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BIOCHEMICAL ENGINEERING & BIOTECHNOLOGY

For Young Scientists

BOOK of ABSTRACTS

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CONFERENCE AGENDA

Thursday, 7th December

10:00-10:30	**Registration**	
10:30-10:40	**Opening ceremony**	
10:40-11:00	**Opening lecture**	

Coffee break and posters

Biotechnology Applications in Biomedical Sciences,
Chair: Ellen H. van den Bogaard and Patrick Zeeuwen

11:30-12:00	**Invited speaker - Aryl hydrocarbon receptor signaling in health and disease: the potential and threats of targeting environmental sensors in human skin**	**Ellen H. van den Bogaard (RadboudUMC, The Netherlands)**
12:00-12:15	**Expanding the possibilities of the stratum corneum model for bacterial growth**	**Noor van Hout**
12:15-12:30	**3D in vitro skin models: a toolbox to study skin biology, disease pathophysiology, and therapeutics**	**Jaimy Klijnhout**
12:30-12:45	**Bacteria X: studying microbe-microbe interaction**	**Mona Fayyazi Jolandan**
12:45-13:00	**Advancing the studies of physiological and pathological bone conditions by using a 3D in vitro cell culture model based on bone-like biomaterial and a perfusion bioreactor**	**Ivana Banićević**

Lunch break and posters

Biotechnological Production and Assessment of Bioactive Compounds
Chair: Patrick Jansen and Nevena Luković

14:00-14:30	**Invited speaker - The Spicy Solution: Capsaicin's Therapeutic Potential in Hepatocarcinoma through AMPK activation.**	**Alicia C. Bort (Alcalá University, Spain)**
14:30-15:00	**Invited speaker - Customized LNPs for targeted transfection**	**Marcus Janschel (Fraunhofer IAP Center, Germany)**
15:00-15:15	**Galactomannan extraction and characterization from Ceratonia siliqua seeds**	**Iván Benito**
15:15-15:30	**By-products from the processing of herbs as sources of antioxidants**	**Mihailo Mladenović**

15:30-15:45	**Discovering potential of polyphenol compounds from blueberry, cranberry and chokeberry extracts as skin prebiotics**	**Anja Petrov Ivanković**
15:45-16:00	**Sugar functionalized superparamagnetic nanoparticles for capturing of cancer cells in liquid biopsy**	**Ivana Banićević**

Coffee break and posters

Enzyme Engineering and Immobilization
Chair: Jose Migel Palomo

16:00-16:30	**Invited speaker - Enzyme-metal nanoparticle hybrids for modulating enzyme-like activity and chemoenzymatic cascade processes for sugar building blocks synthesis**	**Noelia Garcia Losada (University of Lisboa, Portugal)**
16:30-16:45	**Immobilization of xylanase on magnetic nanoparticles modified with polyethyleneimine and its application in xylooligosaccharides synthesis**	**Katarina Banjanac**
16:45-17:00	**New life of waste material: immobilized horseradish peroxidase for degradation of antraquinone dye**	**Tanja Nedeljkov**
17:00-17:15	**Determining the potential of submerged fermentation on wheat bran for production of xylanase**	**Ivana Gazikalović**
17:15-17:30	**Study and preparation of artificial manganese metalloenzymes with laccase-like activity**	**Ana Vukoičić**

Friday, 8th December

Environmental Biotechnology
Chair: Maja Đolić

09:30-10:00	**Registration**	
10:00-10:15	**Characterization of emission from the combustion of solid biofuels in the residential heating appliances**	**Vasilije Matijašević**
10:15-10:30	**Removal of critical metals leached from fly ash using naturally derived cellulose-adsorbent**	**Vanja Lukić**
10:30-10:45	**Utilization of fibrous textile wastes for adsorption of Inorganic and organic pollutants from water**	**Nataša Karić**
10:45-11:00	**Chemometric modelling of the adsorption parameters of drug residues from water using modified fly ash as adsorbent**	**Dušan Trajković**

Coffee break and posters		
Bio-based products and industrial biotechnology Chair: Mirjana Rajilić-Stojanović		
11:30-12:00	**Invited speaker - Biobased polymer materials as promising tool for efficient drug delivery**	**Maja Marković (ICFTM, Serbia)**
12:00-12:15	**The employment of pullulan and collagen in the preparation of electrospun nanofibers loaded with *Teucrium montanum* L. extract**	**Ana Mandura Jarić**
12:15-12:30	**The processing, bioactivity and biocompatibility of scaffolds based on multi-ion doped calcium-phosphates coated with chitosan**	**Teodora Jakovljević**
12:30-12:45	**The use of starch and *β*-lactoglobulin composite hydrogels as frameworks for preserving c-phycocyanin**	**Zorana Jovanović**
12:45-13:00	**Different treatments of lignocellulosic biomass for enhanced delignification and enzymatic hydrolysis**	**Jovana Grbić**

Lunch break and posters		
Functional food and feed Chair: Oswaldo Hernández-Hernández		
14:00-14:30	**Invited speaker - The chemical features of dietary fibers with prebiotic potential - why is it important?**	**Kahlile Youssef Abboud (Maastricht University, The Netherlands)**
14:30-14:45	**Edible flowers of marigold (Calendula officinalis L.) as functional food**	**Sofia Kilibarda**
14:45-15:00	**Sensory analysis of nutritionally improved corn-based snack product with addition of protein- and fiber-rich ingredients**	**Jovana Delić**
15:00-15:15	**Exploring the microbial degradation profile of 3 different dietary fibers via bacterial monoculture and an in vitro fermentation model of the colon (TIM-2)**	**Yanyun Zhang**
15:15-15:30	**Broccoli microgreens-apple juice as novel beverages: total phenolic, flavonoids and antioxidant activity**	**Spasoje Belošević**
15:30-15:45	**Nanofiltration as a tool for high-yield purification of dietary oligosaccharides**	**Milica Veljković**
15:45-16:00	**Valorization of soybean meal for production of high protein animal feed and value-added products using new strain of *Aureobasidium pullulans***	**Slađana Davidović**
16:00-16:30	**Closing ceremony**	

-BOOK OF ABSTRACTS-

Session 1

Biotechnology Applications in Biomedical Sciences

Chair: Ellen H. van den Bogaard and Patrick Zeeuwen

GRAM-POSITIVE ANAEROBIC COCCI GUARD SKIN HOMEOSTASIS BY REGULATING HOST-DEFENSE MECHANISMS

Danique A. van der Krieken[1], Gijs Rikken[1], Thomas H.A. Ederveen[2], Patrick A.M. Jansen[1], Diana Rodijk-Olthuis[1], Luca D. Meesters[1], Ivonne M.J.J. van Vlijmen-Willems[1], Bram van Cranenbroek[3], Renate G. van der Molen[3], Joost Schalkwijk[1], Ellen H. van den Bogaard[1], and Patrick L.J.M. Zeeuwen[1*]

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In atopic dermatitis (AD), chronic skin inflammation is associated to skin barrier defects and skin microbiome dysbiosis including a lower abundance of Gram-positive anaerobic cocci (GPAC). We here report that through secreted soluble factors, GPAC rapidly and directly induced epidermal host-defense molecules in cultured human keratinocytes and indirectly via immune-cell activation and cytokines derived thereof. Host-derived antimicrobial peptides known to limit the growth of Staphylococcus aureus-a skin pathogen involved in AD pathology were strongly upregulated by GPAC-induced signaling through aryl hydrocarbon receptor (AHR)-independent mechanisms, with a concomitant AHR-dependent induction of epidermal differentiation genes and control of pro-inflammatory gene expression in organotypic human epidermis. By these *modes of operandi*, GPAC may act as an 'alarm signal' and protect the skin from pathogenic colonization and infection in the event of skin barrier disruption. Fostering growth or survival of GPAC may be starting point for microbiome-targeted therapeutics in AD.

Keywords: atopic dermatitis; skin microbiome; Gram-positive anaerobic cocci (GPAC); aryl hydrocarbon receptor (AHR)

Acknowledgements: This study is in part funded by a TOP grant from ZonMw (91211052). The skin-microbiome 3D culture system was developed under a MKMD grant from ZonMw (114021503). The fractionation of GPAC and investigating the AHR activating potential was funded by an Off-Road grant from ZonMw (451001028). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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EXPANDING THE POSSIBILITIES OF THE STRATUM CORNEUM MODEL FOR BACTERIAL GROWTH

N.E. van Hout[1*], P.A.M. Jansen[1], P.L.J.M. Zeeuwen[1], E.H. van den Bogaard[1]

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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 project, we examine the interplay between *Staphylococcus aureus* and *Cutibacterium acnes* in the context of atopic dermatitis.

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.

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* seems to take 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.

Keywords: atopic dermatitis; stratum corneum; microbiome; *in vitro* model

Acknowledgements: This study is funded by the European Union.

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3D *IN VITRO* SKIN MODELS: A TOOLBOX TO STUDY SKIN BIOLOGY, DISEASE PATHOPHYSIOLOGY, AND THERAPEUTICS

Jaimy A. Klijnhout[1*], Luca D. Meesters[1], Jos P.H. Smits[1], Patrick L. J. M. Zeeuwen[1], Ellen H. van den Bogaard[1]

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Atopic dermatitis (AD) is a common inflammatory skin disease with heterogeneous clinical features, such as eczematous lesions and itch. The multifactorial cause of AD involves three interacting components: epidermal barrier deficiency; Th2-mediated immune dysregulation; and skin microbiome dysbiosis. The gene encoding filaggrin (*FLG*) has a key role in the formation of the epidermal barrier. Loss-of-Function variants in the *FLG* gene are strongly associated with AD onset and disease severity. Immunological, microbial or environmental cues can trigger AD-related intracellular keratinocyte signalling cascades contributing to disease phenotype. To study complex inflammatory skin disease pathophysiology, meet the societal urge to reduce animal experiments, and overcome the differences between rodent and human skin, the call for an extensive *in vitro* skin model is high. Our three-dimensional, *in vitro* cultured human epidermal equivalents (HEEs) faithfully mimic *in vivo* human epidermis and serves as an alternative for animal experiments in skin biology research. HEEs are generated from patient-derived or immortalized keratinocytes which differentiate into a fully stratified epidermis upon culturing at an air-liquid interface. Skin diseases, host-microbe interplay, and therapeutic interventions can be modeled within this interface. Multiparameter analyses include tissue morphology, gene-and protein expression, barrier function, and (spatial) transcriptomics. Our group showed CRISPR/Cas9 genome engineering in difficult-to-transfect immortalized keratinocytes is feasible by generating a full *FLG* knockout. Inflammatory skin disease hallmarks, such as hyperproliferation and aberrant epidermal differentiation in AD and psoriasis were induced via treatment with disease-associated cytokines. To mimic the skin microbiome and study host-microbe interactions and antibiotic interventions, standardized bacterial inoculation of the HEE was recently optimized. Altogether, this toolbox can be used to study the genetical, immunological and microbial components of skin biology, disease pathophysiology, and potential therapeutic approaches.

