its health, beauty, medicinal, and skin care potential. The plant contains 75 potentially active constituents: enzymes, sugars, lignin, saponins, anthraquinones, salicylic acid, amino acids, vitamins, and minerals. *A. vera* possesses healing, anti-inflammatory, antiseptic, laxative, antiviral, immunostimulant, moisturizing, anti-aging, and antitumor properties. With the aim to protect its bioactives from degradation and contribute to control release technology, as well as the development of better-quality formulations, aloe extract can be encapsulated in liposomes. Encapsulation is defined as a process that entraps substances, particularly thermosensitive or bioactive compounds in liquid extract, into a shell of wall material or matrix to produce particles with different sizes, ranging from nanometres to millimeter scale.

Experimental: Extraction from aloe leaves was done on the shaker at 80°C, using a solid-to-solvent ratio of 1:30 g/mL and 70% v/v ethanol for 30 min. The liposomes with encapsulated extract were developed in the proliposome procedure. A mixture of lipids (1 g) and *A. vera* extract (4 mL) was stirred at 50°C. After cooling to 25°C, 20 mL of the aqueous phase was transferred and stirred for 2 h. To investigate the influence of UV irradiation on the extract-loaded liposome, a liposomal population (5 mL) was UV-irradiated by UV-C irradiation at a wavelength of 253.7 nm in a quartz tube at 25°C for 30 min. Then, the non-treated and UV-irradiated liposomes were freeze-dried at -75°C (in a vacuum) for 24 h. Fourier transform infrared (FT-IR) spectra were recorded in a range of 400 to 4000 cm^{-1}.

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Results: FT-IR spectroscopy is used to get insights into the occurrence of interaction between extract compounds and phospholipids, as well as changes in the obtained liposomes caused by UV irradiation. One of the most dominant peaks of the FT-IR spectra of aloe leaf extracts-loaded liposomes is at 2922 cm^{-1} (asymmetric stretching vibration in the CH_3 groups). The dominant mode at 1735 cm^{-1} represents the stretching vibrations of the C=O functional group. The bands at 1240 and 1171 cm^{-1} correspond to the symmetric and asymmetric stretching of the PO_2^- functional groups, respectively, whereas the peak at 1055 cm^{-1} is related to the C-O-P-O-C stretching. The bands at 968 and 923 cm^{-1} are associated with the symmetric and asymmetric stretching of the C–N bonds, respectively. At the same time, the mode at 721 cm^{-1} corresponds to the C-N symmetric stretching of choline.

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Development of Active-Intelligent Biodegradable Films Based on Bacterial Nanocellulose and Bilberry Pomace Anthocyanins

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Introduction: As the demand for sustainable packaging solutions continues to rise, biodegradable biopolymer films have emerged as promising alternatives to plastic materials. In this regard, bacterial nanocellulose (BNC), a highly pure form of cellulose produced by certain bacterial strains, is gaining significant attention. Thanks to its highly crystalline nanofiber structure, BNC is characterized by excellent mechanical properties, while its GRAS (Generally Recognized As Safe) status makes it suitable for food applications. Different bioactive compounds, such as those extracted from plants, can be incorporated to improve the functional properties of biopolymer films. Anthocyanins (ACN), a highly diverse subclass of polyphenols and natural pigments, show great potential for active-intelligent packaging due to their antioxidant and pH-induced color-changing properties.

Experimental: This study focuses on developing BNC films enriched with anthocyanin extract that could serve as active and/or intelligent food packaging. Bilberry pomace, a juice industry byproduct, was used as a source of anthocyanins. Acidified ethanol was used to extract ACN, while BNC films were produced through the activity of acetic acid bacteria after 4 days of growth in a suitable medium. The ACN were incorporated by submerging BNC in the extracts prepared in four different concentrations (samples E2, E5, E10, and E15), followed by drying. The functional properties of the films were evaluated in terms of DPPH radical scavenging activity, thickness, UV-Vis barrier properties, and pH responsiveness (pH 3–10).

Results: The addition of ACN extract significantly enhanced the properties of BNC films. All samples demonstrated a higher ($p < 0.05$) DPPH radical scavenging activity and improved light barrier properties compared to pure BNC, with the extract content being the main contributing factor. The pH-

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induced color changes occurred in all formulations and were the most notable in samples E5 and E10. These findings suggest great promise of BNC-ACN films for food packaging applications. The color changes could be applied to visually detect food spoilage, often manifested by changes in pH, while the antioxidant and light barrier properties may help extend the oxidative stability of the products. Furthermore, utilizing food industry byproducts is meant to benefit the environment and reduce film production costs. Future research should focus on the films' mechanical properties and food application studies.

Acknowledgments: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-137/2025-03/200116).

Sustainable Valorization of Conifer Cones: A Potent Source of Antioxidants and Bioactive Compounds

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Introduction: Antioxidants are critical in mitigating oxidative stress-related diseases and play a fundamental role in industrial applications by inhibiting oxidation, rancidity, and degradation, thereby enhancing product stability and quality. The identification of waste biomass with potent antioxidant properties and a high concentration of biologically active compounds is essential for the sustainable utilization of natural resources. Such biomass-derived antioxidants offer a promising, environmentally sustainable alternative to their synthetic counterparts, contributing to both human health and industrial sustainability.

Experimental: In this study, the optimization of microwave-assisted extraction (MAE) parameters for the recovery of bioactive compounds from mature *Pinus nigra* and *Thuja orientalis* cones, a lignocellulosic waste produced in large quantities, with insufficiently explored biological potential, was conducted. The extraction process was carried out under constant microwave power, while ethanol concentration (as the extraction solvent), solid-to-liquid ratio, and extraction duration were systematically varied. The resulting extracts were evaluated for their antioxidant activity using the TPTZ (ferric reducing antioxidant power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays. Furthermore, the extracts were characterized for their total phenolic content (TPC), as well as total flavonoids, hydrolyzable tannins, and terpenoids, along with their dry matter (DM) content.

Results: The results demonstrated that antioxidant activity and bioactive compound content increased with the intensification of extraction parameters up to a critical threshold, beyond which a decline was observed. The optimal extraction conditions included a microwave power of 90 W, an ethanol concentration of 40%, an extraction time of 60 seconds, and a biomass-to-

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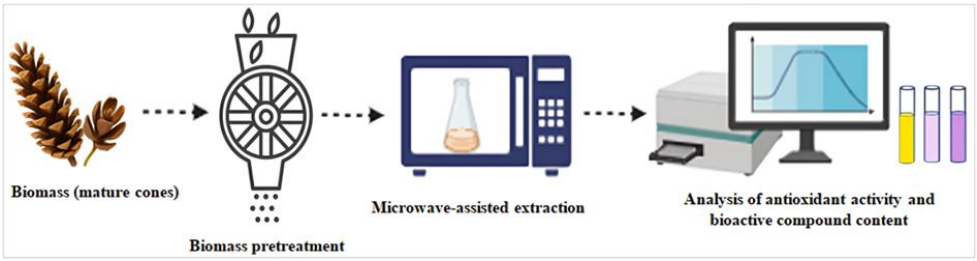
solvent ratio of 1:40 (*w/v*). Mature *P. nigra* cones extract exhibited the highest antioxidant activity, as determined by the FRAP assay (3.1629 mmol Fe^{2+}/g DM), which was further confirmed by the DPPH method. Additionally, mature *P. nigra* cones extract contained higher total phenolic content (328.1226 mg GAE/g DM) and total flavonoid content (62.0985 mg QE/g DM), while mature *T. orientalis* cones extract exhibited a higher content of hydrolyzable tannins (1.6321 mg GAE/g DM) and total terpenoids (8.0391 mg LIN/g DM). This study demonstrates the innovative potential of mature pine cones by revealing their relatively high content of key bioactive compounds and strong antioxidant activity. The research follows sustainable principles by optimizing mild extraction conditions, avoiding toxic solvents, and minimizing energy consumption. Moreover, the proven presence of bioactive compounds encourages further investigation into additional biological activities, such as anti-inflammatory and anti-diabetic effects, expanding the potential utilization of this natural resource.

Acknowledgment: This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-136/2025-03/200135).



Influence of Zirconia Surface Characterization on Its Wettability and Cell Proliferation

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Introduction: Zirconium dioxide (ZrO_2) ceramic has become an increasingly important biomaterial in dental applications due to its high mechanical strength, biocompatibility, and abrasion resistance. However, there is a lack of knowledge on how different surface topographies of ZrO_2 ceramics influence the biological response of adhered cells. Therefore, this study aimed to compare the physical properties and proliferation of fibroblasts that adhered to the surfaces of polished and glazed ZrO_2 ceramics.

Experimental: The materials used in the present study were ZrO_2 discs, Sagemaxnexx Zr T Multi, *Sagemax Bioceramics, Federal Way, WA*. Presintered discs were cut by dry milling method. Milling was performed using a dental milling machine VHF-K5. After sintering, the samples underwent a surface treatment according to which they were devided into subgroups: 1) Polished specimens - underwent dry polishing performed with SagemaxNexxZrShine Kit-diamond rubber polishers (rubber polishers for pre-polishing and high-gloss polish with diamond paste) and 2) Glazed specimens- underwent a transparent aluminosilicate glass deposition on a surface.

Wettability was measured using a standardized volume of liquid (2 μL distilled water droplets) applied from 4 mm at a 90° angle, at 22.5 ± 0.2 °C. Contact angles were recorded with a Canon 77D camera (EF 100 mm lens) and analyzed using ImageJ software.

Fibroblasts isolated from the gingiva were cultured in 25 cm² plastic plates, using cell culture medium (DMEM/F12 with the addition of 10% bovine serum and 1% antibiotic/antimycotic *Gibco, ThermoFisher*). Incubation was carried out at a temperature of 37 °C in an atmosphere of 5% CO_2 and 100%

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air humidity. The fibroblast culture was cultivated until reaching 80-90% confluency, after which the cells were passaged three times. After confirmation of viability and cell counting, an analysis of the cytotoxicity of the material was performed through direct contact of cells with ceramic samples. Cytotoxicity was measured by an indirect method using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test. The ZrO_2 specimens were placed onto 24-well plates; 20,000 cells/well were seeded onto discs and incubated in freshly prepared growth medium at 37 °C in a humidified 5% CO_2 atmosphere for up to 7 days. The medium was changed every 3rd day. Mitochondrial activity was assessed after 3 days of treatment.

Results: The water-wetting angle measurements of polished and glazed zirconia samples showed higher values for polished (58°) than glazed (33°). After 3 days of exposure of the cells to the materials, higher mitochondrial activity was recorded in direct contact with the polished surface compared to the glazed zirconium ceramic. However, this difference was not statistically significant ($p>0.05$). These results indicate a slight advantage of the polished surface in supporting the mitochondrial activity of fibroblasts. Although the polished surface may offer a slight advantage in promoting fibroblast viability, further research is needed to understand this interaction better and confirm the results.