Keywords: atopic dermatitis; organoid; genome engineering; inflammation; microbiome

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ELECTRICAL IMPEDANCE SPECTROSCOPY QUANTIFIES SKIN BARRIER FUNCTION IN ORGANOTYPIC *IN VITRO* EPIDERMIS MODELS

Noa J.M van den Brink[1,†], Felicitas Pardow[1,2,†], Luca D. Meesters[1,2], Hanna Niehues[1], Ivonne van Vlijmen-Willems[1], Susan H. Roelofs[3] , Matthew G. Brewer[4], Diana Rodijk-Olthuis[1], Jos P.H. Smits[1,5,#], Ellen H. van den Bogaard[1,#,*]

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3D human epidermal equivalents (HEEs) are a state-of-the-art organotypic culture model in pre–clinical investigative dermatology and regulatory toxicology. Here, we investigated the utility of electrical impedance spectroscopy (EIS) for non–invasive measurement of HEE epidermal barrier function. Our setup comprised a custom–made lid fit with 12 electrode pairs aligned on the standard 24–transwell cell culture system. Serial EIS measurements for seven consecutive days did not impact epidermal morphology and readouts showed comparable trends to HEEs measured only once. We determined two frequency ranges in the resulting impedance spectra: a lower frequency range termed EIS^{diff} correlated with keratinocyte terminal differentiation independent of epidermal thickness and a higher frequency range termed EIS^{SC} correlated with stratum corneum thickness. HEEs generated from CRISPR/Cas9 engineered keratinocytes that lack key differentiation genes FLG, TFAP2A, AHR and CLDN1 or confirmed that keratinocyte terminal differentiation is the major parameter defining EIS^{diff}. Exposure to pro–inflammatory psoriasis– or atopic dermatitis–associated cytokine cocktails lowered the expression of keratinocyte differentiation markers and reduced EIS^{diff}. This cytokine–associated decrease in EIS^{diff} was normalized after stimulation with therapeutic molecules. In conclusion, EIS provides a non–invasive system to consecutively and quantitatively assess HEE barrier function and to sensitively and objectively measure barrier development, defects and repair.

Keywords: Human epidermal equivalents; epidermal barrier; skin barrier function; electrical impedance spectroscopy; TEER

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OAT-DERIVED AVENANTHRAMIDES AS ANTI-INFLAMMATORIES IN HUMAN KERATINOCYTES

Alba Díaz-Yuste[1,2], Belén G. Sánchez[1,2], José María Mora-Rodríguez[1,2], Nerea Muñoz-Almagro[3], Alba Sebastián-Martin[1,2], Cristina Julio-González[3], Inés Díaz-Laviada[1,2,4], Oswaldo Hernández-Hernández[3], Alicia Bort[2*]

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The oat (Avena sativa) is one of the most widely consumed whole grains and is well known for its many health benefits. It contains soluble, low molecular weight, phenolic alkaloid compounds named avenanthramides (Avns). They are present in oats at approximately 300 parts per million (ppm) and have been found to exhibit antioxidant activity in various cell types. In this study, we have tested whether avenanthramides exhibit anti-inflammatory effect in human HaCaT keratinocytes. Our results have shown that cells treated with some of the avenanthramides were able to reduce the levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1 beta (IL-1β) secreted into the medium. This effect was more highly observed when an inflammatory state was induced by lipopolysaccharide (LPS). The down-regulating role of Avns on these proinflammatory factors was demonstrated in the levels of messenger RNA, in which we found that avenanthramides significantly reduced their expression. Moreover, in our research we observed that avenanthramides regulated NF-κB signalling in keratinocytes by decreasing the activity of the pathway. Taken together, these results do demonstrate that avenanthramides function as anti-inflammatory agents in human keratinocytes.

Keywords: avenanthramides; inflammation; keratinocytes

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TECHNICAL ADVANCE: BACTERIAL COLONIZATION OF 3D ORGANOTYPIC EPIDERMAL MODELS FOR LONG-TERM HOST-MICROBE INTERACTION AND INTERVENTION STUDIES

Gijs Rikken[1], Luca D. Meesters[1], Patrick A.M. Jansen[1*], Diana Rodijk-Olthuis[1], Ivonne M.J.J. van Vlijmen-Willems[1], Hanna Niehues[1], Peter Oláh[2], Bernhard Homey[2], Joost Schalkwijk[1], Patrick L.J.M. Zeeuwen[1], Ellen H. van den Bogaard[1]

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Following descriptive studies on skin microbiota in health and disease, mechanistic studies on the interplay between skin and microbes are on the rise, for which experimental models are in great demand. Here, we present a novel methodology for microbial colonization of organotypic skin and analysis thereof. An inoculation device ensured a standardized application area on the stratum corneum and a homogenous distribution of bacteria, while preventing infection of the basolateral culture medium even during prolonged co-culture periods for up to two weeks at a specific culture temperature and humidity. Hereby, host-microbe interactions and antibiotic interventions could be studied, revealing diverse host responses to various skin-related bacteria and pathogens. Our methodology is easily transferable to a wide variety of organotypic skin or mucosal models and different microbes at every cell culture facility at low costs. We envision that this study will kick-start skin microbiome studies using human organotypic skin cultures, providing a powerful alternative to experimental animal models in pre-clinical research.

Keywords: co-culture; host-microbe interactions

Acknowledgements: Supported by Dutch Research Council ("Meer Kennis met Minder Dieren"-programme).

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BACTERIA X: STUDYING MICROBE-MICROBE INTERACTION

Mona Fayyazi Jolandan[1], Jos P.H. Smits, Patrick L.J.M. Zeeuwen, Ellen H. van den Bogaard, Patrick A.M. Jansen[1*], Patrick A.M. Jansen

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For microbiome studies, we regularly swab healthy volunteers. During one of these experiments an unexpected outcome was caused by the presence of an unknown bacterial strain. This strain seems to prevent the growth of bacteria from the *Staphylococcus* genus. This serendipity we would like to investigate further.

Investigate the antimicrobial properties this bacterial strain harbors towards other bacteria. We would also want to isolate and identify the antimicrobial factor that is present in this strain. Methods: Various methods were extracted from literature to study microbe-microbe interactions which includes the well diffusion assay. To study the factor that is produced by the strain, we tested multiple culturing conditions with the aim that the unknown factor will be secreted into the culture medium for further analysis.

Using the well diffusion assay, we found that our bacterial strain showed high antimicrobial activity against *Staphylococcus hominis*. Also, other *Staphylococcal species*, *Streptococcus pyogenes* and *Micrococcus luteus* are sensitive. It did not show activity against *Cutibacterium aurimucosum*, *Pseudomonas aeruginosa* and *Candida albicans*. Our efforts to isolate and identify the factor that is responsible for the antimicrobial activity remains unsuccessful.

By accident, we picked up an unknown bacterium that shows activity against a broad spectrum of potentially pathogenic bacteria. Further research is needed to further validate the antimicrobial potential of this bacterial strain.

Keywords: antimicrobial activity, well diffusion assay

Acknowledgements: No acknowledgements

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ADVANCING THE STUDIES OF PHYSIOLOGICAL AND PATHOLOGICAL BONE CONDITIONS BY USING A 3D IN VITRO CELL CULTURE MODEL BASED ON BONE-LIKE BIOMATERIAL AND A PERFUSION BIOREACTOR

Ivana Banicević[1]*, Mia Milosević[1,2], Jelena Petrović[1,2], Ksenia Menshikh[3], Milena Milivojević[4], Milena Stevanović[4], Radmila Janković[5], Andrea Cochis[3], Jasmina Stojkovska[1], Lia Rimondini[3], Bojana Obradovic[1]

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Cell monolayers and animals are still predominately used as traditional models for preclinical anti-tumor drug testing and the investigation of pathological and physiological bone conditions, despite their inherent limitations and inadequacy. Namely, cell monolayers do not recapitulate complex cell microenvironments whereas animals are physiologically different from humans. Three-dimensional (3D) in vitro culture models aim to overcome the shortcomings of traditional models by acknowledging the complex and dynamic cell microenvironment. Our study aimed to develop a 3D in vitro model for bone cell cultivation based on macroporous composite alginate hydrogel scaffolds with incorporated hydroxyapatite particles (2 wt.% alginate, 2 wt.% hydroxyapatite) in conjunction with a perfusion bioreactor ("3D Perfuse", Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia). Scaffolds provide cells with a three-dimensional arrangement and artificial extracellular matrix (ECM) whereas perfusion bioreactor provides efficient mass transport of nutrients and hydrodynamic shear stresses. Murine osteosarcoma cells (K7M2-wt) were used as model cells for imitation of bone tumor osteosarcoma whereas human mesenchymal stem cells were used to imitate healthy bone. Cells were manually seeded onto the scaffolds and then cultivated in perfusion bioreactors for 7 days (medium flow rate 0.27 cm3/min, superficial velocity 40 μm/s). Static cultures served as controls. The 3D in vitro culture model supported both cell types: osteosarcoma cells exhibited the hallmarks of tumor cells (increased proliferation, spontaneous self-assembly into spheroid-like forms, excessive secretion of ECM and pluripotency gene expression), while mesenchymal stem cells were viable and proliferated over the culture time. Moreover, perfusion cultures were superior compared to the controls owing to enhanced mass transport and adequate shear stresses estimated to be up to 5 mPa acting on pore walls. Overall, we envisage broad applications of our 3D in vitro culture model as it was shown to be promising in both bone tumor and tissue engineering.

Keywords: osteosarcoma; tumour engineering; bone tissue engineering; macroporous alginate scaffolds

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Session 2

Biotechnological Production and Assessment of Bioactive Compounds

Chair: Patrick Jansen and Nevena Luković

ENZYMATIC EXTRACTION PROVIDES ARABINOXYLAN FROM WHEAT CHAFF IN HIGHER YIELD AND WITH SUPERIOR PROPERTIES

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Arabinoxylans (AX) are an important group of branched carbohydrate polymers in the outer layer and endosperm of the cell wall of cereals. Previous studies have shown numerous AX health benefits such as the lowering of cholesterol and blood sugar levels, the antioxidant activity, and the strengthening the immune system. The yield and the properties of AX are highly dependent on the choice of extraction technique. Since arabinoxylan is linked to other components in the cell walls, the application of enzymes to enhance the release of AX from plant cells by cell wall disruption has attracted much attention.

In this study, amylase, protease, cellulase, and amyloglucosidase were used for the extraction of AX from wheat chaff, which represents waste from agro-industrial complex. Yield and properties of extracted AXs were determined and compared to those of hydrothermally extracted AX. Results showed that the yield of AX from hydrothermal extraction was 12 mg/g dry matter (DM) while by using individual enzymes or their combinations for enzymatic extraction yield of arabinoxylan was higher and ranged from 16 mg/g DM to 25 mg/g DM. Applied enzymatic extraction affected *in vitro* antioxidant activity of AX; ABTS radical scavenging activity of hydrothermally extracted AX was 23 μmoL TE/g DM while when enzymatic extractions were applied it reached up to 79 μmoL TE/g DM. In comparison to hydrothermally extracted, water and oil holding capacities of AXs from enzymatic extraction were also improved from 3 to 6.97 mg water/g and from 1 to 9 mg oil/g, respectively. Besides being more efficient than hydrothermal, enzymatic extraction of AX from wheat chaff provided polymers of superior properties. This might be the result of highly preserved AX structure allowed by using enzymes under mild conditions, in contrast to hydrothermal extraction where the structure can be damaged under harsher conditions regarding temperatures and pressure.

Keywords: arabinoxylan; enzymatic extraction; antioxidant; functional properties; wheat chaff

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GALACTOMANNAN EXTRACTION AND CHARACTERIZATION FROM CERATONIA SILIQUA SEEDS

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Carob (*Ceratonia siliqua*) is a leguminous plant of Mediterranean origin that consists of two main parts. A pod is rich in polyphenols, predominantly gallic acid, while the seeds are rich in galactomannan. This compound is a soluble carbohydrate of high molecular weight formed by β-D-mannopyranose units linked by (1→4) bonds, with α-D-galactopyranose branches linked by (1→6) bonds. Carob galactomannan is widely used both in food as a thickener and in pharmaceuticals as a carrier of many drugs. Carob flours are sold in different formats including raw flour directly from the seed, flour obtained from the endosperms of the peeled seed, or the galactomannan purified from the latter to obtain an extract with a galactomannan concentration of between 50 - 80 % by weight. In this work, the production of different types of flour from the whole seed (including with and without husk by milling and/or precipitation with absolute ethanol, as well as their characterization using enzymatic kits, gas chromatography, molecular exclusion and SDS-PAGE) have been carried out. The different carob flours differ in their galactomannan concentration from lower to higher as elements such as husk or other impurities are removed during peeling and/or ethanol precipitation. On the other hand, the degree of purity of the galactomannan in these flours does not substantially affect the quality of the carbohydrate, maintaining a mannose/galactose ratio characteristic of this plant between 3-4, as well as a constant molecular size of ~ 1 000 kDa.

The protein from the germ is also maintained in all types of flour obtained in this work. These flours differ from the commercial ones in some additional fractionation steps in the latter, such as the separation of the endosperm from the germ, which makes the product purer and more expensive.

Keywords: carob seed flour; gas chromatography; size exclusion chromatography; SDS-PAGE

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COMPARATIVE ANALYSIS OF BIOFORTIFICATION EFFECTS ON PHENOLIC AND GLUCOSINOLATE PROFILES IN *DIPLOPTAXIS TENUIFOLIA* (L.) DC T&T HYBRID

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Wild rocket, scientifically known as *Diplotaxis tenuifolia* (L.) DC is a leafy green plant from the Brassicaceae family. Native to the Medditerian region, today this perennial plant is cultivated worldwide, owing to the distinctive pungent taste of its deeply lobed green leaves. Beyond their culinary uses, the leaves of wild rocket exhibit potential for pharmaceutical applications due to their rich bioactive compounds profile. These include vitamins, pigments (chlorophyll a and b, carotenoids), phenolics, and glucosinolates (GLSs), all exhibiting strong antioxidant and anti-cancer activities. This study aimed to evaluate the effects of biofortification, employing biostimulant Kelpak and two distinct foliar fertilizers (iron and potassium-enriched) in comparison to a control group, on the phenolic and glucosinolate content in the leaves of wild rocket hybrid T&T F1. Using an ultra-high-performance liquid chromatography (UHPLC) system, coupled with a quadrupole time-of-flight mass spectrometry (Q-ToF-MS), a total of twenty-three plant phenolics was quantified, and the relative content of four glucosinolates was evaluated in 70% methanol extracts. Concerning the overall content of phenolic compounds, treatment involving iron application showed the highest levels at 3549.1 mg/kg of fresh weight (FW), while the control group exhibited the lowest content (2171.4 mg/kg FW). Moreover, the most prominent phenolics included quercetin-3,4'-di-*O*-hexoside-3'-*O*-(6"-sinapoyl)-hexoside (ranging from 527.2 to 651.8 mg/kg FW among groups) and 1,8-dipropoxyanthraquinone (278.3-558.5 mg/kg FW). The control and iron-enriched samples exhibited the presence of kaempferol-3,7,4'-tri-O-hexoside + HCOOH and isorhamnetin-3-O-hexoside-4'-O-gentobioside, in addition to the non-identified sinapic acid hydroxide in the Kelpak treated group. Additionally, the determination of relative GLSs content of glucosativin, glucoerucin, neoglucobrassicin, and DMB-GLS revealed that glucosativin was the dominant compound, with proportions ranging from 79.8% (Kelpak treatments) to 89.1% (iron treatments). In conclusion, selected hybrid biofortification treatments, notably through the use of iron foliar fertilizers, enhanced phenolic and glucosinolate leaf content, highlighting its potential significance in nutrition and medicine.

Keywords: glucosinolates; phenolics; UHPLC-Q-ToF-MS; wild rocket

Acknowledgements: This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant numbers 451-03-47/2023-01/200116 and 451-03-47/2023-01/200054 as well as the Science Fund of the Republic of Serbia, #GRANT No. 7744714.

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PROTEIN-ASSISTED LARGE-SCALE ASSEMBLY AND DIFFERENTIAL PATTERNING OF DNA ORIGAMI

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Nanofabrication has experienced a major boost thanks to the invention of DNA origami. It enables the production and assembly of complex nanoscale structures that could be able to open up completely new functions in biology and beyond. However, the remarkable precision with which these structures can be designed and fabricated is not yet matched by their assembly dynamics, which can be extremely slow, especially when attached to biological templates such as membranes. Here we demonstrate the rapid and controlled formation of DNA origami gates on the order of hundreds of micrometers in just 30 minutes. We utilize active patterning by the Min protein system of Escherichia coli, achieving a remarkable improvement over conventional assembly methods based on passive diffusion. Different patterns such as patches, inverse patches, labyrinths and networks were produced at different scales, the shape and density of the assembled structures were adjusted and analyzed using confocal and atomic force microscopy. The differential positioning achieved by Min-induced diffusiophoresis even enables the introduction of "pseudo-colors", i.e. complex core-shell patterns through the simultaneous structuring of different types of DNA origami. In addition to the targeted functionalization of biological surfaces, this could also be a promising approach for the production of nanomaterials, with potential applications in plasmonics, catalysis and molecular sensor technology.

Keywords: nanofabrication, self-assembly, patterning, atomic force microscopy

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TiO_2-BASED NANOHYBRID MATERIAL WITH ENHANCED ANTIMICROBIAL ACTIVITY

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The growing threat of infectious diseases to human health posed by bio-contaminated surfaces has prompted global efforts to mitigate these risks. The main underlying problem is the establishment of biofilms on surfaces exposed to contaminated environments, which can harbor microbial populations of disease-causing viruses and bacteria, often promoters of outbreaks. Hitherto, available antimicrobial strategies remain ineffective in preventing this biothreat under current demand and health and environmental guidelines, recalling urgent alternative solutions. This study aims to contribute to these mitigation challenges by introducing a novel approach based on the grafting of bioactive compounds on the surface of metal oxide nanoparticles. The viability of this approach is demonstrated through the grafting of Econea® bioactive compound onto the TiO_2 anatase nano-surface, to create an antimicrobial TiO_2-based nanohybrid material. This was achieved using a two-step methodology involving a bifunctional isocyanate-based coupling agent. The grafting of the bioactive compound onto the nanoparticles' surface and the maintenance of their characteristic crystalline structure were confirmed through X-ray diffraction and diffuse reflectance infrared Fourier transform spectra. Morphological properties assessment of the nanohybrid material using scanning electron microscopy revealed spherical nanoparticles with increased agglomeration supported by an organic/polymer matrix. Furthermore, the nanohybrid material hampered the growth of three Gram-positive and four Gram-negative pathogens, significant to human health. The study also provides insights into the morphology and biophysical properties of inactivated methicillin-resistant S. aureus (MRSA) and *V. cholerae* bacteria following treatment with the nanohybrid material. Treatment with the new nanohybrid material resulted in altered cell size, membrane roughness, and a loss of flagella. Synergistic antimicrobial effects from both the biocide and TiO_2 may be involved in this enhanced activity. Our study highlights the potential for tailored nano-supports to serve as a promising antimicrobial strategy with broad-spectrum activity, paving the way for further research and exploration in this field.

Keywords: microbial threats; grafting antimicrobials; nano-anatase; bacterial cell morphology

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PLANT VIRUS NANOPARTICLES GENETICALLY MODIFIED TO PRESENT BIOACTIVE PEPTIDES SIMULTANEOUSLY ENHANCE OSTEOGENESIS AND ANGIOGENESIS

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Bone tissue engineering represents one of the strategies employed to address the ever increasing need for resolving the problem of large bone defects. Both naturally-derived and synthetic hydrogels are being utilized to mimic the microenvironment of cells in vivo and guide them towards the desirable cell fate. However, the lack of appropriate combination of biological cues, present in the natural extracellular matrix, requires further improvement of the hydrogel-based scaffolds. In order to functionalize hydrogels, plant virus nanoparticles (VNPs) have been proposed as an effective addition to the cell-laden 3D hydrogel-based matrices. In a novel approach, presented here, VNPs are used to present high local concentration of cues for simultaneous osteogenesis and angiogenesis.

An osteopontin-derived peptide sequence was presented on the surface of the viral nanoparticles, which were investigated with human bone marrow-derived mesenchymal stem cells (hMSCs) and human umbilical vein endothelial cells (HUVECs). Stem cells were induced to differentiate in the presence of the virus and Alizarin red, Alkaline phosphatase activity and qPCR were used to quantify the extent of differentiation towards osteogenic lineage. At the same time wound healing migration assay, Boyden chamber migration assay and qPCR were used to inspect the angiogenic effect of the viral nanoparticle in combination with HUVECs. Stem cell experimental groups cultivated with 1 ng/µl VNPs showed increase in Alizarin red staining, ALP, and upregulation of osteogenesis related genes, when compared to the groups cultivated without any VNPs. Migration studies showed an increased rate of wound closure and transmembrane migration of HUVECs when VNPs were used at only 1 ng/µl. In addition, qPCR analysis revealed that this concentration of the VNP was enough to cause upregulation of the angiogenesis related genes. The preliminary results showed encouraging results for the usage of biotechnologically obtained nanoparticle in bone tissue engineering to simultaneously stimulate osteogenesis and angiogenesis.

Keywords: bone tissue engineering; plant virus nanoparticles; osteogenesis; angiogenesis

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OPTIMIZATION OF NATURAL DEEP EUTECTIC SOLVENTS (NADES) EXTRACTION BY RESPONSE SURFACE METHODOLOGY FROM SOUR CHERRY (*PRUNUS CERASUS* L.) KERNELS

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Sour cherry kernels are well known as food production by-products with a high potential of phenolic compounds. The main goal of this work was to determine the most influential parameters on NADES extraction and optimization of this process. NADES extraction was applied with N4 solvent (Lactic acid:Glucose=5:1). Optimization was performed with response surface methodology with central composite design. Temperature (50, 60, 70 °C), extraction time (60, 120, 180 min) and S/L radio (10, 20, 30 g NADES/g sample) were independent variables. Total phenolic yield (TP), DPPH, ABTS and FRAP assays were selected responses. Regression coefficients had high values for TP and DPPH (0.9317 and 0.9208, respectively), and the values of lack-of-fit were insignificant ($p > 0.05$) for all responses, except of FRAP assay. For all responses, the most dominant parameter was temperature, where both TP content and antioxidant activity increased with increasing temperature. Only in case of TP, interaction between temperature and time of extraction had significant effect, and quadratic term of S/L ratio had an influential factor on TP. The highest TP (10.27 mg GAE/g) was obtained at the highest levels of independent variables (70 °C, 180 min and 30 g NADES/g sample). In case of antioxidant activity, DPPH assay showed the highest result (12.08 μM TE/g DW) under conditions: 60 °C, 120 min, 30 g NADES/g sample. While the highest value of ABTS (21.10 μM TE/g DW) was obtained at 70 °C, 180 min and 10 g NADES/g sample. The best result for FRAP assay (80.10 μM Fe2+/g) was obtained under extraction conditions: 70 °C, 60 min, 30 g NADES/g sample. Optimized set of NADES conditions was: 70 °C, 161 min, S/L ratio 1:25 m/m. In conclusion, NADES extraction proves effective for cherry kernel application, which are a great source of antioxidant-rich bioactive compounds.