Viscoelastic properties of whole grain buckwheat flour dough substituted with wheat flour hydrolysates

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Introduction: This study aimed to assess the effect of substituting whole grain buckwheat flour (WGBF) with wheat flour hydrolysate (WFH) with reduced content of allergenic epitopes (< 20 ppm). The amount of added hydrolysate was varied, with the final substitutions being 1% and 5%. Whole grain buckwheat flour is considered gluten-free and beneficial for human diet due to high protein and fiber content. It is rich in minerals and nutrients which are considered bioactive. The lack of gluten proteins in WGBF hinders the formation of a stable protein network, resulting in decreased viscoelastic properties of dough. Therefore, the addition of WFH and the effects on the dough properties were investigated.

Experimental: Thermo-mechanic properties were evaluated using Mixolab® by applying a modified *Chopin+* protocol. In order to obtain dough of good consistency, the mixing speed was set to 60 rpm and the weight of the formed dough was increased from 75 to 90 g. The protocol consisted of 5 different phases (mixing, protein weakening, starch gelatinization, amylase activity and starch retrogradation), where each significant value points were measured (C1–5).

Results: In order to achieve a standard dough consistency (1.1 ± 0.05 Nm), degree of hydration varied among the control (WGBF) and WGBF substituted with 1% and 5% of WFH, where the control required greater amount of added water. The lowest mechanical weakening of proteins (9.57%) and the highest dough stability were recorded for the WGBF substituted with 5% of WFH. During the second phase, the temperature in the mixing block increased and the proteins underwent structural changes. During this phase, C2 value of WGBF substituted with 5% of WFH showed the least decrease in value. Throughout the gelatinization, the measured values for C3 showed no significant differences among the tested samples.

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Constant high temperature and mixing in the fourth phase deteriorated the starch granules and therefore impacted the starchy gel stability. The least reduction in C4 value was recorded for the WGBF substituted with 5% of WFH (0.803 Nm). In the cooling phase, the C5 values of the tested doughs increased due to starch retrogradation, where the highest value was recorded for WGBF substituted with 5% of WFH (0.996 Nm). In conclusion, the addition of WFH affected the overall rheological properties of WGBF, amylase activity and starch retrogradation in the last phase of the experiment.

Acknowledgment: This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-136/2025-03/200135 and Contract No. 451-03-136/2025-03/200287).

Evaluation of Fermentation Effect on Antioxidants Amplification in Dandelion Root Extracts

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Introduction: Fermentation is a process of partial decomposition of the fermented substrate due to the activity of various microorganisms involved. It is one of the most useful biocatalytic processes that may beneficially improve the bioactivity of plant extracts. Accordingly, this study investigates the fermentation effects of underutilized dandelion root dust (*Taraxacum officinale* L.) on phenolic content and antioxidant activity potential in the produced extracts. The dandelion root was collected as dust remaining after the processing for the industrial tea blends preparation, thus is classified as a second-rank raw material, possessing low or no commercial value. Regarding the fermentation effects, the focus was on the contributions of various microorganisms, including the yeasts *Saccharomyces cerevisiae* and *Saccharomyces cerevisiae var. boulardii*, a yeast-like fungus *Aerobasidium pullulans,* and lactic acid bacteria (LAB) *Lactobacillus rhamnosus* ATCC® 7469™.

Experimental: Fermentation is a process of partial decomposition of the fermented substrate due to the activity of various microorganisms involved. It is one of the most useful biocatalytic processes that may beneficially improve the bioactivity of plant extracts. Accordingly, this study investigates the fermentation effects of underutilized dandelion root dust (*Taraxacum officinale* L.) on phenolic content and antioxidant activity potential in the produced extracts. The dandelion root was collected as dust remaining after the processing for the industrial tea blends preparation, thus is classified as a second-rank raw material, possessing low or no commercial value. Regarding the fermentation effects, the focus was on the contributions of various microorganisms, including the yeasts *Saccharomyces*

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cerevisiae and *Saccharomyces cerevisiae var. boulardii*, a yeast-like fungus *Aerobasidium pullulans*, and lactic acid bacteria (LAB) *Lactobacillus rhamnosus* ATCC® 7469™.

Results: During the fermentation of dandelion root, a single yeast culture demonstrated the highest efficiency in enhancing the levels of antioxidants in the resulting extracts, significantly outperforming the control sample. Specifically, the probiotic yeast *S. boulardii* induced a substantial increase in TPC, with a 2.5-fold rise to 68.4 mg GAE/g dm. Additionally, TFC levels increased 2.8 times, reaching 44.9 mg QE/g dm, while the FRAP showed a 3.9-fold increase, totalling 417.2 µmol Fe^{2+}/g dm, when compared to the control. Following *S. boulardii*, *S. cerevisiae* emerged as the next most effective yeast for dandelion root fermentation. A combination of *L. rhamnosus* and *S. cerevisiae* also showed good performance, although the fermentation with *L. rhamnosus* alone led to only a negligible increase in the content of bioactive compounds and antioxidant activity. The results of this study are valuable in promoting the value-added fermentation effect in the utilization of dandelion root extracts as supplements in food and pharmaceuticals that offer enhanced health benefits. The approach taps into readily available industrial residues to be creatively exploited in useful and profitable ways.

Acknowledgment: This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-136/2025-03/200135, 451-03-136/2025-03/200287, 451-03-136/2025-03/200011 and 451-03-136/2025-03/200053).

Biodegradable protein-based films incorporated with quercetin and quercetin/β-cyclodextrin complex

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Introduction: Following the current trends and necessities for biodegradable products, protein-based films were developed to serve as carriers for cosmetic active ingredients, such as quercetin, plant-based compound with potent antioxidant and antimicrobial activity.

Experimental: Novel biodegradable films based on complex coacervate of gelatin type A and sodium caseinate were obtained by casting method and successfully incorporated with quercetin and quercetin/*β*-cyclodextrin complex (1, 3 and 5%, in relation to total protein content). Molecular complex of quercetin and *β*-cyclodextrin was prepared by kneading method and characterized with ATR FTIR. Optical and barrier properties of films were determined, as well as release of quercetin from films in 60% ethanol solution.

Key findings: Thickness of films increased with increase in quercetin and complex content, as it would be expected for films with higher solid content. Opacity of films with complex was significantly higher than those with pure quercetin, and it increased with concentration, probably because of vibrant yellow hue of quercetin. Moisture content of films with 3 and 5% both quercetin and complex decreased, while for 1% films, slight increase was observed. Solubility in water and water vapor permeability of films increased with complex content, while it decreased with quercetin content due to its hydrophobicity. Amount of quercetin extracted from films was significantly higher for films with complex, because complexation with *β*-cyclodextrin increases quercetin solubility.

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National Lactic Acid Database development: current status and future direction

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Introduction: Lactic acid bacteria (LAB) are gram-positive bacteria that primarily utilise carbohydrates as a carbon source and exhibit high tolerance to low pH. They are commonly found in raw milk and dairy products. Although LAB encompasses over 60 genera, the most prevalent in food fermentation include *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Pediococcus*, *Leuconostoc*, and *Weissella*. The key metabolic characteristics of LAB consist of acid and aroma production, protein hydrolysis, exopolysaccharide synthesis, and antimicrobial compound production. The indigenous microbiota of raw milk directly influences the sensory properties of its products. LAB are classified as Generally Recognized as Safe (GRAS) and are widely employed in fermented foods. The WHO recommends including fermented dairy, meat, and vegetable products in the daily diet because of LAB's beneficial impact on human health.

Experimental: Our decades of research demonstrate that each LAB strain possesses unique characteristics, offering specific health benefits for both humans and animals. Given the limited biodiversity of commercial starter cultures used in the fermented product industries, which have reached their technological and probiotic potential, our distinct collection of natural LAB isolates from Serbia (and the surrounding region) provides essential resources for addressing future challenges in human and animal health, agriculture, and the food industry. The IMGGE's LAB collection comprises approximately 5,000 different isolates collected from artisanal dairy, meat, and other fermented products across Serbia, Montenegro, Bosnia and Herzegovina, Croatia, Bulgaria, Iran, Azerbaijan, and the Russian Federation. Our LAB strains are utilised in scientific research (doctoral and master's theses) and industry (spinout companies, licensing agreements), fostering both scientific excellence and innovation.

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Key findings: Our strategic project aims to establish the LABbank database, which will catalog the technological and probiotic characteristics of each LAB strain in our collection. The project's first phase involved developing the LABbank database for the first 1,000 strains, implementing administration features, and adding filter and search options based on strain characteristics and taxonomy. Among these 1000 strains, the most dominant genera are *Lactobacillus* sp. (52.85%), *Enterococcus* sp. (26.53%), *Lactococcus* sp. (8.5%), *Streptococcus* sp. (6.22%) and *Leuconostoc* sp. (5.49%). For now, eighteen strains are deposited in the BCCM culture collection, and three are under international patent protection (PCT). Our database will support researchers in their studies and provide easier access for companies in the fermented food and pharmaceutical industries to identify our LAB strains suitable for industrial applications.

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Green extraction of blackthorn (*Prunus spinosa* L.) polyphenols with natural deep eutectic solvents

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Introduction: In recent years, the scientific community's interest has been directed towards developing and applying green solvents that can be used to obtain high-quality and safe extracts. These solvents not only aim to reduce the environmental footprint of extraction processes but also ensure the recovery of high-quality, bioactive compounds with enhanced safety for human use. NADES are generally composed of binary mixtures, but they can also be ternary, quaternary, or even multi-component mixtures to make desirable physicochemical properties of the solvent [1]. Blackthorn, a wild perennial plant, produces fruit rich in bioactive compounds, particularly polyphenols. Among the phenolic compounds present in blackthorn anthocyanins, phenolic acids, and flavones are considered to be the most abundant and representative groups [2].

Experimental: The main objective of the research is to determine and compare the effects of various natural deep eutectic solvents on extracts obtained from the mesocarp of blackthorn and to identify the most efficient solvents for extracting the highest content of total polyphenols with antioxidant activity. The experiment was conducted with 10 different NADESs.

Results: Based on the obtained results, it can be concluded that the NADES solvent N4 (choline chloride–citric acid–urea in a molar ratio of 2:1:1) exhibited the highest extraction efficiency, achieving the highest total polyphenol content, which amounted to 28.61 mg GAE/g DW. On the other hand, the NADES solvent N12 (L-proline–lactic acid in a molar ratio of 1:2) demonstrated the highest antioxidant activity, as determined by the DPPH test, reaching a value of 38.56 mg TE/g DW. Based on the obtained results, future research will focus on the optimization of key extraction parameters, including extraction time, temperature, and the molar ratio between the raw

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material and the solvent, as well as the interactions among these factors, in order to establish the most favorable extraction conditions. The aim of this optimization is to maximize the yield of bioactive compounds, which possess significant potential for application in the pharmaceutical, food, and cosmetic industries, owing to their proven antioxidant properties and health-promoting effects.