Keywords: NADES extraction; optimization; response surface methodology; central composite design; polyphenols

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MULTI-RESPONSE OPTIMIZATION OF NATURAL DEEP EUTECTIC SOLVENT (NADES) EXTRACTION OF POLYPHENOLS FROM STRAWBERRY TREE FRUIT (*Arbutus unedo* L.)

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Arbutus unedo is very popular due to its high content of phenolic compounds. The main goal of this research was to isolate the phenolic compounds and enhance the antioxidant activity by applying an optimized NADES extraction. Response Surface Methodology in combination with face-centered central composite experimental design was performed with temperature (40-70 °C), extraction times (60-180 min) and L/S ratio (10-30 g NADES/g sample). Obtained responses were total phenolics (TP), total flavonoids (TF), DPPH, FRAP and ABTS assays. Optimization under were applied for two different NADES solvents (N6-Betaine:glycerine:water and N9-Choline:chloride:glycerine). For N6 extraction optimized conditions were: 1:30 m/m S/L ratio, 65 °C, and 150 min, and for N9: 1:20 m/m S/L ratio, 56 °C, and 180 min. The experimental values were in accordance with the predicted values for all responses, and HPLC-DAD was performed to quantify polyphenols from the optimized extracts. High coefficients of determination were obtained for TF (0.999) and DPPH assay (0.926) in N6 extracts, and in N9 extracts for DPPH (0.941) and FRAP (0.920) assays. The linear term of S/L ratio had the main impact on phenolic content and antioxidant activity in the N6 extracts, while linear term of extraction time and quadratic term of S/L ratio were predominant in the N9 extracts. Both solvents showed considerable ability to recover phenolic compounds (N6 TP: 26.47 mg GAE/g; and TF: 8.78 mg CE/g), and antioxidant capacity (N9 DPPH: 35.19 mg TE/g; ABTS: 49.45 mg TE/g). The main compound in N6 extracts was ellagic acid derivative 1 (0.021 mg/g NADES extract), gallic acid (0.042 mg/g NADES extract) dominated in the N9 extracts. Thanks to the successful NADES extraction of bioactive compounds, *A. unedo* was identified as a highly valuable source of TP that could be used in many functional products in the future.

Keywords: *Arbutus unedo* L.; NADES extraction; phenolic compounds; antioxidant activity; RSM optimization

Acknowledgements: This research was supported by the Science Fund of the Republic of Serbia, 7750168, Novel extracts and bioactive compounds from under-utilized resources for high-value applications–BioUtilize and by Croatian Science Foundation through the funding of the Hurdle Technology and 3D Printing for Sustainable Fruit Juice Processing and Preservation project, IP 2019–04-2105.

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CHARACTERIZATION OF ANTHOCYANIN PROFILES IN ACAI, PURPLE CABBAGE AND BLACK RICE

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pH-Sensitive films would serve as indicators for food spoilage, presenting information for the current state of the foods and providing an innovative approach to food safety. Three materials were chosen for preparing the films such as: purple cabbage, which is usually found in European regions, black rice, which is commonly found in Asian regions, and acai which is predominantly prevalent in Brazil. The purpose was to create a solution that transcends geographical limitations, making use of materials available in specific areas, but adaptable and replicable on a global scale. The selection of these diverse materials was based not only on their geographical abundance, but also on their distinct anthocyanin profiles. Anthocyanins are water soluble pigments that are present in various fruits and vegetables. They are responsible for their vibrant red, purple and blue colors. Since anthocyanins are sensitive to pH changes, as the pH value of the food product changes over time, it triggers a distinct change in the color of anthocyanin solutions, thus serving as a reliable indicator for assessing the freshness and quality of the food. The main goal of the work was to study the complex anthocyanin composition of acai, purple cabbage, and black rice. For this purpose, a method using high-performance liquid chromatography with diode array detection and tandem mass spectrometry with positive electrospray ionization (HPLC-DADESI/MSn) with an ion-trap mass analyzer was developed and optimized. This detailed analysis was fundamental in understanding the specific color changes corresponding to variations in pH levels. By comprehending these variations, we are able to determine the precise pH range at which these films change color, therefore indicating different stages of food spoilage. This knowledge provided guidance for adapting the films to suit various food products based on their individual pH characteristics.

Keywords: anthocyanins; HPLC-DADESI/MSn; pH – sensitive films; cyanidin derivatives

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BY-PRODUCTS FROM THE PROCESSING OF HERBS AS SOURCES OF ANTIOXIDANTS

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During the processing of herbs, a significant amount of chopped biomass by-products is left behind, often discarded and underutilized. Since they are important sources of different groups of bioactives like polyphenolics, essential oils or terpenes and structural compounds like lignocellulose, they could be exploited in fermentation by different microorganisms. However, fractionation and extractions could significantly increase the effectiveness and productivity of the valorization process. We examined the possibility of extracting antioxidants from by-products of hoary willowherb (Epilobium spp.), sage (Salvia spp.), and basil (Ocimum spp.), as medicinal herbs with significant pharmaceutical and market value. Extracts of hoary willowherb, sage and basil residues were obtained by a conventional, Soxhlet extraction for polar compounds and supercritical carbon(IV)-oxide extraction as an unconventional extraction method for non-polar compounds. We measured the antioxidant content in the obtained extracts by the ABTS and DPPH spectrophotometric methods. In addition, we measured the total polyphenol content of the extracts by the Folin-Coicalteu method. According to the ABTS method, the antioxidant activity in the sage extracts obtained from Soxhlet extraction is 2.2 times greater than in the extracts obtained from supercritical carbon(IV)-oxide extraction. The antioxidant activity in the basil extracts obtained from Soxhlet extraction is 9.1 times greater than in the extracts obtained from supercritical carbon(IV)-oxide extraction, according to the DPPH method. The total polyphenol content in the hoary willowherb extracts obtained from Soxhlet extraction is 4.6 times greater than in the extracts obtained from supercritical carbon(IV)-oxide extraction. The antioxidant activity is greater in the extracts obtained by Soxhlet extraction than in the extracts obtained with supercritical carbon(IV)-oxide extraction. Because we examined residues from the tea processing industry, the content of easily volatile non-polar components is expected to be lower. The results confirm that valorization of these residues should be focused on the polar components and their microbial biotransformations.

Keywords: hoary willowherb; sage; basil; waste; circular economy

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PLANT FLAVONOID TAXIFOLIN AGAINST THE BIOFILMS OF CANDIDA SPP.

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Resistance to a wide range of antimicrobial drugs is becoming more common, so there is a need to find new ones. A highly resistant biofilm, which is a virulence factor of Candida albicans, is a major challenge in the treatment of infections produced by this species. The strains used in study were *C. albicans* 10/15, *C. albicans* 13/15, *C. albicans* ATCC 10231, *C. albicans* 475/15, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750, *C. krusei* H1/16, and *C. glabrata* 4/6/15. The antimicrobial potential of taxifolin was investigated by the microdilution method. Crystal violet assay was used to determine the potential of this compound to inhibit biofilm formation. In addition, the inhibition of exopolysaccharide (EPS) production in the biofilm matrix was monitored by the Congo red binding assay. Moreover, the cytotoxicity of this flavonoid on selected human cell lines (lung fibroblasts) was examined to establish whether it is selectively toxic to species to Candida species or induces non-selective toxicity in eukaryotic cells (MTT cytotoxicity assay). The antimicrobial potential of taxifolin is the same for all tested strains, MIC value is 0.165 mg/mL, MFC 0.33 mg/mL. The most pronounced antibiofilm effect of taxifolin is exhibited towards C. glabrata 4/6/15 (61.6%), while the percentage of inhibition is not higher than 50% for other strains. Furthermore, this compound showed a good potential on the destruction of the previously formed biofilm of *C. albicans* ATCC 10231 (50.94% inhibition), and *C. albicans* 475/15 (47.55%). Contrary, the effect on the reduction of EPS production in the biofilm matrix of the mentioned strains was weaker (*C. albicans ATCC* 10231 27%, and *C. albicans* 475/15 18%). Importantly, this compound was shown non-toxic possibility, which warrants further testing as a potential antifungal agent. Further studies of this compound are needed to determine its potential to be part of new antifungal therapies.

Keywords: taxifolin; antimicrobial; virulence factors; antibiofilm; Candida.

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ANTIBACTERIAL PROPERTIES AND UHPLC-QTOF-MS ANALYSIS OF KIWANO FRUIT EXOCARP

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In accordance with the growing popularity of circular economy, the main objective of our research was to establish the best extraction method for lyophilized fruit exocarp from kiwano – *Cucumis metuliferus* E. Mey. (Cucurbitaceae) using different variables like extraction time (min), ethanol/water ratio (%), and ultrasonic bath power (%). The yields of the dry extracts varied between 26.37% and 43.98% under different extraction conditions. The best extraction conditions for the highest yield were as follows: EtOH (%): 50; Amplitude (%): 40; Time (min): 30. Furthermore, of 25 different ethanolic and hydro-ethanolic, extracts revealed the presence of the following compounds: trihydroxybenzoic acid pentoside vanillic acid hexoside, hydroxybenzoic acid pentoside, vanillic acid hexosyl-hexoside, dihydroxybenzoic acid, vanillic acid rhamnosy-hexoside, hydroxybenzoic acid hexoside, dihydroxybenzoic acid pentosyl-pentoside, hydroxybenzoic acid, citric and homocitric acid, catalpol, dimethylcitric acid, phenyllactic acid hexoside, phenylethyl hexosyl-hexoside, vanilloloside, decaffeoyl-acteoside, hydroxymethyl-phenyl hexosyl-hexoside, benzyl pentosyl-hexoside and hydroxy-dimethyl-decadienedioic acid hexoside. Antibacterial properties of these extracts were also investigated and the most successful extracts against the PAO1 strain of *P. aeruginosa* had MBC and MIC values of 0.5 mg/mL and 0.25 mg/mL, respectively. Considering the obtained results, our future research will focus on the bioactive properties of the obtained extracts and their incorporation into food matrices, as well as the chemical identification of bioactive compounds.

Keywords: *C. metuliferus* exocarp; ultrasound-assisted extraction; antibacterial activity; phytochemicals

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USE OF ORANGE PEEL FOR THE PRODUCTION OF GALACTURONIC ACID

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In a circular economy, resources are kept within a closed-loop system, aiming to maximize their use, minimize waste production, extend material lifespan, and foster sustainable practices. In recent years, the food and animal feed industry has adopted various strategies to convert agri-food waste and by-products into high-value ingredients. The citrus processing industry holds significant importance for European agriculture. However, the environmental and economic impact of waste, particularly orange peel, raises concerns. Adhering to the principles of the circular economy, these residues are repurposed for extracting functional ingredients like antioxidants and fiber. Moreover, they are bioconverted into specific compounds, reducing the reliance on more aggressive processes.

This study aimed to employ a bioconversion strategy for producing galacturonic acid (GalA) from citrus waste, the primary monomer in pectin's structure with numerous industrial applications. Two commercial pectins and an orange by-product were utilized. Physicochemical and structural characterization, along with an assessment of the biological activity of the first two pectins, were conducted.

GalA production involved enzymatic hydrolysis. Enzymes exhibiting the highest polygalacturonase activity were selected, and incubations of the three samples were performed under varying conditions of substrate concentration, enzyme activity, and time. The enzyme Pectinex® Ultra SP-L yielded the highest amount of GalA, achieved after 2 hours for the commercial pectins and 8 hours for the orange by-product, using the highest substrate ratio and the lowest enzyme ratio. Subsequently, these conditions were replicated, and the monomeric composition was analyzed before and after hydrolysis, revealing a significant increase in GalA concentration in all cases.

In conclusion, utilizing citrus by-products and/or residues proves to be an effective means of obtaining galacturonic acid through sustainable bioconversion.

Keywords: Galacturonic acid; circular economy; orange peel

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ENZYMATIC EXTRACTION OF PECTIN FROM TOMATO PEELS AND SEEDS. OPTIMISATION AND CHARACTERISATION

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Tomato (*Lycopersicum esculentum* L.) is one of the world's most widely produced vegetables (200 million tonnes per year). The tomato processing industry produces large quantities of waste such as peels and seeds. The need to reduce the amount of these wastes has led to the emergence of new strategies for the utilisation of these wastes, which are now considered as by-products. Some of these strategies focus on obtaining functional compounds of interest such as pectins, a complex group of polysaccharides present in the plant cell wall of fruits and vegetables. They are composed of three domains:i) homogalacturonan, a linear chain of D-galacturonic acid (GalA), with α-(1,4) bonds, partially methylesterified at C-6; ii) rhamnogalacturonan I (RG-I), a chain of GalA and L-rhamnose, with α-(1, 2) and α-(1, 4) bonds and branches of D-galactose and/or L-arabinose, linked to rhamnose; and, iii) RG-II, a very complex, conserved and minor domain, consisting of a chain of HG with branches formed up to 12 different sugars. This work has focused on the optimisation of the extraction of pectin from the industrial waste peels and seeds of tomato using the commercial cellulase Celluclast®1.5L based on an experimental design (DisExp) further analysed by artificial neural networks (ANN). The independent variables studied in the 17 trials were Celluclast (45-250 U/g), extraction time (6-24 h) and solids concentration (2-7%). The dependent variables were extraction yield, GalA, and glucose content that was considered as impurity. The extracted pectins were subsequently characterised by determining molecular mass (HPSEC-ELSD), monomer composition (GC-FID), and degree of methyl esterification (FT-IR). The variables studied in the DisExp presented the following ranges of values: yield 3.0-6.4%, GalA 54.6-64.6% and glucose 2.2-6.1%. In the modelling, the regression coefficients obtained by ANN were higher (R2 and R2adj > 0.87) than those presented by RSM (R2 and R2adj > 0.63). The optimal conditions for maximising yield and GalA content, while minimising glucose, were 6 h, 42.9 U/g and a substrate concentration of 1.9%. Under these conditions, 6.4% pectin was obtained, with 62.1% GalA and 4.4% glucose, close to the theoretical optimum values of the ANN model, yield 7.1%, GalA 65.6% and glucose 3.1%.

Keywords: Pectin extraction; ANN; Celluclast®1.5L; tomato by-products

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SEQUENTIAL EXTRACTION OF PHYTOCHEMICAL COMPOUNDS AND PECTIN FROM ARTICHOKE BY-PRODUCTS

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According to general data, consumption of fresh and processed fruits and vegetables is growing. Among the vegetables, artichoke (*Cynara cardunculus* L. var. *scolymus*) has generated a great interest. Spain is the world's third largest producer (200 million tonnes/year) of artichoke, having doubled production in the last 10 years. Approximately, half of the artichoke production is consumed in conserve, leaving the bracts and outer stems as wastes that can be used for the extraction of polyphenols, inulin or pectin, or as a source of energy. Furthermore, to our knowledge, there are no studies on the sequential use of these by-products to obtain bioactive compounds such as phytochemicals and pectin. For this reason, the present work focused on the selection of the most favorable method for the extraction of phytochemicals before obtaining pectin with citric acid. Four different devices were used for the extraction of phytochemicals with 70% ethanol as food grade extractant, at 50 °C for 30 min: internal plate agitation (500 rpm), high- speed homogeniser (Ultraturrax, IKA, 4000 rpm), ultrasound probe (20 kHz, 450 Digital Sonifier Branson, 400 W, 6 mm tip, 30% amplitude, on/off 2/1 s) and ultrasonic bath (45 kHz, Sonica Sweep System, power density 0.17 W/cm3). Then, phytochemical-enriched extracts (FCEx) and pectins were characterised. In the FCEx, as bioactive compounds, total phenols were quantified with values between 98.6 to 119.1 mg/g of lyophilised extract (LEx), total flavonoids from 13.9 to 17.0 mg/g of LEx, tannins from 4.2 to 7.0 mg/g of LEx and vitamin C from 0.54 to 0.60 mg/g of LEx. In addition, antioxidant activity was determined by the ABTS method (0.68 to 0.79 mmoles Trolox/g LEx) and the DPPH method (1.08 to 1.27 mmoles Trolox/g LEx). The total content of low molecular weight carbohydrates (fructose, glucose, kestose, nystose and myo-inositol) ranged from 93 to 137 mg/g LEx. In general, there were no significant differences in any of the parameters determined for the different phytochemical extraction methods, except for the quantification of carbohydrates. Regarding the yields, the highest yields were obtained with the plate agitation (19.1%) and the high-speed homogeniser (19.6%), so these methods were selected as a previous step to obtain pectin with citric acid (3.5%, 95 ºC, 30 min) from the solid residues resulting after the ethanolic extraction. In this study functional extracts rich in phytochemicals and sugars were obtained sequentially and efficiently, and pectin, from artichoke by-products, following a more sustainable methodology than the traditionally used for pectin extraction.

Keywords: artichoke, pectin, phytochemicals, citric acid; antioxidant activity

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EVALUATION OF BIOLOGICAL ACTIVITY OF ENZYMATICALLY SYNTHESIZED PHLORIDZIN OLIGOMERS

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Dihydrochalcones are a class of flavonoids found in apple trees (*Malus* sp) generally present in high levels in young leaves and immature fruits. Phloridzin is a member of the dihydrochalcones which possess good antioxidant activity, as well as a protective role against UV radiation, hence it is a constituent of various commercial cosmetic preparations. These natural low-molecular weight phenolic compounds with diverse physicochemical characteristics are substrates for laccases, copper-containing polyphenol oxidases whose activity can yield compounds that vary in structure and biological activities. The positive effect of laccase-catalyzed polymerization of flavonoids on the functionality of produced oligomers or polymers has been already shown on some flavonoids' representatives.

In this work, fungal laccase from *Trametes versicolor* was applied as a biocatalyst in the reaction of phloridzin oligomerization. Synthesized phloridzin oligomers were separated from the reaction mixture and lyophilized in order to test their antioxidant and antimicrobial activity. Different antioxidant assays, such as ABTS (2,2'-azino-bis(3-ethylbenzenothiazoline-6-sulfonic acid) diammonium salt) and DPPH (2,2-diphenyl-1-picrylhydrazyl) showed that in comparison with the monomer, synthesized oligomers had the weaker ability to scavenge the free $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals, since for both assays, examined IC50 values of phloridzin oligomers (0.46 mg/ml and 1.52 mg/ml, respectively) was higher than the values for parent molecule (0.13 mg/ml and 1.07 mg/ml, respectively). This could be ascribed to the fact that some of the functional groups responsible for the antioxidant activity probably participate in the formation of linkage between phloridzin units in the structure of oligomers. On the other hand, synthesized phloridzin oligomers demonstrated a significantly higher antimicrobial effect on the Gram-positive bacteria *Staphylococcus aureus* compared to the parent molecule phloridzin. Moreover, the greater inhibition of bacterial growth was achieved by increasing the oligomers' concentration. Given the obtained results, synthesized compounds show potential to be further examined as dermal prebiotics.

Keywords: phloridzin**;** phloridzin oligomers; laccase; antioxidant activity; antimicrobial activity

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EFFECT OF THE PHYSICAL STATE OF BROCCOLI WASTE ON THE EXTRACTION OF PECTIN AND PHYTOCHEMICAL COMPOUNDS

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Spain is the fifth worldwide broccoli producer, increasing tenfold the production in the last 10 years, so in the same way waste has also increased. Broccoli byproducts have been studied as a source of polyphenols, but rarely as a pectin one. Moreover, to the best of our knowledge, there are no studies on the integral use of these by-products, neither on pectin extraction from fresh broccoli, in such a way that previous drying, an energetically expensive process, is needed.

Therefore, the present work focused on sequential extraction of pectin and phytochemicals, and its posterior characterization, from dried broccoli (DB) and fresh broccoli (FB) by-products. First of all, own broccoli enzymes were inactivated by blanching at 95 ºC during 5 min. For pectin extraction, cellulase Cellic® (0.3 mL/g total dry matter) was used in a 5% solid concentration.

With regard to pectin extraction, we obtained pectin yields of 5.8% (DB) and 4.9% (FB). Pectins extracted had a galacturonic acid content (GalA) of 25.0% for pectin extracted from DB and 35.2% for pectin extracted from FB, so the compound extracted is not pectin but pectic polysaccharides with a high neutral monosaccharides proportion. Monomeric relation GalA:rhamnose:arabinose:galactose:xilose:manose for both pectic polysaccharides was 1:0.31:0.70:0.94:0.06:0.50 for DB and 1:0.28:0.33:0.61:0.10:0.25 for FB. Apart from that, glucose content, which could come from cellulose, was 9.4% for polysaccharides extracted from DB and 12.3% for polysaccharides extracted from FB. Furthermore, phytochemical-rich extracts were characterized by determining total polyphenol content and antioxidant activity (DPPH and ABTS methods).

In this study, pectin and an extract rich in phytochemicals have been efficiently obtained from broccoli by-products following a sequential methodology, with special relevance of results of fresh broccoli extraction, that shows that previous drying could be suppressed, in line with the principles of sustainable development.

Keywords: fresh broccoli; freeze-dried broccoli; integral use; sequential extraction; antioxidant activity

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PREBIOTIC POTENTIAL OF PHENOLIC COMPOUNDS FOR MYCOBIOTA MODULATION

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Traditionally used medicinal herbs, such as yarrow (lat. *Achillea millefolium*), represent a source of diverse phenolic compounds. The prebiotic potential of phenolic compounds has been recently acknowledged, as their beneficial effects are mediated through interactions with the gut microbiota. However, the bidirectional relationship between phenolic compounds and mycobiota remains underresearched. This study aimed to investigate the effects of phenolic compounds, identified in yarrow extracts, on the growth of the probiotic yeast *Saccharomyces boulardii*, *Saccharomyces cerevisiae* and the opportunistic pathogen *Candida albicans*.

Effects were tested by the addition of commercially available phenolic acids and polyphenols to tryptone soy broth (TSB) in a concentration range, followed by the broth dilution method and inoculation on tryptone soy agar (TSA) or *HiCrome™ Candida* differential plates. Growth stimulatory activity was assessed as a ratio between the number of colony-forming units (CFU/ml) in the samples and pure TSB controls, with experiments performed at least in duplicate (biological replicate). The results indicate that several tested phenolic compounds stimulated *S. boulardii* growth and inhibited the growth of *C. albicans*. Effects on *S. boulardii* were dose-dependent, with lower concentrations of polyphenols (≤12 μg/mL) exhibiting a higher degree of stimulation. Synergistic effects on *S. boulardii* growth were observed after the addition of several phenolic combinations, with almost complete inhibition of *C. albicans* growth. Additionally, phenolic compounds exerted mixed effects on the growth of *S. cerevisiae*, highlighting the strain-specific differences in the effects of polyphenols.