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Optimisation of Green Extraction of Antioxidant Compounds From Blackthorn Pomace Using Natural Deep Eutectic Solvents

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Introduction: Blackthorn (*Prunus spinosa* L.) is an indigenous fruit species widespread across Europe, known for its richness in polyphenols and anthocyanins, which contribute to its antioxidant properties [1]. Optimization of extraction conditions using natural deep eutectic solvents (NADES) has been shown to significantly increase polyphenol content and antioxidant activity of blackthorn extracts [2]. Aiming to maximize the use of blackthorn fruit and minimize industrial waste through green extraction techniques, ultrasound-assisted extraction (UAE) using NADES was optimized.

Experimental: In the first optimization step, UAE of lyophilized blackthorn pomace was performed using 10 different NADES at 40 °C for 30 minutes, with a solid-to-liquid ratio of 1:10 (w/v) and 20% water content in NADES. Total phenolic content (TPC) and antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay were determined for the resulting extracts. Based on the results, the second step included variations in temperature (40 °C and 50 °C), extraction time (30 and 60 min), solid-to-liquid ratios (1:10 and 1:20 w/v), and water content in NADES (20% and 15%), using two solvents: N12 (Proline–Lactic Acid in a molar ratio 1:2) and N14 (Choline Chloride–Glycerol in a molar ratio 1:1). Sixteen extract combinations were analyzed for TPC and DPPH.

Results and conclusions: N12 was clearly the superior solvent. The highest TPC values were found in R12 (29.8134 ± 0.6722 mg GAE/g DW) and R1 (29.6757 ± 0.6050 mg GAE/g DW), both using N12, at 50 °C for 60 minutes. The difference was in the solid-to-liquid ratio (R1: 1:20; R12: 1:10 w/v) and water content (R1: 15%; R12: 20%). The best antioxidant activity was observed in R8 (48.3193 ± 2.5679 mg TE/g DW), R6 (45.2149 ± 1.3738 mg

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TE/g DW), and R10 (44.2394 ± 2.0909 mg TE/g DW), all of which shared the solid-to-liquid ratio of 1:20 (w/v), though extraction time, temperature, and water content varied. Based on these findings, the third optimization step focused on N12 with 20% water content, and the following extraction parameters were selected: solid-to-liquid ratios of 1:15, 1:20, and 1:25 (w/v); temperatures of 45, 55, and 65 °C; and extraction times of 30, 60, and 90 minutes.

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Isolation, Identification and Potential Applications of Pigment-Producing Purple Non-Sulfur Bacterium

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Introduction: Purple non-sulfur bacteria (PNSB) are a diverse group of phototrophic microorganisms capable of thriving under anaerobic-light conditions. These bacteria produce a variety of pigments, including bacteriochlorophylls and carotenoids, making them valuable in multiple biotechnological applications. Recent interest has focused on their use in wastewater treatment and natural pigment production, with added potential in recycling organic waste while generating biologically active compounds. This study investigates the isolation and characterization of a pigment-producing PNSB strain from environmental samples, with a focus on its pigment production and response to different light conditions. Particular attention is given to the potential for bioprocessing organic waste while obtaining high-value compounds like spheroidene and spheroidenone.

Experimental: Environmental samples were collected over three weeks and cultured using a selective medium tailored to support the growth of purple phototrophic bacteria. Initial isolation was performed using a Winogradsky column, which provided a gradient-based enrichment system. Isolated strains were cultivated in anaerobic-light conditions and their growth was monitored using spectrophotometry (OD 660 nm). A custom-designed photobioreactor equipped with green, orange, and infrared light sources was employed to evaluate growth and pigment production. The new isolate was incubated for a period of 150 hours and monitored spectrophotometrically within the 330-900 nm wavelength range. As an extraction solvent, a mixture of acetone and methanol in 7:3 ratio was used. Following the extraction, a complete decolorization of the bacterial cells was observed. Tentative species identification was based on pigment-specific absorption peaks and morphological observations.

Results: Spectral analysis of the bacterial cell suspension, as well as the microscopical slide, supported the identification of the strain as belonging to the *Rhodobacter* spp. The spectra of the pigment extracts indicated the

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presence of bacteriochlorophylls absorbing in the region around 770 nm, while carotenoids were detected in the range of approximately 400–500 nm. Peak signals around 486 nm and 454 nm suggested the presence of spheroidene or its derivative, spheroidenone. It was noted that the growth of the isolate was relatively fast concerning the main carbon source was acetate. The optical density as a marker for the cell growth over time of 150 hours achieved its highest peak at around the 70th hour.

These findings indicate the strain's potential for integrated applications such as organic waste conversion and production of natural pigments with antioxidant properties, that can be implemented in the food and the pharmaceutical industry.

From plastic waste to nutraceutical-conversion of PET containing hydrolysates to bacterial nanocellulose with pomegranate extract

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Introduction: Bio-upcycling is an innovative end-of-life strategy for polymer waste management that use the activity of microorganisms to convert monomers and products of pre-treated polymer waste into high-value materials. Poly(ethylene terephthalate) (PET), a leading synthetic polyester made from petroleum-based feedstocks, currently lacks a fully sustainable alternative that can meet global demand. Recently, biotechnological approaches and enzymatic recycling of PET containing plastic waste have offered the sustainable route in PET waste management. Enhancing recycling and upcycling rates remains the most effective strategy for achieving plastic circularity.

Experimental: This study explores the conversion of mixed plastic waste containing PET hydrolysates—obtained through thermal pretreatment—into bacterial nanocellulose (BNC), a promising and sustainably produced biopolymer. Following optimization of BNC production under various static culture conditions using PET hydrolysates as carbon source for *Komagataeibacter medellinensis* ID13488, the most effective conditions, in terms of yield, were identified and subsequently scaled up for larger production. Elemental analysis and HPLC analysis were used for the identification of elements and monomers and dimers, respectively. To expand the applicative potential of BNC derived from PET-containing plastic waste, BNC was further used for the adsorption of pomegranate peel extract (PPE), creating a novel bioactive formulation suitable as functional food.

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Key findings: Structural and compositional analyses (FTIR, HPLC) confirmed the successful incorporation of PPE, while FESEM analysis gave an insight into its morphology. *In vitro* release studies of ellagic acid and punicalagin were performed, as well as, pH-dependent release from BNC. Antioxidant potential was assessed using DPPH and FRAP assays, and α-glucosidase inhibition was tested to evaluate the potential in the regulation of blood sugar levels. Overall, this study emphasizes a circular approach in transforming PET containing plastic waste into bacterial nanocellulose (BNC) and further opening its potential as a nutraceutical.

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Antioxidant potential of medically unknown plants from Asteraceae family

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Introduction: There are a large number of less researched medical plants within Asteraceae family. According to that fact, a lot of space for finding the plants with significant biological activity such as antioxidant potential exists. The major groups of secondary metabolites in plants from Asteraceae family are: phenolic acids (chlorogenic, caffeic, ferulic), flavonoids (kaempferol, luteolin, quercetin, apigenin), sesquiterpene lactones, alkaloids, tannins, saponins, essential oils, carotenoids and from there originates the strong physiological activity (anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, diuretic). Their use for traditional purposes to relax smooth muscles, to treat wounds, headache, pain, and hemorrhoids have a long history

Experimental: This research was focused on determination of antioxidant activity from less researched plans which belongs to Asteraceae family. The antioxidant potential was expressed through 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-(-3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and reducing capacity of Fe^{3+} ions (FRAP) assays. Thirteen samples from 9 plants were subjected to investigation. Herb of the plants was used as a raw material however, for four plants root was also investigated. Eight different plant genera were covered, and within *Inula* genus two plant varieties were researched. The extracts were obtained by applying traditional solid/liquid extraction where the following extraction conditions were set: solid/liquid ratio was 1:20 m/v, 60% ethanol was used as solvent, at room temperature and extraction process lasted 24 h with constant shaking (180 rpm).

Results: Antioxidant potential for DPPH assay was ranged from 14.7518 to 475.6997 μM/g. Asteraceae plants such as *Solidago virgaurea* (475.6997±12.44 μM/g), *Tussilago farfara* (399.7905±11.64 μM/g), *Cota tinctoria* (205.3327±7.37 μM/g), *Tanacetum vulgare* (197.8063±5.10 μM/g),

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and *Inula ensifolia* (197.5298±3.33 µM/g) expressed the highest ability to neutralize DPPH radicals. In the case of ABTS assay, the Asteraceae plants antioxidant ability was between 33.0179 and 1013.7647 µM/g. *Inula ensifolia* (1013.7647±22.34 µM/g), *Tanacetum vulgare* (975.7102±45.89 µM/g), *Tussilago farfara* (964.7644±31.03 µM/g), *Solidago virgaurea* (945.7371±27.95 µM/g), and *Cota tinctoria* (544.2208±38.47 µM/g) stood out as plants with highest ability to neutralize ABTS radicals. The ability to reduce Fe^{3+} ions was in range from 19.8167 to 774.4365 by Asteraceae plants. Five plants showed strong capacity to reduce Fe^{3+} ions: *Tanacetum vulgare* (774.4365±33.19 µM Fe^{2+}/g), *Solidago virgaurea* (744.3895±14.04 µM Fe^{2+}/g), *Tussilago farfara* (722.8466±27.99 µM Fe^{2+}/g), *Inula ensifolia* (724.5140±8.75 µM Fe^{2+}/g), and *Cota tinctoria* (424.6846±21.13 µM Fe^{2+}/g). In general, for all three assays the lowest antioxidant capacity was observed in roots from *Helianthus tuberosus, Carlina acanthufolia utzuka, Cichorium inthybus*, and *Inula helenium.* However, the herb of those four plants showed moderate antioxidant activity. Further investigation will be focused on green extraction technique implementation and chemical profile and correlation between bioactive compounds and antioxidant activity determination. Moreover, the application of this type of extracts in food, cosmetic and medicinal products should lead to the creation of functional products.

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Alternative source of high antioxidant potency bioactives from different parts of blue poppy (*Papaver somniferum*)

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Introduction: Poppy (*Papaver somniferum*) represents one of the most ancient plant, which belongs to the Papaveraceae family, and is mostly cultivated in the Mediterranean region. Due to its nutritional and medicinal properties, its widely used in medicine, cosmetics and food industries. It is also extensively used in alternative medicine due to its rich bioactive content. The most common secondary poppy metabolites are alkaloids. Besides alkaloids, polyphenols, flavonoids, tocopherols, vitamins and minerals are also present in different parts of the poppy. Poppy has wide application in medicine due to the presence of alkaloids, which have been confirmed to have analgesic, antidiarrheal, narcotic and antitussive properties. It is also well known for its application in the food and flavor industry. The aim of this research was to examine the antioxidant potential of bioactive compounds in different parts of poppy (leaves, stems and roots).

Experimental: For isolation of poppy extracts, conventional solid/liquid extraction (SLE) was performed using different concentration of ethanol (0, 20, 40, 60, 80 and 96% w/v). The extraction was performed at room temperature for 24 h, with a shaking speed of 150 rpm, with molar ratio 1:10. The obtained extracts were analyzed for antioxidant activity. The antioxidant potential of poppy extracts was assessed using *in vitro* tests: 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic) cation (ABTS) and ferric reducing antioxidant power (FRAP).