To our knowledge, this is the first time that the stimulatory activity of phenolic compounds on the growth of *S. boulardii* was demonstrated, along with the antimicrobial effects towards *C. albicans*, confirming the great prebiotic potential of phenolic compounds identified in yarrow in restoring the mycobiota balance. The results indicate the possibility of formulating prebiotic or synbiotic preparations which would be used for mycobiota modulation.

Keywords: phenolic compounds; mycobiota; prebiotics

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DISCOVERING POTENTIAL OF POLYPHENOL COMPOUNDS FROM BLUEBERRY, CRANBERRY AND CHOKEBERRY EXTRACTS AS SKIN PREBIOTICS

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Berries are known to be one of the richest sources of polyphenols which can offer various health benefits. Dietary supplementation with berries has a positive influence on the gut microbiota, which directly affects overall health, including skin health. However, topical application of berry polyphenols has been used mainly for its antioxidant activity to prevent premature aging and improve the skin's appearance. Therefore, this study examined the content of different polyphenol classes of cranberry (*Vaccinium macrocarpa*), chokeberry (*Aronia melanocarpa*) and blueberry (*Vaccinium angustifolium*) extracts, investigating their antioxidant properties and potential impact on skin as topical prebiotics. The prebiotic capacity of these extracts in applied concentrations range of 0.015-0.05 mg GAE/mL, was determined against two cutaneous bacteria - beneficial *Staphylococcus epidermidis* and opportunistic pathogen *Staphylococcus aureus*, since the disrupted balance between them may contribute worsening of atopic dermatitis. The obtained results showed that the total polyphenol content was highest in chokeberry extract (9.88 mg GAE/g DM), followed by cranberry extract (8.78 mg GAE/g DM), and the lowest in blueberry extract (6.48 mg GAE/g DM). Chokeberry extract was also richest in flavonoids, flavonols, anthocyanins and phenolic acids. Notably, cranberry extract had the highest concentration of tannins, almost three times higher compared to blueberry extract. According to DPPH and ABTS methods, the antioxidant activity was significantly high in chokeberry extract, while FRAP method revealed that cranberry extract is the most potent antioxidant. Regarding prebiotic capacity, positive values (0.10-0.48) were observed at all concentrations of cranberry extract, with a trend indicating a decrease in prebiotic capacity as polyphenol concentration increases. Prebiotic capacities of blueberry and chokeberry extracts had either negative values or values equal to zero, indicating that these extracts do not positively impact the microorganisms' ratio. Obtained findings suggest cranberry extract potential for enhancing both antioxidant defense of skin and rebalancing cutaneous microbiota.

Keywords: cranberry extract; chokeberry extract; blueberry extract; antioxidants; skin microbiota

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IN VITRO RELEASE AND (TRANS)DERMAL DELIVERY STUDY OF BLACKCURRANT ANTHOCYANINS INCORPORATED IN COSMETIC FORMULATION

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Blackcurrant is anthocyanin-rich berry with proven antioxidant and photoprotective activity and emerging prebiotic potential, widely applied in cosmetic products. Although beneficial effects of its extract are well known, release from skin care products and (trans)dermal permeation were not previously investigated.

In this study, enzyme-assisted extraction of polyphenols from lyophilized blackcurrant was performed and obtained extract was incorporated into an Aristoflex® AVC based hydrogel. Furthermore, Franz diffusion cell experiments were conducted in order to examine the release of these molecules from the prepared formulation using cellulose acetate membrane and transdermal and dermal delivery using human skin mimicking membrane (Strat® M).

Obtained results revealed that all four dominant anthocyanins readily permeated from hydrogel since 17.5%, 32.8%, 33.8% and 39.2% of delphinidin 3-glucoside, delphinidin 3-rutinoside, cyanidin 3-glucoside and cyanidin 3-rutinoside, respectively, were detected in receptor fluid after 24 h. Experimental values were successfully fitted with the Peppas and Sahlin diffusion model. On the other hand, after 72 h long experiment with transdermal skin diffusion model (Strat® M membrane), no detectable amounts of anthocyanins were present in receptor fluid and only 0.5% of the initial quantity from donor compartment was extracted from the membrane itself, indicating weak dermal delivery.

Present study revealed that hydrogel is suitable carrier system for the topical delivery of bioactive anthocyanins from blackcurrant, while dermal and transdermal delivery of these molecules is very limited. This implies applicability of blackcurrant extract for treatments targeting skin surface (i.e. antioxidant, prebiotic, photoprotective).

Keywords: blackcurrant; anthocyanins; hydrogel; diffusion

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SUGAR FUNCTIONALIZED SUPERPARAMAGNETIC NANOPARTICLES FOR CAPTURING OF CANCER CELLS IN LIQUID BIOPSY

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Liquid biopsy is a promising and minimally invasive procedure for the detection, staging and study of cancer and cancer metastasis. This method takes advantage of the fact that several different cells and cell products derived from the primary tumor and different metastatic sites can be found in patient blood samples. Upon isolation and analysis of such derivatives like circulating tumor cells (CTCs) new and complementary therapeutic and scientific insights into the course of the disease can be obtained. A major challenge, however, is the very low number of tumor cells in the blood. Therefore, it is essential to enrich them before further analysis. We have developed superparamagnetic iron oxide nanoparticles (SPIONs) bio-functionalized with sugar, aiming for a specific enrichment in cancer cells using the Warburg effect and subsequent magnetic separation of labelled cells. The particles, synthesized in the organic solvent, were transferred into the aqueous phase using an amphiphilic polymer and consequently conjugated via carbodiimide-cosslinking with maltose or glucose. Enrichment of the SPIONs in cells was measured using inductively coupled plasma mass spectrometry (ICP-MS) and labelled cells were magnetically retained using a magnetic column. The produced particles showed non-toxic behavior and an improved cellular uptake. Cancerous cells could be retained depending on their internalized amount of SPIONs with an efficiency up to 100%. Enrichment among non-labelled cells was analyzed using flow cytometry and could be shown even at a ratio of one target cell in 1000 non-labelled cells. Sugar-functionalized magnetic nanoparticles have therefore shown a great potential towards separation of cancerous cells in blood samples and need to be further investigated by competitive studies including non-cancerous cell lines and patient blood samples.

Keywords: liquid biopsy; nanoparticles; magnetic separation; nanomedicine

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Session 3

Enzyme Engineering and Immobilization

Chair: Jose Migel Palomo

DESIGN OF CANDIDA RUGOSA LIPASE HYBRID NANOFLOWERS AND VALORIZATION OF THEIR POTENTIAL USE

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Lipases from *Candida rugosa* (CRL) are renowned industrial biocatalysts in fats and oils processing, biosynthesis of ester-based nutraceuticals, pharmaceuticals and biofuels. Nevertheless, the full exploitation of CRL biocatalytic potential is hampered by their low stability in industrial reaction settings. Enzyme immobilization is an essential technology for commercializing biocatalysis, encompassing their stabilization and reusability for an efficient application. Organic-inorganic enzyme hybrid nanoflowers are unique class of nanobioatalysts with distinguished morphology and versatile biotechnological applications. This study reports a cost-effective and environmentally friendly method for production of CRL nanoflowers (CRL-NF) and evaluation of their applicative potential. In the first phase of our research critical reaction parameters were optimized: metal type (calcium or copper), CRL and metal concentration, incubation time, pH and reaction temperature. When calcium was used as inorganic component, significantly higher CRL encapsulation yield was achieved, compared to copper-containing CRL-NF (95% and 75.7%, respectively). Likewise, activity yield of calcium-containing CRL-NF was two times higher than for their copper-containing counterparts (40.3% and 20.2%, respectively). Accordingly, calcium was selected as inorganic component for the design of CRL-NF in all further experiments. Hydrolytic activity of obtained CRL-NF with calcium was completely preserved as confirmed via in gel digestion of *p*-nitrophenyl palmitate and olive oil. Most importantly, immobilization has resulted in significant thermaland pH stabilization of CRL. Obtained CRL-NF could be effectively reused in 4 consecutive cycles of *p*-nitrophenyl palmitate hydrolysis. Thus, our preliminary data indicate the promising applicative potential of calcium-containing CRL-NF in food industry and set a solid base for further design improvement of these enticing biocatalysts.

Keywords: enzyme immobilization; enzyme hybrid nanoflowers; nanobiocatalysts; food biotechnology

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IMMOBILIZATION OF XYLANASE ON MAGNETIC NANOPARTICLES MODIFIED WITH POLYETHYLENEIMINE AND ITS APPLICATION IN XYLO-OLIGOSACCHARIDES SYNTHESIS

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A food-grade bacterial xylanase enzyme preparation, ROHALASE® SEP-VISCO, was immobilized by adsorption onto polyethyleneimine (PEI) functionalized magnetic nanoparticles (MNPs). This process resulted in nanobiocatalysts with optimal characteristics. Subsequently, this nanobiocatalyst was employed to produce xylo-oligosaccharides (XOS). In the past decade, magnetic nanoparticles (MNPs) have gained significant interest, finding diverse applications in biomedicine, biosensor production, food processing, catalysis, agriculture, and environmental processes. MNPs have proven highly effective for enzyme immobilization because of their unique and distinguished properties such as high specific surface area, biocompatibility, and magnetic characteristics. Magnetic characteristics facilitate the easy removal of MNPs from reaction mixtures, allowing for their efficient reuse. The functionalization of MNPs through with the cationic polymer PEI can further augment their ability for enzyme attachment. Generally, xylanases hydrolyses β-1,4-glycosidic linkages present in xylan which is a major component of the hemicellulosic fraction in the plant cell walls. Furthermore, xylanases can produce XOS composed of xylose units linked by *β*-1,4-xylosidic bonds. XOS have stimulatory effects on the selective growth of human intestinal microbiota and are frequently defined as prebiotics.

In the present work, the xylanase immobilization efficiency on MNPs-PEI is between 100 and 43 % within the wide range of xylanase concentrations (400-2400 mg/g of support). Free and immobilized xylanase showed maximal catalytic activity at pH 6.0 and 60°C in reaction with commercial birchwood xylan (concentration of 1 % w/v). The maximum activity of 1675 IU/g of support was achieved when immobilization was performed at initial enzyme concentration of 1250 mg/g of support during 2 h. Since this immobilized preparation exhibited the activity immobilization yield of 80 % and specific activity of 2.1 mg of proteins/g of support, it has been applied in reaction of XOS synthesis. The MNPs-PEI-xylanase was found to produce high yield of XOS from birchwood xylan, indicating its potential for utilization in feed and food formulations.

Keywords: xylanase; xylo-oligosaccharides; magnetic nanoparticles; immobilization of enzymes; prebiotics

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STUDY AND PREPARATION OF ARTIFICIAL MANGANESE METALLOENZYMES WITH LACCASE LIKE ACTIVITY

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Laccase (EC 1.10.3.2, p-diphenol: dioxygen oxidoreductase) is a blue copper oxidase of great industrial interest due to its ability to catalyseoxidation processes of phenols and persistent organic pollutants. However, it is susceptible to denaturation by high temperatures, and sensitive to pH and the presence of high concentrations of solvents, which is a problem for industrial use. To solve this problem, this project develops the synthesis in aqueous medium of a new Mn metalloenzyme with laccase oxidase mimetic catalytic activity. To do this, *Geobacillus thermocatenulatus* lipase (GTL) is used as a "scaffold" enzyme, which is mixed with a manganese salt at 50 ºC in an aqueous medium. In this way, the *in situ* formation of manganese (IV) oxide nanowires is generated, interacting with the enzyme and obtaining the GTL-Mn bioconjugate. On the other hand, its oxidative activity was evaluated using the ABTS assay, obtaining a specificity 300 times greater than the laccase from *Trametes versicolor* and 2 times more than the laccase from *Myceliophthora thermophila* expressed in *Aspergillus oryzae* (Novozym 51003®). In addition, the new metalloenzyme turned out to be 2 times more stable at 40 ºC, 3 times more stable in thepresence of 10% can, and 10 times more stable at 20% and 30% AcN than laccase (Novozym 51003®) by evaluating it at 2 hour incubation. Moreover, it was shown that the use of immobilization strategies improves the stabilization of this artificial metalloenzyme two times more. Finally, the novel Mn metalloenzymes were effective in the reaction of phlorizin oligomerization. Future research will aim to extend this study for the use of flavonoids as prebiotic molecules in the cosmetics industry.

Keywords: metalloenzyme; Mn; laccase like activity; oxidant

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MECHANOCHEMICAL SYNTHESIS OF ZN BIONANOHYBRIDS WITH ESTERASE-LIKE AND CATALASE-LIKE ACTIVITY

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The mechanochemical synthesis of nanomaterials for catalytic applications is a growing research field due to its simplicity, scalability, and eco-friendliness. In this work, we have developed the synthesis of new zinc bionanohybrids by a mechanochemical method that can improve the enzyme-like activities of the catalysts synthesized in aqueous medium. For this purpose, Candida antarctica Lipase B (CAL-B) enzyme was used as a scaffold, which is added to a zinc salt using a neutral-alkaline medium of 0.1M sodium phosphate (pH 7) or 0.1M sodium bicarbonate (pH 10) at room temperature by means of a "ball milling technology" method. The catalytic performance of the novel Zn@BIC bionanohybrids was evaluated in the p-nitrophenyl pamlitate (p-NPP) hydrolysis with esterase-like activity. On the other hand, the oxidative activity of Zn@PHOS bionanohybrids was studied in the degradation of hydrogen peroxide for their evaluation as mimetics of catalase activity, obtaining a value 12 times greater than for the catalyst synthesized in aqueous medium.

Keywords: mechanochemistry; ball-milling; zinc bionanohybrids; esterase-like activity; catalase-like activity

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NEW LIFE OF WASTE MATERIAL: IMMOBILIZED HORSERADISH PEROXIDASE FOR DEGRADATION OF ANTRAQUINONE DYE

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Various strategies have been developed for the removal of synthetic dyes from the wastewater of the textile industry due to their stability as well as their negative effect on the environment. Enzymatic wastewater treatments as environmentally safe processes have many benefits. An enzyme suitable for use in the treatment of wastewater from the textile industry is horseradish peroxidase (HRP) because it can degrade different types of dyes. Immobilized enzymes have many advantages over free enzymes in solution such as higher stability and the opportunity to be removed easily from the reaction mixtures. Consequently, immobilized enzymes can be used more than once. Many different materials are used as a carrier for enzyme immobilization, so if residual material is used as a carrier, waste valorization will be achieved. In this study immobilized commercial HRP was used for decolorization of anthraquinone dye Acid green 40 (AG 40). Waste material – potato peels were used as a carrier for the immobilization of HRP. Potato peels were oxidized with sodium periodate to introduce aldehyde groups that can react with the primary amino groups of the enzyme to form Schiff bases, resulting in covalently immobilized HRP. The success of the oxidation was verified by the 2,4-dinitrophenylhydrazine (DNPH) test, and subsequently, the oxidized potato peels were characterized by SEM and FTIR. After oxidation, the influence of the mass of the immobilization carrier on enzyme activity and immobilization efficiency was investigated. The optimal conditions of pH, hydrogen peroxide concentration, concentration of AG 40, and enzyme activity were determined for decolorization of AG 40 with immobilized HRP. Adsorption of AG 40 on the carrier was also determined. In addition, the operational stability of the enzyme was determined. The results obtained show that oxidized potato peels are a promising material that can be used as a carrier for the immobilization of HRP.

Keywords: potato peel; horseradish peroxidase; immobilization; anthraquinone dye; Acid green 40

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DETERMINING THE POTENTIAL OF SUBMERGED FERMENTATION ON WHEAT BRAN FOR PRODUCTION OF XYLANASE

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Utilization of agro-industrial byproducts represents a way to broaden the palette of products derived from the less attractive and usually cheaper sources. The underutilized agro-industrial byproducts have attracted attention in recent years due to their effect on the environment. Wheat bran (WB) represents a byproduct of wheat industry and is a good source of fiber. Valorization of industry byproducts leads to reduction in production cost of enzymes due to high expenditure of expensive substrates required for microbial growth during the production process. Hydrolytic enzyme xylanase which transforms xylan into xylose is possible to produce using various microbial strains.

Production of xylanase was investigated *via* submerged fermentation by *Penicillium chrysogenum* using a defined fermentation broth supplemented with adequate amount of WB (1 and 2.5% (*w/v*)). Submerged fermentation was carried out in a shaking incubator (100 rpm, 30 °C, n=2) over the course of 10 days. Crude enzyme samples were investigated each day for xylanase enzyme activity via 3,5-dinitrosalicylic acid (DNS) assay for reducing sugars using 1% (*w/v*) beechwood xylan solution in 50 mM Sodium Citrate buffer, pH 5.0 at 37 °C. Maximum activity was achieved on the 4th (1% (*w/v*) WB) and 5th (2.5% (*w/v*) WB) day of fermentation, 3.93±0.31 IU/ml and 4.48±0.067 IU/ml, respectively. pH (4–10) and temperature (25–60 °C) optima were determined *via* DNS method, and the optimal conditions were determined to be pH 5.0–5.5 and 37 °C. The influence of the addition of different metal ions and reagents on the enzyme activity was determined. The most beneficial effect was noted for the addition of $MnCl_2$ and dithiothreitol. The increase of enzyme activities recorded was 22–29% for $MnCl_2$ and 53–59% for dithiothreitol. It was concluded that *P. chrysogenum* can be further exploited in an enzyme production optimization process to increase xylanase activity.

Keywords: xylanase; wheat bran; submerged fermentation; enzyme; valorization

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NOVEL Cu/Ag-ENZYME BIOHYBRIDS AS ARTIFICIAL METALLOENZYME

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In recent years, the synthesis of novel artificial metalloenzymes by the combination of metal or complex organometallic systems and enzymes is rapidly growing field of research in catalytic applications. In this study, new artificial metalloenzymes have been designed and developed based on the combination of an enzyme and different metal salts. For this purpose, novel synthesis method has been used, where the enzyme, Lipase B from *Candida antarctica* (CALB), acts as an inducer of metal nanoparticles of copper and silver. Obtained biohybrids show improved stability properties compared to natural enzymes. Moreover, the synergetic effect between copper and silver has been evaluated, looking for an improvement in the catalytic efficiency of an only copper-metalloenzyme previously developed.

The copper-silver-CALB metalloenzymes have been characterized by X-Ray diffraction analysis, mass spectrometry, and SEM and TEM as microscopies techniques. After that, their catalytic efficiency was tested in different reactions, in particular in the phloridzin oligomerization as laccase-like activity.

Keywords: metalloenzymes; copper; silver; phloridzin; lacasse-like activity

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INFLUENCE OF DIFFERENT IMMOBILIZATION TECHNIQUES ON IMMOBILIZED LACCASE ACTIVITY

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In this work, immobilization of laccase from Trametes versicolor using different immobilization techniques was investigated and obtained results regarding immobilized enzyme activity were compared. The enzyme was successfully immobilized onto different nanoparticles such as magnetic (MNPs) as well as cellulose (CNP) and silica nanoparticles (SNP). Functionalized MNPs were prepared by co-precipitation of Fe2+ and Fe3+ ions, which were initially coated with a layer of citric acid to prevent particle agglomeration. Further, they were coated with sodium silicate and functionalized with aminosilane. MNPs were activated with the crosslinking reagent glutaraldehyde and laccase was immobilized onto functionalized MNPs. On the other hand, CNPs were prepared by ultrasonic treatment of microcellulose while the SNPs were obtained from rice. Functionalization of these nanoparticles was performed by introducing amino groups onto their surface, by modification of CNPs with poly(ethyleneimine) and with organosilane in case of SNPs. Additionally, the immobilized laccase in the form of cross-linked enzyme aggregates (Lac-CLEAs) was prepared by simple immobilization method involving precipitation of the enzyme from aqueous buffer using ethanol as precipitation solvent followed by cross-linking of aggregates of enzyme molecules by glutaraldehyde. When the magnetic cross-linked enzyme aggregates from laccase (Lac-mCLEAs) were synthesized, magnetic nanoparticles were added to the synthesis process. The immobilization yield of laccase immobilized onto nanoparticles was 95%, 83% and 84% for MNPs, CNPs and SNPs, respectively, followed by activity yield of 77%, 73% and 66%, respectively. Some lower immobilization yield was achieved for the Lac-CLEAs and Lac-mCLEAs, 74% and 75%, respectively. Lac-mCLEAs showed the highest activity yield (85%) while activity yield of 76% was achieved for Lac-CLEAs when BSA, as proteic feeder was used. Obtained results suggest that laccase could be successfully immobilized using different supports and immobilization techniques.

Keywords: laccase; immobilization; nanoparticles; cross-linked enzyme aggregates

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Session 4

Environmental Biotechnology

Chair: Maja Đolić

POSSIBILITY OF BIODEGRADATION OF COTTON MEMBRANE CONTAINING TEMPO RADICAL AND CITRIC ACID

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Cotton-based membranes, due to their exceptional biocompatibility and sustainability, have attracted considerable attention in various applications, especially in the field of bio and green technologies. This study investigates the biodegradation potential of cotton membranes modified with TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) radical and citric acid (CA), with the aim of improving their properties and facilitating environmentally friendly disposal. TEMPO radicals, in conjunction with the crosslinker citric acid, are incorporated into the cellulose structure through a novel modification process. The citric acid component acted as a plasticizer, increasing the amorphous parts of the cellulose and promoting enzymatic attack. The TEMPO radical, with its nitroxyl group, contributed to the oxidation of cellulose, further facilitating biodegradation.

The biodegradation aspect of these modified membranes was investigated in controlled environmental conditions (Soil Burial test), simulating natural scenarios (humidity, influence of enzymes, and bacteria). Biodegradation parameters such as weight loss, structural changes, and degradation kinetics were examined during 90 days. Characterization of the structure was performed using FTIR and SEM methods.

Our findings suggest that cellulosic membranes possess complete (100%) biodegradability after 70 days compared to unmodified membranes. Obtained result shed light on the potential of membranes modified in this way as sustainable and biodegradable alternatives in various applications. The results emphasize their ecological nature and ability to reduce environmental stress. Such cellulose-based materials promise a much greener future in biotechnology, healthcare, and environmental protection.