Results: The ethanol concentration had an important effect on the potential of antioxidant activity. According to DPPH assay, 60% ethanol for all three parts of the poppy extract showed the greatest power to remove free radicals. The best antioxidant activity was shown by the stem extract and it was 8.06 µM TE/g DW. ABTS showed different results compared to DPPH. In the case of stem and root extracts, 40% ethanol stood out as the best and the ABTS results were 22.30 µM TE/g DW and 20.27 µM TE/g DW, respectively, while in the case of leaves, the extract obtained with water as a solvent showed the strongest antioxidant activity (43.67 µM TE/g DW). The

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FRAP test had the same trend as ABTS, so 40% ethanol in the case of stems and roots proved to be the best solvent for the extraction of bioactive components with the highest ferric reducing antioxidant power.

It can be concluded that different parts of the poppy could be used as an alternative source of antioxidant-reach extracts, and could also have a wide range of applications in industries such as food, cosmetics, and pharmaceuticals.

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Impact of carrier composition and probiotic metabolic activity on mechanical properties of extruded spheres

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Introduction: Cold-pressed oil production is a rapidly expanding industry, though it currently operates with low resource efficiency and generates significant amounts of waste. Flaxseed cake, a byproduct of this process, is notably rich in fiber, protein (~35%), and various bioactive or potentially bioactive compounds. Due to its high fiber and protein content, flaxseed cake holds promise as an effective carrier for probiotic immobilization - either on its own or in combination with other materials such as alginate and pectin. The strength of the support plays an important role in the production process.

Experimental: Flaxseed cake, sourced from the cold-pressed oil industry, was utilized as a substrate for the encapsulation of probiotics. The material was then ground using a ball mill and passed through a 0.6 mm mesh sieve. The resulting flaxseed powder was sterilized in an autoclave at 120 °C under 1.5 bar pressure for 30 minutes. Alginate and pectin from citrus were diluted in water and pasteurized at 60 °C for 30 min. Four carriers were obtained: AP- 1% alginate and 0.67% of pectin, AL - 1% of alginate and 2.5% of flaxseed, APL- 1% alginate, 0.67% of pectin, and 2.5% of flaxseed, and AL2- 0.6% alginate and 2.5% flaxseed. The encapsulation efficiency of the extrusion technique was analyzed by comparing probiotic viability before and after encapsulation. Mechanical properties of carriers were analyzed before and after 4.5 h of coconut milk fermentation. The tests were performed using a Universal Testing Machine, AG-Xplus (Shimadzu, Japan), equipped with a 100 N force load cell.

Results: Encapsulation efficiency was about 100% for all carriers. The maximal force used for AP beads was 0.62209 ± 0.05052 N before and 0.22827 ± 0.00950 N after fermentation. The maximal force for AL carrier was 0.34506 ± 0.05036 N before and 0.22389 ± 0.01694 N after fermentation. The maximal force of APL beads was 0.18833 ± 0.01582 N before and 0.23816 ± 0.04075 N after fermentation. The maximal force for

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AL2 was 0.27904 ± 0.04667 N before and 0.46992 ± 0.10059 N after fermentation. As the results show, alginate and pectin produced the strongest beads, which were also the most affected by fermentation. During fermentation, the mechanical strength of these beads decreased significantly. Adding flaxseed powder to the carrier notably reduced the beads' mechanical strength initially, but the beads became stronger as fermentation progressed. Particles lacking pectin showed the weakest strength after fermentation. Overall, the best performance was observed in particles containing alginate, pectin, and flaxseed powder.

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Impact of the composition of alginate-yeast hydrogel beads on dye biosorption

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Introduction: Wastewater from the dyeing industry poses a significant environmental challenge. Among the various treatment methods, biosorption is considered both cost-effective and environmentally friendly. In this study, spent brewery yeast is proposed as a promising biosorbent. Yeast cells possess a cell wall structure composed of polysaccharides, proteins, and lipids, which contain various functional groups such as hydroxyl, carboxyl, phosphate, and amino groups. These groups actively participate in the binding of contaminants, including heavy metals and synthetic dyes, through mechanisms like ion exchange, complexation, and physical adsorption. Alginate, a naturally occurring polysaccharide derived primarily from brown seaweed, is widely recognized for its biocompatibility, non-toxicity, and gel-forming ability. These properties make it an excellent candidate for biosorption applications. Its porous structure and functional groups allow for the effective binding dyes. Moreover, alginate beads are easy to produce via simple techniques like extrusion, making the material both cost-effective and environmentally sustainable for large-scale applications.

Experimental: Four types of biosorbents were prepared using the extrusion technique: beads containing 6.0 % spent yeast and varying concentrations of alginate and chitosan (1.0% alginate, 1.3% alginate and 0.03% chitosan, 1.6% alginate and 1.6% alginate coated with 0.4% chitosan). Experiments were carried out in four Erlenmeyer flasks, each containing a dye solution (25 mg/L) and 40 g/L of the respective adsorbent. Dye concentrations were measured using a UV/Vis spectrophotometer (Ultrospec 3300 pro, Amerischam Bioscienc) at a wavelength of 624 nm. Interactions between the dye and biosorbents were analyzed via Fourier-transform infrared spectroscopy (FTIR) using a Nicolet iS10 spectrometer (Thermo Scientific, Sweden)

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Results: Light microscopy analysis revealed that reducing the alginate content in the biosorbents had no significant effect on particle size or sphericity. All samples formed spherical beads with an average diameter of approximately 3.1-3.4 mm. The combination of alginate and spent yeast proved to be an effective biosorbent, capable of removing up to 90% of dye from aqueous solutions within a short period. Among the tested formulations, the biosorbent containing 1.3% alginate, 0.03% chitosan and 6.0% yeast exhibited the highest adsorption capacity. The results suggest that yeast contributes more significantly to dye adsorption than alginate. Furthermore, coating alginate beads with chitosan and incorporating it increased absorption capacity and decreased absorption time. The findings of this study suggest that the immobilization technique enables the development of cell-based biosorbents that are easy to handle, stable, cost-effective, environmentally friendly, and suitable for treating dye-contaminated water.

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Enzymatic oligomerization of phloridzin in betaine-based deep eutectic solvent

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Introduction: Laccase-catalyzed oligomerization of flavonoids has been investigated in many studies where the requirement of certain amount of organic solvent to provide the solubility of the monomer and products has been noted. Recently, the usage of more environmentally friendly medium has attracted significant attention. Deep eutectic solvents (DES) are a new type of solvents which have gained much attention and have been tested for many applications, including biocatalysis. As they are considered as green solvents, their combined use with laccases can enhance the greenness of the oligomerization process. In this work, DES betaine:sorbitol:water (B-S) was used in different concentrations as co-solvents in oligomerization of flavonoid phloridzin catalyzed by laccase from *Trametes versicolor*.

Experimental: Betaine:sorbitol DES was prepared by heating and stirring method until the formation of a clear liquid. Subsequently DES was mixed with water to obtain DES-water mixtures (10, 25, 50 and 75 wt% DES) which were used as medium for phloridzin oligomerization reaction. Laccase activity was evaluated during 24h of incubation at 30 °C, and compared to its initial activity in each medium. Laccase catalyzed oligomerization of phloridzin was performed at phloridzin concentration 5 mg/ml, laccase concentration 0.5 mg/ml, temperature 40 °C and under stirring of 130 rpm. Reaction progress and products' yield were determined by reverse phase-high performance liquid chromatography (RP-HPLC) and compared to the results obtained in the organic solvent.

Results: Initially, results showed that laccase activity was greatly influenced by the B-S concentration. At lower concentrations of 10 and 25 wt% DES, similar enhancements of laccase activity during the time (3.26 and 5.89%) were measured. The major positive effect was noted in case of 50 wt% of B-S, where the increase of activity reached 41.52%. However, further increase of DES concentration negatively influenced the catalyst activity, since

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almost 40% of activity was lost when laccase was incubated in 75 wt% of DES. Upon the selection of the best DES concentration regarding laccase activity (10, 25 and 50 wt%), phloridzin oligomerization was performed at these conditions. Although initial testing showed that laccase activity was greatly enhanced in 50 wt% DES, at these conditions, 17.46% lower conversion was achieved, in comparison with the reaction in organic solvent. However, the other two DES:water media led to an increase of phloridzin conversion in both cases, reaching around 96% after 24h and thus higher products yield. Obtained results indicate that lower concentrations of B-S can replace organic solvent in phloridzin oligomerization catalyzed by laccase from *Trametes versicolor* and contribute to the overall greenness of the process.

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Effect of conifer cones extracts on biomass and polysaccharide production using *Fomes fomentarius* TMF2 mycelium

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Introduction: Medicinal fungi have a long tradition of usage in Asia. They have been recognized as sources of immunomodulators, prebiotics, antimicrobials, antioxidants, anticarcinogenic, and antidiabetic substances. The Western scientific community has expressed interest in this topic in recent years. It has been proven that many of those beneficial properties are owed to polysaccharides. The primary source of polysaccharides is the fruiting body of the mushroom. However, submerged-cultivated mycelia produce exopolysaccharides (EPS). They are secreted into the liquid medium and easily isolated by ethanolic precipitation. Moreover, intracellular polysaccharides (IPS) can be isolated from mycelia. Plant extracts could stimulate polysaccharide production. Therefore, the present study aimed to investigate the effect of three different conifer cone extracts, i.e., thuja (*Thuja orientalis*), spruce (*Picea excelsa*), and cypress (*Cupressus sempervirens*), on the biomass and polysaccharide production by the mycelium of the medicinal fungus *Fomes fomentarius* TMF2 during submerged fermentation.

Experimental: Mature conifer cones were dried, minced, and extracted with 50% ethanol by maceration for 7 days in the dark at 25 °C. In the first experiment, the thuja (TCE), spruce (SCE), and cypress (CCE) conifer cone extracts were added separately to the liquid medium in a concentration of 0.5% (v/v). In the second experiment, TCE and CCE extracts were added in concentrations of 1.0 – 3.0% (v/v). All media, including the control one (without any extract), were inoculated with 1% (v/v) of a 6-day-old culture of *Fomes fomentarius* TMF2 mycelium and incubated for 10 days at 30 ± 2 °C and 120 rpm. Biomass was separated by centrifugation, washed with distilled water, dried, and minced. EPS and IPS were obtained by cold ethanol precipitation from supernatant, i.e., hot water mycelia extract, respectively.

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Results: The extracts of mature conifer cones were rich in bioactive compounds, i.e., phenolic components, flavonoids, terpenoids, and hydrolyzable tannins that stimulated mycelia biomass and polysaccharides production by *F. fomentarius* TMF2. The results showed a positive impact of all three extracts, although the effect of SCE was not significant. The addition of 0.5% (v/v) conifer extracts improved EPS yield by 8.5%, 15.0%, and 36.3%, while IPS yield was enhanced by 1.5%, 19.4%, and 4.1% compared to the control by SCE, TCE, and CCE, respectively. Biomass increased by 2.7% (SCE), 13.6% (TCE), and 19.1% (CCE). Increasing concentrations of TCE and CCE up to 2% (v/v), i.e., 1% (v/v), respectively, additionally improved biomass and polysaccharides production, while higher concentrations had a lower stimulation effect. The highest achieved biomass enhancement of 214.0% was obtained with 1% (v/v) of CCE. The same extract had the most noticeable outcome on the IPS production, with a 58.2% higher yield compared to the control. The highest EPS yield was obtained with 2% (v/v) TCE, and it was 196.3% higher than in the control. These findings indicate that conifer cone extracts positively affect polysaccharide production, with further research needed on their structure and biological properties.

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Screening for polysaccharide producers among four different natural isolates of fungi

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Introduction: The traditional medicine of many cultures uses mushrooms to maintain and improve vitality and health. Their application as food is spreading even more around the globe. Of the many biologically active compounds present in them, the most abundant are polysaccharides, terpenes, phenols, lectin, etc. Polysaccharides are complex carbohydrates that play a key role in various biological processes. They are found in diverse forms across the natural world, with higher fungi being a notable source. The polysaccharides and mycelium biomass derived from these fungi have attracted considerable interest due to their various applications in the food and pharmaceutical sectors. Mycelial growth is greatly dependent on fermentation media and physical conditions. The present study aims to test the ability of four new isolates of higher fungi to produce intracellular (IPS) and extracellular (EPS) polysaccharides during submerged fermentation.

Experimental: Mycelia of fungi named *Ganoderma resinaceum* NMKSS, *Fomes fomentarius* TMF2, *Bjerkardera adusta* TMF1, and *Ganoderma* sp. were assessed for polysaccharide production from glucose as a substrate. They were grown in medium containing 50 g/L glucose for 10 days on an orbital shaker (120 rpm) at 30±2 °C. Afterward, the biomasses were separated by centrifugation, washed, dried, weighed, and ground. These were used to extract IPS, while EPS were obtained directly from the supernatants. Polysaccharides content was estimated by phenol-sulphuric acid method. In the next phase of research, the selected strains were grown under the same conditions, with varying sources of organic nitrogen.

Results: The first screening of polysaccharide production showed that three of four analyzed strains produced polysaccharides under applied conditions. The EPS yield was in the range (0.44 – 0.84 mg/mL), while IPS was (33.8 –

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113.3 mg/g dry biomass). *F. fomentarius* TMF2 and *B. adusta* TMF1 exhibited a significantly higher yield of polysaccharides compared to the others, thus, they were selected for testing the effect of inorganic nitrogen source. The highest yield of both EPS and IPS was obtained in the medium containing only peptone. *F. fomentarius* TMF2 gave the maximum yield of EPS (0.84 mg/mL). The enhancement of EPS produced by *F. fomentarius* TMF2 was 45% and 22% compared to media containing only yeast extract or yeast extract and peptone, respectively. The maximum IPS yield (134.12 mg/g dry biomass) was achieved by *B. adusta* TMF1. IPS production by *B. adusta* TMF1 was increased by 40% compared to the medium with yeast extract as a sole nitrogen source and by 33% compared to yeast extract and peptone combination. The highest amount of biomass for both isolates was achieved in the medium with yeast extract only. That leads us to the conclusion that medium with yeast extract is more suitable for biomass production while medium with peptone is the best for stimulation of polysaccharide production. Further tests are needed to enhance EPS and IPS production from new natural isolates of higher fungi.

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Utilizing the natural advantages of potato peels: Peroxidase and polyphenol oxidase for tackling dye pollution

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Introduction: Finding new sources of peroxidase and polyphenol oxidase for use in wastewater treatment is essential. Isolating peroxidase and polyphenol oxidase from waste materials is particularly convenient. Therefore, this study focuses on isolating these enzymes from food industry waste, specifically potato peels, purifying them, characterizing them, and applying them to wastewater treatment.

Experimental: Peroxidase and polyphenol oxidase were extracted from potato peels using a mechanical screw press. To optimize enzyme yield during extraction, ground potato peels were mixed with 20 mM phosphate buffer at pH 6 in various mass ratios: 2:1, 1:1, 1:2, and 1:4. This mixture was incubated for 1 hour at 4°C. The enzyme activity in these extracts was then compared to the enzyme activity in the potato peel juice, which was obtained through mechanical processing. Precipitation was chosen as the first purification step. To determine the optimal concentration of ammonium sulphate required for enzyme precipitation within 24 hours at 4 °C, the potato peel juice was mixed with ammonium sulphate (to obtain 30, 40, 50, 60, 70, 80, and 90% of the saturation at a given temperature). In addition to ammonium sulphate, the optimal concentration of organic solvents (ethanol and acetone) for precipitation was also determined. Therefore, the potato peels juice and organic solvent were mixed in the volume ratio 1:1, 1:2, 1:3 and 1:4 for 1 h at 4°C. In the obtained potato peels juice, extracts, precipitates and supernatants, the activity was measured using several substrates, pyrogallol, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and catechol. The concentration of proteins was determined by the modified Lowry method. The precipitates obtained after precipitation

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with ammonium sulphate were subjected to electrophoretic separation. Non-reducing SDS-PAGE electrophoresis was used to separate proteins by their molecular weight and non-reducing SDS-PAGE electrophoresis, followed by renaturation using TRITON X-100 and the standard substrate pyrogallol, was used to determine which bands are the target enzymes. The obtained enzyme preparation was used for the decolorization of different types of dyes.

Results: The process of isolating peroxidase and polyphenol oxidase from potato peel is considered solvent-free because varying the solid-liquid ratio did not increase the enzyme activity yield. Based on the activity yield and purification factor, it was determined that ammonium sulphate is more effective than organic solvents for precipitation. Following the renaturation of non-reducing SDS-PAGE electrophoresis, bands were observed at approximately 40 kDa across the entire tested range of ammonium sulfate saturation (30-90%). Additionally, bands at a molecular weight of approximately 70 kDa appeared in the precipitate when the ammonium sulphate saturation was between 70% and 90%. These results suggest that adjusting the ammonium sulphate saturation enhances the separation of the fraction rich in peroxidase from the one containing polyphenol oxidase. Both fractions showed high efficiency (biodegradation efficiency > 60%) in degrading a different class of synthetic dyes.

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Probiotic as wound dressing: Probiotic immobilization and release from AC pads

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Introduction: One of the key challenges in developing efficient probiotic systems for various applications, such as wound dressing, is ensuring effective cell preservation. Immobilization is a well-established technique widely used across various industries. A fundamental requirement of any immobilization technology is maintaining the viability of bacterial cells, which plays a key role in selecting the appropriate method. The freeze-drying is widely regarded as the gold standard in the biopharmaceutical industry. However, the freezing phase can cause cellular damage due to ice crystal formation and osmotic stress. To minimize such damage, various protectants are often added to the drying medium. Another crucial factor is the controlled release of cells into the surrounding environment. Depending on the application, it may be necessary to release a large number of cells rapidly, or in some cases, a sustained and gradual release is more desirable. Here, we selected 100% activated carbon fabric (AC pads), which is usually used in medicine to neutralize odors, as support for probiotic immobilization.

Experimental: *Lactobacillus plantarum* (Lp299v) from the frozen stock was subsequently incubated under anaerobic conditions in MRS broth at 37 °C for 18 h, twice in succession, washed three times with NS (normal saline solution, 0.9% w/v NaCl), centrifuged and diluted in 10% trehalose, and a mixture of 5% trehalose with 5% skim milk. Concretely, a freeze-drying media mixed with cells was aseptically added on top of each AC pad, frozen at −80 °C, and subsequently freeze-dried for 5 h using a Beta 2-8 LD plus freeze dryer (Christ, Germany). The pads containing immobilized probiotics were incubated in 2 mL of normal saline at 37 °C for 10 minutes, 3 h, and 24 h. At each time point, the release of viable cells into the solution was assessed.

Results: AC pads made by punching were approximately 12 mm in diameter and weighed 0.017 ± 0.001 g. Both cryoprotectants - trehalose alone and in combination with skim milk - provided comparable protection during the

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freeze-drying process, with probiotic viability maintained at 81% of the initial value prior to immobilization in both cases compared to 71% of the control (probiotic immobilized without cryoprotectant). However, differences emerged in probiotic release over time. The combination of trehalose and skim milk demonstrated superior performance, preserving 78% viability after 24 hours, compared to 45% viability observed with trehalose alone. When probiotics are used in wound dressings, their controlled release is crucial for the antimicrobial activity. It can be concluded that the AC pad with probiotic protected by trehalose and skim milk is a better choice for wound dressing compared to the AC pad with probiotic protected by trehalose alone.

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Valorization of Brewery Spent Grain through Chitin Extraction from Black Soldier Fly Larvae

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Introduction: Organic waste, such as solid kitchen residues and crop-by products, represents an opportunity for valorization though bioconversion. Insects, particularly the larvae of *Hermetia illucens* (Black Soldier Fly Larvae, BSFL), are highly efficient in converting organic matter into value-added by-products. This insect is highly adapted to organic residues and grows rapidly under optimal conditions. Thus, the by-products that can be obtained from BSFL are oil for the production of biofuels (biodiesel and green diesel), as well as protein, amino acids and chitin [1]–[4] — a biopolymer with different uses in the industrial sector. This by-product recovery encourages the principles of circular economy [5]. This work analyses the performance in the extraction of chitin. In addition, the spectrum of BSFL chitin was qualitatively analyzed.

Experimental: The method for the extraction of chitin was carried following a methodology similar to the one described in [4], using the centrifugation method (1500 rpm at 15 min) for filtration after demineralization and deproteinization. In the demineralization process, 1 M HCl with a 1:10 w/v ratio was used, using 10 grams of BSFL sample with operating conditions of 100 °C with a duration of 30 min. Likewise, for the deproteinization process, 1 M NaOH was used at a temperature of 80 °C for 24 h. The filtration process yielded chitin with a significantly higher efficiency compared to the yield reported by [4] using prepupal stage larvae of BSFL, with a yield of 13.9 ± 0.12 % chitin. On the other hand, in the Fourier transform infrared spectroscopy (FTIR) analysis was used to qualitatively analyze the chitin structure, with spectral similarity to those reported by [4], [6], [7].

Key findings: These findings highlight the potential for a comprehensive use of the *Hermetia illucens* within a circular economy framework. Likewise,

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areas of opportunity include quantitative analysis of the chitin to determine its purity, and a life cycle assessment to estimate potential reductions in greenhouse gas emissions from future BSFL-based biorefineries. Experimental work for the further valorisation of spent BSFL is also underway. These findings indicate the strain's potential for integrated applications such as organic waste conversion and production of natural pigments with antioxidant properties, that can be implemented in the food and the pharmaceutical industry.

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Fermented common nettle by-product extracts in natural deep eutectic solvents, a sustainable additive for skincare

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Introduction: Nettle extracts are rich in bioactive compounds such as polyphenols, flavonoids and vitamins, known for their antioxidant, anti-inflammatory, antimicrobial, and anti-aging properties. By-product of the nettle processing for the teabags industry has potential as a source of a wide range of biologically active compounds for different applications, including in skin care. Their efficacy may be limited by poor solubility, bioavailability or stability. Natural deep eutectic solvents (NADES) are innovative green solvents that can improve the selectivity of the extraction of bioactive compounds. In addition, NADES, usually composed of natural components such as sugars, organic acids and amino acids, can act as functional ingredients in cosmetic formulations by stabilizing biologically active compounds and providing moisturizing properties. Fermentation with microorganisms can generate postbiotic metabolites beneficial to the skin and skin microbiome. Also it can potentially enable the biotransformation of biologically active compounds into more potent active forms. The combination of fermented NADES extracts of nettle could be a very powerful and multifunctional cosmetic ingredient with improved antioxidant capacity, improved bioavailability and extended shelf life. This study was aimed to find optimal biocompatible NADES for the extraction of antioxidants from nettle by-product and their biotransformation by *Ligilactobacillus salivarius*.

Experimental: Initially, six biocompatible and skin care compatible NADES were synthesized. To improve the viscosity, 30% distilled water was added to the NADES, and the extraction of nettle by-products was performed at 70 °C. Total phenolic content (TPC) and total flavonoid content (TFC)

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were used as criteria to examine NADES extraction effectiveness. After selecting the most efficient NADES for extraction, fermentation with *Ligilactobacillus salivarius* was performed for 24h. Samples were taken at 0h, 6h, 19h and 24h of fermentation. Viable cell count, TPC, TFC and DPPH (2,2-diphenyl-1-picrylhydrazyl) were utilized to determine microbial growth and impact of fermentation on extract composition and antioxidant activity, respectively. Upon fermentation, HPLC/MS analysis was performed to examine the effect of fermentation on the concentration of key biologically active compounds.

Results: NADES composed of betaine/glycerol in a molar ratio of 1:2 was shown as the most efficient for the extraction of antioxidants from common nettle by-products. The highest viable cell count (10^{10} CFU/ml) of *L. salivarius* was examined at the 24th hour of fermentation. TPC and TFC decreased during fermentation as well as antioxidant activity measured by DPPH. HPLC analysis revealed an increase of chlorogenic acid, caffeoylmalonic acid, and rutin. Based on this preliminary study, fermented extracts of nettle by-products in NADES offer a potential symbiotic additive for diverse applications. The integrated approach of green extraction and fermentation supports the development of environmentally conscious, skin care products that are aligned with the goals of the circular bioeconomy by valorising plant by-products, reducing application of synthetic additives and promotes natural products.

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Silver-Decorated TiO_2-Based Nanohybrids Functionalized with Bioactive Plant Extracts: Antimicrobial and Cytotoxic Properties

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Introduction: Due to the intensive and often inappropriate use of conventional antimicrobial agents, many pathogenic microorganisms have developed resistance, rendering these treatments less effective. In recent years, increasing interest has been in developing nanoparticles with potent antimicrobial activity. However, the cytotoxic effects of several nanoparticles, including TiO_2, on human cells have raised concerns and limited their broader biomedical applications. Surface modification of TiO_2 nanoparticles with bioactive components derived from plant extracts presents a promising strategy to reduce cytotoxicity while enhancing their antimicrobial performance. Additionally, incorporating silver into TiO_2-based nanohybrids further improves their antimicrobial potential, owing to silver's well-documented broad-spectrum activity and synergistic interactions with TiO_2. This dual-functionalization approach enhances performance while aiming to maintain biocompatibility.

Experimental: The first step in this research is the synthesis of a hybrid nanocomposite. Before synthesis, microwave-assisted extraction (MAE) of phenolic compounds from green tea and horsetail tea production waste material is performed using water. After that, TiO_2 nanoparticles are dispersed in the obtained extracts, forming an interfacial charge transfer (ICT) complex. HPLC analysis is performed to identify the phenolic compounds present in the extracts before and after synthesizing the nanocomposite. To further enhance the antimicrobial properties of the nanocomposite, an additional modification step is carried out by adding

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silver to the surface. Then the obtained hybrid nanomaterial is characterized using methods such as UV/Vis, FTIR, and XRD. After characterization of the nanocomposite, antimicrobial activity and cytotoxicity are evaluated.

Key findings: During the synthesis of the nano-hybrid, a noticeable color change of the powder from white to brown indicated successful surface modification. UV/Vis diffuse reflectance spectroscopy revealed a shift in the absorption spectrum from the UV to the visible region. FTIR spectra evidenced the presence of characteristic functional groups originating from the bioactive components on the TiO_2 surface. XRD patterns further demonstrated the successful incorporation of silver into the nanocomposite.

The antimicrobial activity of the synthesized nano-hybrid was tested on pathogenic microorganisms: the Gram-negative bacterium *Escherichia coli*, the Gram-positive bacterium *Staphylococcus aureus*, and the fungus *Candida albicans*. Functionalization of TiO_2 with polyphenols extracted from plants enhanced its antimicrobial properties compared to the unmodified TiO_2. However, the most pronounced reduction in microbial viability was achieved only after additional modification with silver, indicating a strong synergistic effect between the polyphenols and silver nanoparticles. Cytotoxicity was assessed using the MTT assay on human lung fibroblasts (MRC-5) and cervical cancer cells (HeLa), revealing lower toxicity levels for the functionalized nanocomposites compared to pure TiO_2, thereby confirming improved biocompatibility. These results highlight the potential of the synthesized TiO_2–Ag nano-hybrids as effective alternative antimicrobial agents, with possible applications in industrial-scale wastewater treatment currently under investigation.

Functionalized Fe_3O_4 Nanoparticles for Stable Immobilization of Horseradish Peroxidase

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Introduction: Horseradish peroxidase (HRP) is a widely used enzyme in industrial and analytical applications due to its high catalytic efficiency and substrate specificity. However, its practical application is often limited by poor stability and the inability to be reused. Enzyme immobilization on solid supports presents a promising strategy to enhance enzyme stability, reusability, and operational efficiency. This study is dedicated to developing a stable and efficient biocatalyst by immobilizing horseradish peroxidase (HRP) onto magnetite (Fe_3O_4) nanoparticles functionalized with specific organic ligands. The functionalization strategy aims to improve enzyme-support interactions, thereby increasing immobilization efficiency and maintaining enzymatic activity over multiple usage cycles. The effect of ligand type on catalytic activity retention and overall biocatalyst performance was systematically investigated under varying experimental conditions.

Experimental: Fe_3O_4 nanoparticles were synthesized via a standard co-precipitation method using ferrous and ferric salts in an alkaline medium. The surface of the synthesized nanoparticles was then functionalized with two ligands: caffeic acid (containing a free carboxyl group) and 5-aminosalicylic acid (containing a free amino group). These ligands were selected based on their potential to form stable interactions with enzyme molecules through electrostatic attraction or covalent bonding. The immobilization of HRP onto the functionalized nanoparticles was carried out under controlled conditions, with variations in enzyme concentration and the quantity of the carrier material. The pyrogallol assay was used to evaluate enzymatic activity after immobilization, where horseradish peroxidase

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(HRP) catalyzes the oxidation of pyrogallol in the presence of hydrogen peroxide. The reaction was monitored spectrophotometrically by measuring the absorbance at 420 nm at 30-second intervals. The operational stability and reusability of the immobilized enzyme were evaluated over multiple catalytic cycles.

Key findings: The functionalization of Fe_3O_4 nanoparticles significantly influenced immobilization efficiency and enzymatic performance. Materials modified with 5-aminosalicylic acid exhibited excellent retention of peroxidase activity over five consecutive cycles, with only a minor decrease in catalytic efficiency. This suggests that amino groups on the nanoparticle surface promote stable enzyme binding and favorable orientation for catalysis. In contrast, nanoparticles functionalized with caffeic acid, which contains a carboxyl group, showed a marked decline in activity as early as the second usage cycle. This reduction is attributed to weaker enzyme-carrier interactions at the working pH of 8.5, likely due to the pKa values of the ligand affecting charge distribution and binding affinity. These findings demonstrate the critical role of surface chemistry in enzyme immobilization and highlight the importance of tailoring functional groups to optimize biocatalyst stability and performance. The results contribute to the broader understanding of how ligand selection can be used to design efficient and reusable nanostructured enzyme carriers for various industrial and biomedical applications.

Lactic acid fermentation of industrial artichoke by-products to increase the availability of soluble fibre

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Introduction: The transition to sustainable development is a key priority in the EU and requires a shift in food production systems toward circular economy models. In this context, incorporating *food loss and waste* (FLW) into circular economic models is of vital importance. *Cynara cardunculus* L. var. *scolymus* (artichoke), native to the Mediterranean region, is largely processed industrially in Spain, the world's third largest producer (0.2 million t/year, FAO 2021). Approximately half of its production is consumed in canned form, leaving the outer bracts and stems as blanched artichoke by-products (BAB), which are rich (60-70%) in cell wall polysaccharides (cellulose, hemicellulose and pectin). Despite their potential, BAB valorisation strategies, especially those enhancing the solubility of these polysaccharides, remain underexplored. Fermentation has shown promise in increasing the bioavailability of compounds of interest. In this study, spontaneous BAB fermentation was used to isolate strains with desirable enzymatic profiles, identified *in silico*. These were then employed individually and in consortia to target ferment BAB, aiming to increase soluble fibre content with potentially improved prebiotic properties.

Experimental: During fermentation, the pH, total microorganism count and changes in the soluble carbohydrate fraction were evaluated. Monomeric composition was determined by GC-FID, and the amount and molecular weight (M_w) distribution by HPSEC-ELSD.

Results and conclusions: In fermentations with individual isolates, the pH decreased, especially with *Lactobacillus paraplantarum* B184, while total

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bacterial counts increased (from 0.5 to 3.3 logarithmic units, mean 1.93), especially with *Levilactobacillus brevis* B143. Carbohydrate profiles varied considerably: glucose dropped from 54% in the control to an average of 14%, reaching just 7.8% in the fermented sample of *L. paraplantarum* B184. Notably, *Lactobacillus kimchii* B191 released significant amounts of xylose, arabinose and galactorunic acid. Total soluble carbohydrate yields ranged from 46 mg/g DM (with *Lactiplantibacillus pentosus* DSM20314), which showed high metabolic activity, to 124 mg/g DM (with *Leuconostoc kimchi* A156), compared to 84 mg/g DM in the non-fermented control.

In consortium fermentations, pH decreased less (0.19 and 0.87 points) but microbial growth was greater (0.98 and 3.65 log units, mean 2.28) than in single-strain trials. Although total soluble carbohydrates remained unchanged, the relative abundance of low-M_w compounds (<0.3 kDa) fell to ~34% in selected consortia. In terms of monomeric composition, consortia containing *Lactobacillus paraplantarum* B181 showed lower xylose and arabinose levels, likely because this strain consumes the pentoses released by the other microorganisms. This fact is consistent with the monomeric profile of BAB fermented solely with *L. paraplantarum* B181, which also displays a marked decrease in xylose and arabinose content.

In summary, shifts in the carbohydrate fraction reflect each isolate's unique enzymatic activities, generating pronounced variability in pH, growth and carbohydrate composition. By contrast, defined consortia deliver more uniform acidification and enhanced overall metabolism, driven by cross-feeding interactions among member strains.

Preliminary study of effects on bacteria of *Myrtus communis* L. Extracts

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Introduction: The exessive use of synthetic pesticides in agriculture has a negative impact on the environment and human health. Furthermore, their continuous use has led to the emergence of pesticide-resistant phytopathogenic bacteria. This research focuses on the potential of *Myrtus communis* L. as a source of bioactive compounds with antimicrobial activity against key phytopathogenic bacteria. *Myrtus communis* L., is an evergreen bush native to southern Europe and western Asia. This plant has been traditionally used in folk medicine for various diseases.

Experimental: In the present work, extracts from different parts of the plant (seeds and leaves) were formed by means of ultrasound-assisted extraction (HAE), using different solvents (ethanol, methanol and *n*-hexane). The solvents were evaporated and the extracts were collected in a dry form. The solids formed were dissolved in suitable solvent which did not have any antimicrobial activity and the antimicrobial activity of the solution was studied on three phytopathogenic bacteria namely *Clavibacter michiganensis*, *Pseudomonas syringae* and *Xanthomonas campestris*, using the disk diffusion method (DDM).

Results: Different extracts displayed varying degrees of inhibition against the tested phytopathogenic bacteria. All leaf extracts demonstrated notable activity against *C. michiganensis*, with the methanol leaf extract (ML1) showing the largest inhibition zone (21 mm), followed by the hexane (18 mm) and ethanol (17 mm) leaf extracts. The hexane seed extract (MS3) also exhibited moderate activity (10 mm) against *C. michiganensis*, while the ethanol seed extract (MS2) showed a smaller inhibition zone (8 mm), and the methanol seed extract (MS1) showed no inhibition. For *P. syringae*, the hexane leaf extract (ML3) presented the highest antibacterial activity (11 mm), followed by the methanol leaf extract (ML1) (9 mm). Against *X. campestris*, the leaf extracts again demonstrated the most potent

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antibacterial effects, with the methanol leaf extract (ML1) and hexane leaf extract (ML3) exhibiting the largest inhibition zones (21 mm and 19 mm, respectively), followed by the ethanol leaf extract (ML2) (17 mm). Overall, the methanol and hexane extracts of *Myrtus communis* L. leaves consistently demonstrated the most promising antibacterial activity across the tested phytopathogenic bacteria, suggesting their potential as a source of bio-pesticides.

Development and characterisation of soy protein-based biodegradable food packaging

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Introduction: Providing enough food and adequate nutrition for the growing world population will be challenging, since millions face hunger each year. At the same time, tonnes of food are wasted annually, posing both ethical and environmental concerns. Consequently, finding effective solutions that would substantially reduce food waste has become an imperative. Among various strategies, packaging emerges as promising approach. However, petroleum-based polymers, which dominate food-packaging market, pose a serious environmental burden, thus turning one solution into a new problem. Hence, the development of novel packaging materials based on natural polymers offers a promising compromise between reducing food waste and achieving sustainability goals. Protein-based packaging materials offer several advantages compared to those obtained from polysaccharides and lipids, primarily in terms of superior oxygen barrier and mechanical properties. This work focuses on the development of sustainable soy protein-based films and coatings.

Experimental: Film-forming solutions (FFS) contained varying amounts (4–8% w/w) of heat-pretreated soy protein concentrate (SPC) (80 °C, 1 h, pH adjusted to 11, and centrifuged at 1000 rpm for 5 min), along with glycerol (Gly) in the range of 0–3.8% w/w. Ultrasound treatment (30% amplitude for 1 min) was applied for FFS homogenization, after which 30 ml of FFS was cast into 90 mm diameter Petri dish and dried at 55 °C for 18 h. Mechanical properties (tensile strength and elongation at break) of films were determined using AGXplus Universal Testing Machine. Films were cut into strips of 60 mm length and 10 mm width. The initial grip separation and crosshead speed were set at 30 mm and 30 mm/min, respectively.

Results: The resulting soy protein-based films were homogeneous, pale yellow, and transparent. Differential scanning calorimetry revealed that

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pretreatment caused denaturation of soy proteins, while the electrophoresis confirmed presence of all characteristic bands of glycinin and β-conglycinin. Films without Gly were brittle and could not be further analysed due to cracking. Increasing concentration of SPC, while maintaining the same SPC/Gly ratio, improved mechanical properties, with the best results observed at 8% SPC. Optimisation of Gly content further enhanced film performance. Films with 1.9% Gly had the highest tensile strength of 3.68±0.14 MPa, but significantly lower elongation at break (185.8±5%), while those with 2.8% Gly had the opposite trend (2.67±0.23 MPa, 352.3±10.4%). The highest tested Gly content led to a substantial decrease in tensile strength (1.25±0.03 MPa). Thermogravimetric analysis revealed that the degradation of films occurs in three stages, with the optimal processing temperature being up to 150 °C, as significant mass loss was observed beyond this point. Optimal formulation (8% SPC, 1.9% Gly) was applied as a coating for cherries and strawberries, however obtained results showed negligible difference in weight loss between coated and uncoated fruits. Nevertheless, these formulations pose as a promising material for the development of natural active packaging by incorporating bioactive ingredients.

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Antimicrobial properties of probiotic edible films based on whey proteins

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Introduction: The development of biodegradable food packaging materials has gained widespread attention due to growing environmental concerns associated with plastic pollution. Additionally, food safety remains a serious global issue, resulting in numerous deaths annually. Consequently, development of not only biodegradable, but also active packaging materials based on natural components, that would guarantee food safety and prolong food shelf-life became an imperative. Incorporation of probiotic bacteria in protein-based films presents a promising way to create edible active food packaging that also enables consumption of health-promoting probiotics. Namely, probiotics within films produce numerous bioactive compounds that contribute to improved functional properties of films, particularly their antioxidative and antimicrobial activities. However, controlling the synthesis and release of bioactive compounds in probiotic films presents a difficult task. In this paper, films obtained from whey protein concentrate (WPC) and inulin were used as carriers for the probiotic strain *Lactiplantibacillus plantarum* 299v.

Experimental: Film-forming solutions (FFS) contained heat-pretreated (9% w/w; 90 °C, 20 min pH adjusted at 8) WPC, glycerol (50% w/w of WPC+inulin), inulin (0-33% w/w of WPC), and 0.35 g *L. plantarum* 299v. Prepared FFS were cast in silicon shape (5.5 x 8 cm). Volumes were adjusted so that each film has the same amount of dry matter. Films were dried at 37 °C for 18 h. For testing antimicrobial potential, films were cut into 1x1 cm pieces and immersed into tryptic soy broth inoculated with: Gram-negative bacteria *Escherichia coli* ATCC 25922, Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, and fungi *Candida albicans* ATCC 24433. After 24 h of incubation at 37 °C, optical density (OD) was measured at 600 nm and percent of inhibition was calculated as: (ODcontrol –

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ODsample)/ODcontrol * 100, where control and sample correspond to films without and with *L. plantarum*, respectively.

Results: The results indicated that the obtained WPC and WPC-inulin composite films are suitable carriers for probiotics, as all formulations achieved the targeted viable cell count per gram of film (10^6 CFU/g). The film without inulin had the lowest cell count, which was still acceptable, providing approximately 10^6 CFU/g. The hypothesis that the addition of inulin would enhance the survival of lactic acid bacteria was confirmed. Films containing inulin showed an increased count of viable bacteria, reaching 10^7 CFU/g. Moreover, increase in inulin content did not have significant effect on bacterial survival over the tested storage period. Further testing were conducted to see potential antimicrobial properties of obtained films. Results showed that all films possess higher antimicrobial activity compared to their counterpart without bacteria. Namely, the highest inhibition was obtained against *E. coli* (48% without inulin, 44% with inulin), followed by *S. aureus* and *C. albicans*. Interestingly, films with inulin generally exhibited lower inhibition compared to WPC films, expect for *S. aureus* where no difference was observed. These results suggest that WPC and WPC-inulin composite films have great potential as active, sustainable food packaging.

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Development of multifunctional polyvinyl alcohol–activated charcoal dressings for advanced wound management

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Introduction: Wound care continues to pose a significant clinical and socioeconomic challenge. Around 2% of the global population suffers from "hard-to-heal" wounds that are frequently colonized by microorganisms and are prone to infection. Efficient management of these wounds has shifted toward polymer-based dressings that maintain a moist environment through fluid absorption, while also enabling controlled drug release, antimicrobial protection, and sometimes odor control. Synthetic polymers such as polyvinyl alcohol (PVA) are commonly used components of wound dressings due to their reproducibility, tunable properties, and mechanical stability. The incorporation of activated charcoal (AC) has gained attention for its ability to remove odors and microbial toxins. In this study, we developed a PVA–AC-based dressing functionalized with either silver ions (SI) or povidone-iodine (PI), aiming to combine exudate management, sustained antimicrobial activity, malodor control, and structural integrity.

Experimental: PVA–AC hydrogels were prepared by dissolving 10 wt% fully hydrolyzed PVA (Sigma-Aldrich) in deionized water at 90 °C overnight. Activated charcoal (5 wt%) was dry-sterilized at 200 °C for 1 h and added to the PVA solution. After homogenization, the mixture was cast into Petri dishes and crosslinked through four freezing–thawing cycles (−18 °C for 20 h, then +4 °C for 4 h). Hydrogels were freeze-dried for 24 h at 0.011 mbar, and dried films (~1×1 cm) were immersed for 3 h in SI or PI solutions (100 or 1000 ppm), followed by a second freeze-drying step. Swelling and release were assessed in simulated wound exudate (SWE) after 3 h, while antimicrobial activity was investigated after 3 and 24 h against clinical strain of *Klebsiella pneumoniae* in suspension. Control samples of 10 wt% PVA were prepared using the same protocol.

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Results: The obtained results have shown that both SI and PI were successfully adsorbed onto AC, as confirmed by the decreased concentration of antimicrobial agents in functionalization solutions. Functionalized samples showed moderate swelling: 2.9-fold for PVA–AC and 3.3-fold for PVA controls, relative to their dry weight. Silver-loaded PVA–AC (100 and 1000 ppm) reduced bacterial cell counts approximately 4-fold and 3-fold, respectively, which aligned with silver release into SWE. In contrast, PI-functionalized composites showed negligible antimicrobial activity, and iodine was not detected in the surrounding fluid. These results align with literature indicating strong affinity of AC for iodine and its limited release under physiological conditions. The findings support PVA–AC–SI as a multifunctional wound dressing, while PVA–AC–PI requires further optimization to improve iodine release and antimicrobial function. In addition, the system allows future functionalization with other antimicrobial agents, extending its applicability in wound care.

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Characterization of *β*-Glucans and a Partially Methylated *α*-Galactan from the Mosaic Puffball Mushroom *Bovistella utriformis*

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Introduction: Puffballs are a group of fungi that produce characteristic enclosed, globose fruiting bodies. As they mature, they undergo autolysis, which transforms their interior (gleba) into a dark, powdery spore-bearing mass. Puffballs have been used in folk medicine worldwide, primarily as wound dressings. However, there is still limited knowledge about their chemical composition, biological activity, and how they are affected by autolysis. The aim of the study was to characterize the water soluble polysaccharide fraction of the immature and mature mosaic puffballs (*Bovistella utriformis*).

Experimental: The dry mushroom gleba was subjected to hot water extraction (drug-to-solvent ratio = 1:30) under high pressure in an autoclave. The extract volume was reduced, and the crude extract was obtained as a precipitate by adding ethanol. The extract was dialyzed, enzymatically deproteinized, and freeze-dried, yielding the “polysaccharide extract”. The extract was analyzed qualitatively, quantitatively, and structurally using gel-exclusion chromatography, NMR, HPLC, and GC-MS. Samples were hydrolyzed with 4M TFA to identify and/or quantify sugar monomers by HPLC and NMR. GC-FID-MS analysis of alditol acetates (prepared by hydrolysis, reduction, and acetylation of the samples) was also used to confirm sugar identity. Structural analysis included NMR, as well as GC-

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FID-MS analysis of partially methylated alditol acetates (prepared by methylation, hydrolysis, reduction and acetylation of the samples). The fraction was tested for proinflammatory activity using THP-1 cells and an ELISA assay at two concentrations (20 and 200 µg/mL).

Results: Carbohydrate content of "polysaccharide extracts" of immature and mature puffballs was ~70 and only ~47%, respectively, with glucose being the most dominant (~49 and ~59%), followed by galactose (~38 and ~26%), the rare 3-*O*-methyl galactose (~13 and ~13.9%) and mannose (in traces). Structural analysis revealed the presence of two main polysaccharide fractions – glucans and galactans, which, according to gel-exclusion analysis and previous research, could be forming a loose complex. The evidence indicates that the glucan fraction represents a highly branched (1→6)(1→3)-*β*-D-glucan, forming a stable complex with proteins (~20-25% in immature and >30% in mature puffballs); the glucan content was about 50% lower in mature puffballs due to autolysis and there was an increase in *β*(1→3) linkages, which was also reported for *Agaricus brasiliensis*. The galactan was found to be a linear, partially methylated (1→6)-*α*-D-galactan; methylation, occurring at *O*-3, seems to increase with maturation, from 1 in 4 to 1 in 2 galactose units. Partially methylated *α*-galactans have been reported from several phylogenetically distant species, indicating their presence in fungi is widespread, although puffballs seem to possess an unusually high content of these poorly known polysaccharides. Maturation also led to melanization and increased anti-oxidative activity of the extract. Both extracts exhibited prominent *in vitro* immunomodulatory activity, strongly increasing the release of proinflammatory cytokines TNF*α*, IL-1*β* and IL-6 in THP-1 cells, but also anti-inflammatory IL-10 and IL-27 to some degree; the activity was correlated with *β*-glucan content, and the biological potential of partially methylated galactans has yet to be clarified.

Development of Multi-Doped Mesoporous Bioactive Glass and 3D-Printed Composite Scaffolds for Bone Tissue Engineering

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Introduction: The repair of large bone defects remains a significant clinical challenge, driving the development of advanced 3D-printed composite scaffolds that provide both mechanical stability and promote tissue regeneration. The incorporation of mesoporous bioactive glass (MBG), known for its mesoporosity, biocompatibility, and ability to load and release drugs and ions, enhances the functional properties of these scaffolds, positioning them as promising candidates for bone tissue engineering applications.

Experimental: In this study, multi-doped MBG particles with a nominal composition of $70SiO_2$–20CaO–3MgO–5SrO–1CuO–1ZnO (mol.%) were synthesized via a modified microemulsion-assisted sol-gel method with simultaneous ultrasound application. Ciprofloxacin drug was successfully loaded onto MBG particles. After comprehensive physicochemical and biological characterization, MBG particles were incorporated into a photopolymerizable resin based on polyethylene glycol diacrylate (PEGDA) and methacrylic acid and used for the 3D printing of macroporous scaffolds via mask stereolithography (mSLA).

Key findings: The particles exhibited spherical morphology with an average diameter of ~300 nm, confirmed by scanning electron microscopy (SEM). Inductively coupled plasma optical emission spectroscopy (ICP-OES) verified the successful incorporation and release of all dopant ions. X-ray diffraction (XRD) analysis confirmed the amorphous structure of MBG particles, while Brunauer–Emmett–Teller (BET) analysis revealed a high

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specific surface area (609.78 m^2/g) and well-developed mesoporosity, further supported by high-resolution transmission electron microscopy (HR-TEM).

In vitro biocompatibility testing using murine bone marrow-derived mesenchymal stem cells (BM-MSCs) showed preserved cell viability, with low levels of apoptosis and minimal necrosis, indicating cytocompatibility and absence of harmful cellular responses. Ciprofloxacin was released in a sustained manner, significantly enhancing antibacterial activity by completely inhibiting Staphylococcus aureus growth.

SEM confirmed uniform distribution of MBG particles within the printed scaffolds, which increased surface roughness due to their presence in the hydrogel matrix. The combined use of a modified sol-gel method and photopolymer-based 3D printing resulted in composite scaffolds with optimized physicochemical and biological properties, establishing them as strong candidates for future regenerative therapies, particularly in bone tissue engineering.

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Application of Lion's Mane Mushroom in Cosmetics: The Influence of Drying Method and Solvent

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Introduction: Mushrooms have traditionally been studied for their nutritional and medicinal properties; however, growing interest has recently emerged in their potential applications in cosmetics. Increasing evidence suggests that mushrooms are a rich source of valuable cosmeceuticals, particularly compounds with anti-aging properties, which are among the most sought-after in skincare. As the market for mushroom-based cosmetics continues to expand, there is a clear need for further scientific research in this area. Lion's mane mushroom (*Hericium erinaceus*) is a fungi species that parasites on oaks and produces characteristic irpicoid fruiting bodies; in recent years, it has gained popularity as a commercially cultivated edible species with potential health benefits. The aim of this study was to investigate the potential cosmetic benefits of lion's mane mushroom.

Experimental: The effect of drying was assessed using two methods: heat drying (38°C, 24 h) and freeze-drying (-40°C, 0.12 mbar, 24 h). Additionally, the influence of solvent polarity was evaluated using water and water-ethanol mixtures containing 30%, 50%, and 70% ethanol. The resulting extracts were subjected to chemical and biological characterization, with a focus on compounds and activities beneficial for skincare. Total carbohydrates were quantified via the phenol-sulfuric acid method. Sugars and polyols were analyzed using HPLC, and glucan content was determined with a Megazyme® *β*-Glucan Assay Kit. Antioxidant activity was assessed i*n vitro* using ABTS, CuPRAC, and *β*-carotene bleaching assays. Antityrosinase activity was evaluated via an *in vitro* enzymatic assay.

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Results: All samples demonstrated high extraction yields (28–44%), which decreased with increasing ethanol concentration. A similar trend was observed for carbohydrate content (6.6–14.9%), except in the extract from heat-dried mushrooms with 30% ethanol, which showed the highest carbohydrate content. Glucans were detected only in aqueous and 30% ethanol extracts (3.4–7.5%). The drying method influenced glucan composition: in freeze-dried mushrooms, α- and β-glucans were present in approximately equal proportions, while in heat-dried samples, α-glucans (glycogen) were only detected in trace amounts. Conversely, β-glucan content was higher in the heat-dried extracts, suggesting that α-glucans, as storage carbohydrates, may be catabolized during drying, while β-glucans might either accumulate due to desiccation stress or become more extractable after heat drying. A similar trend was observed in sugar composition: trehalose content was higher in heat-dried samples, while glucose was only detected in freeze-dried samples. The extract obtained with 30% ethanol from heat-dried mushrooms exhibited the highest β-glucan (7.5%) and trehalose (7.3%) levels. Mannitol emerged as not only the dominant sugar alcohol but also the predominant compound in all extracts, ranging from 35–58%. Both antioxidant and antityrosinase activities were generally higher in extracts from heat-dried mushrooms, likely due to the formation of soluble polyphenolic pigments during natural drying. Antityrosinase activity also increased with higher ethanol concentrations, indicating a strong dependence on solvent polarity.

In conclusion, lion's mane mushroom represents a promising source of cosmeceutical agents, including humectants (glycogen, trehalose, mannitol), anti-aging compounds (β-glucans), antioxidants, and potential anti-hyperpigmentation agents. These properties are strongly influenced by the drying method applied to the raw material.

Plant abiotic stress and animal aging might mirror each other, a language model based preliminary examination

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Introduction: Certain signaling pathways related to animal senescence exist in plants, such as reactive species and some nutrient sensors. Manipulation of these may provide an advantage for plant development. Galactose, a pharmacological agent that induces a progeria-like phenotype in animals, according to old literature, also disrupts plant physiology. In addition, a plant protein that protects against both biotic and abiotic stress, osmotin, protects animals against type 2 diabetes and Alzheimer's disease. Its structure is similar to adiponectin, an insulin-sensitizing hormone, even though the two are not homologs. Just as herbal medicines exert their influence on animals, animal-based compounds that slow down the aging process also help to alleviate abiotic stress: taurine has been shown to protect plants from cadmium poisoning, and carnosine has been shown to protect plants from drought and salt stress. We hypothesize that abiotic stress in plants and aging in animals are sufficiently similar that they can serve as models for each other, similar to yeast models of animal aging. On this basis, a testable prediction was made that generative smaller language models based on transformer technology would assign aspects of one context to the other. This prediction is based on perceived linguistic similarity and the way large language models work. They predict the next token, the minimal unit of the text, based on the previous context, the entirety of the preceding text. If a language model assigns tokens that factually belong in one phenomenon to another one, it would not directly provide evidence for the biological similarity of the two processes. It would, however, provide evidence for the similarity of the language used.

Experimental: Large language models have been examined for their tendency to conflate aspects of aging and abiotic stress: Mistral models to test associations between telomerase activity and halotolerance and Llama models to test multiple associations between abiotic stressors and age-related diseases. Two of the possible sources for these findings were examined using

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ChatGPT models to generate plant-derived secondary metabolite methylglyoxal scavengers and antioxidants, both of these implicated in abiotic stress, and evaluating their effects against biotic stress and as caloric restriction mimics, as both phenomena affect animal senescence. The outcome was further checked against the published literature.

Key findings and conclusion: Smaller Mistral models showed a higher tendency to associate telomerase with halotolerance (19/20 successes for the smallest Mistral Mini instruct model, 10/20 for the Mixtral Small instruct model, and 1/20 for the Mistral Medium base model). The Llama-2-70b instruct model conflated abiotic stressors and age-related diseases with perfect outcomes (20/20 successes) for each prediction. This success was replicated with the Llama-3-70b instruct model. Methylglyoxal scavengers and antioxidants generated turned out to have both caloric restriction and suppression of biotic stress properties. The molecular aspects of plant abiotic stress and animal senescence are linguistically similar. Preliminary evidence that plant abiotic stress and animal senescence are similar is established.