Keywords: cotton linters, TOCell membrane, Soil Burial test, biodegradable polymers, cellulase

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PINUS SYLVESTRIS ESSENTIAL OIL AS A GREEN CORROSION INHIBITOR FOR MILD STEEL IN 1M HCl SOLUTION

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With its superior mechanical and physical qualities, carbon steel finds extensive usage in several fields. Its primary flaw is that it easily corrodes, especially in acidic environments. Globally, scientists are researching green corrosion inhibitors, which are primarily defined as plant extracts and essential oils that are cheap, safe, non-toxic, and have a high corrosion inhibition efficiency.

The study of Pinus Sylvestris essential oil as a green corrosion inhibitor of steel in 1M HCl serves as the foundation for our investigation. Through hydrodistillation with a Clevenger-style device, the essential oil of fresh, dried Pinus Sylvestris needles was recovered. Different inhibitor doses and immersion periods in HCl were used to calculate the internal efficiency (IE). Utilizing Polarization Measurements and Electrochemical Impedance Spectroscopy (EIS), the oil's inhibitory effects were estimated.

Using electrochemical methods, it was determined that the optimal inhibitor concentration is 100 ppm. The effectiveness of the inhibition increases during the time of 1 to 4 hours of immersion. Also, this essential oil is a mixed corrosion inhibitor with dominant control of cathodic reaction.

Keywords: corrosion; green inhibitor; electrochemical methods; plants

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STRUCTURAL ADAPTIBILITY OF HAEMATOCOCCUS PLUVIALIS GREEN PHASE CELLS EXPOSED TO MANGANESE EXCESS

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Haematococcus pluvialis is a unicellular green alga with a complex life cycle and a remarkable metabolic and structural adaptability that allows it to thrive in metal-infested environments. *H. pluvialis* could be potentially used in the remediation of waters polluted with metals, such as manganese (Mn). Mn is also an essential element involved in different metabolic processes, such as photosynthesis and antioxidantive defence. Herein, we examined morphological response of metabolically active green cell type of *H. pluvialis* (microzooids) to high Mn concentrations exceeding their physiological quota. When exposed to 1 mM Mn^{2+}, cell viability remained stable over a 3-day period. Inductively coupled plasma atomic emission spectroscopy showed the prompt uptake of Mn by the microzooid cells after 1 h of the treatment, with a modest increase of the concentration of Mn in the biomass at 24 h. Scanning electron microscopy revealed granular deposits on microzooid surfaces after 1 hour, likely Mn deposits, while transmission electron microscopy (TEM) micrographs showed that some cells had wall rupture and degraded intracellular content and damaged organelles. After 24 and 72 h, a different type of cell morphology emerged, characterized by thickened cell wall, preserved intracellular compartments, and reduced total area of lipid droplets. Both cell types exhibited vacuoles containing dark granules, possibly indicative of Mn accumulations. Quantitative TEM analysis demonstrated that an excess of Mn reduced cell cross-section and lipid droplet area while increasing vacuole cross-section and cell wall thickness. The intricate adaptive responses of *H. pluvialis* to elevated Mn concentrations exemplified by cell wall thickening, reduction in lipid droplets total area due to increased energy demand, and the accumulation of Mn in vacuoles, exhibits the impressive structural adaptability. Further investigation using analytical methods will provide a more profound understanding of the metabolic dimensions of adaptive response.

Keywords: microalgae; *Haematococcus pluvialis*; manganese; bioremediation; ICP; electron microscopy

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THE COORDINATION AND STORAGE OF MANGANESE IN THE MICROALGA *HAEMATOCOCCUS PLUVIALIS*

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Haematococcus pluvialis is a unicellular green alga of significant environmental and economic impact, well adapted to life in extreme conditions such as metal pollution. Manganese is a common pollutant of water bodies that is related to mining and industrial drainage, and microalgae have been applied in their bioremediation. To study changes in Mn redox and coordination form upon interaction with *H. pluvialis* cells, we exposed late exponential/early stationary green phase culture to 1 mM $MnCl_2$ (Mn^{2+}) for 72 h. Applied concentration exceeds microalgal physiological quota but it was non-toxic. Structure of coordinated Mn in the cells was analyzed using X-ray absorption near edge structure (XANES) and extended X-ray Absorption Fine Structure (EXAFS) spectroscopy, while visualization and elemental mapping of Mn was performed by micro X-ray fluorescence (μXRF). XANES spectra showed that the oxidation form of Mn in *H. pluvialis* biomass remained 2+. However, EXAFS showed Mn coordination in microalgae is closer to hydrated $MnSO_4$ with minor deviation of the local geometry. The shorter Mn-O bonds in biomass, compared to crystal model, implies somewhat more stable complex in *H. pluvialis*. This suggests that *H. pluvialis* may use sulphated polysaccharides for Mn sequestration, which would be a newly proposed mechanism of metal coordination and storage. μXRF analysis showed co-localization of Mn with O and Na, with particularly good superimposition for Mn and O. O-rich regions may represent vacuoles, filled with organic acids including sulphates, or starch granules and accumulations of sulphated polysaccharides, which are known to be produced by *H. pluvialis*. In addition, vacuoles in plant cells are known to act as sinks for Na+ ions. These findings are in line with structural analysis that showed dark precipitates in vacuoles, which are likely accumulations of Mn.

Keywords: microalgae; *Haematococcus pluvialis*; manganese; XANES; μXRF

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FROM WASTE CELLULOSE TO EFFECTIVE BIOMEMBRANES: WASTEWATER PURIFICATION

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Pollution of water with toxic substances is increased. Therefore, innovative solutions for their treatment are essential. This paper represents results from an adsorption study where novel synthesized biobased membranes were applied in the removal of dye metanil yellow from wastewater solution. Batch adsorption tests were applied, where the different operational impacts including contact time, initial pollutant concentration, temperature, etc. were varied. Fabricated membranes were based on waste cellulose tobacco boxes modified by amino acid lysine with an aim to increase sorption capacity toward azo dye. Structural properties were examined by FE-SEM and ATR-FTIR techniques. The activities of materials prior to and after modification were compared. It was found that the modified material achieved a better sorption capacity. The resulting adsorption capacity for the improved membrane was 65 mg/l compared to 51 mg/l, at 45°C, for the base cellulose membrane. The kinetics of the process follows a pseudo second-order curve. The best agreement of the correlation factor R^2 was shown with the Freundlich isotherm. The obtained results show the success of the modification with a good sorption capacity of the material towards the target pollutant. Overall, it can be concluded that the modified membranes lay a good foundation for potential application in industrial dye wastewater treatment systems.

Keywords: dye removal; metanil yellow; environmental protection; water purification; adsorption

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REMOVAL OF CRITICAL METALS LEACHED FROM FLY ASH USING NATURALLY DERIVED CELLULOSE-ADSORBENT

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Bacterial nanocellulose (BNC) is an exopolysaccharide produced by aerobic bacteria in a form of nanostructured network of glucose molecules. This results in a mechanically resistant, yet soft and elastic material with a high surface area, water holding and gas exchange capacity. BNC is a biomaterial with exceptional physico-chemical and biological properties, finding applications in medicinal and pharmaceutical sciences, such as wound dressing and drug carrier. Adsorbents based on nanocellulose offer an eco-friendly platform for effectively extracting critical metals from various sources. This innovative approach aligns with environmental sustainability goals, presenting a promising avenue for the responsible management of metal pollutants. In this paper, BNC was used for the removal of several critical metals, including heavy metals, from suspended fly ash collected from a thermal power plant. The structure, thermal properties, and crystallinity of naturally derived nanocellulose (BNC) were thoroughly investigated using multiple analytical techniques (FTIR, DCS/TG and XRD).

The effectiveness of BNC in adsorbing critical metals leached from fly ash in water or 0.1 M solution of acetic acid was evaluated using mass spectrometry with inductively coupled plasma (ICP-MS). This assessment considered various parameters such as pH, contact time, and contact area of the adsorbent. The results demonstrated the success of BNC in efficiently removing metals leached from fly ash. However, the efficacy varied depending on the specific metal ion and the combination of factors applied. The promising outcomes suggest that BNC has substantial potential for application in the removal of metals from aqueous mediums. This underscores the need for further research and exploration of BNC in wastewater treatment technologies.

Keywords: bacterial nanocellulose; BNC; biopolymer; adsorption; critical metals; metal removal; fly ash; ICP-MS

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CHARACTERIZATION OF EMISSION FROM THE COMBUSTION OF SOLID BIOFUELS IN THE RESIDENTIAL HEATING APPLIANCES

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Emissions from small-scale heating appliances in the household sector are one of the main contributors to the problem of low air quality Serbia faces. This study examined fuel characteristics and burning emissions inside small-scale combustion unit of two types of beech wood pellets commercially available on the Serbian market, as well as pellets produced from crop residue of wheat and a mixture of wheat and rapeseed as a potential alternative to wood pellets. Characteristics of the test fuels – heating value, moisture and volatile matter content, elemental composition and ash content and melting temperature were analysed in accordance with normative specifications. The results demonstrated that tested beech pellets complied with the requirements for the high-quality ENplus A1 and ENPlus A2 classes, while agropellets from wheat and mixture of wheat and rapeseed straws failed to meet established requirements and did not qualify into any classified category. Emission characteristic tests were conducted by combusting test pellets inside an automatic residential pellet stove. The measured and calculated values of O_2, CO_2, CO, NOx, SO_2, and total organic carbon were discussed in comparison to each other and relevant literature. Examination of the combustion of beech pellets revealed low emissions of SO_2 and NOx, while the emission levels of CO and total organic carbon were found to be dependent on the performance of the appliance. Combustion of agropellets was found to be unsuccessful, presenting them as unsuitable fuel for use in household pellet appliances. These findings have implications for both end-users and policymakers as they highlight the importance of ensuring that only pellets of suitable quality are present on the market and used in domestic appliances.

Keywords: wood pellets; agropellets; solid biofuel; combustion; domestic heating; emission factor

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UTILIZATION OF FIBROUS TEXTILE WASTES FOR ADSORPTION OF INORGANIC AND ORGANIC POLLUTANTS FROM WATER

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The textile industry became one of the main polluters of the environment nowadays since it generates large amounts of waste, which reckless disposal can cause serious soil, water, and air pollution. Implementation of different mechanisms, such as reusing or recycling textile waste, reduces the environmental impact of textile waste and represents the most important link in the chain of sustainable development. In an attempt to reuse fibrous textile waste, waste materials of lignocellulosic, cellulosic, and synthetic origin were used as biosorbents and precursors for carbon adsorbents for the purification of water polluted by organic and inorganic pollutants. Waste cotton and cotton/polyester yarns, and hemp and flax fibers were characterized by scanning electron microscopy, Fourier transform infrared spectroscopy, iodine sorption, water retention, and point of zero charge, as well as through the determination of crystallinity index and degree of surface crystallinity. To improve the adsorption properties, yarns and fibers were modified, chemically using 18 % NaOH, and thermally through classical and hydrothermal carbonization, followed by activation. The adsorption properties of unmodified and modified materials were tested through the adsorption of selected pollutants (lead ions, methylene blue, and pharmaceuticals from the class of sedatives) from water. More heterogeneous chemical composition and the presence of non-cellulosic components in the fiber structure, along with the presence of fibrillation, cavities, and cracks on the surface of the fibers proved to be key factors that affect the efficiency of waste fibers as biosorbents. It was found that the chemical and thermal modification increases the adsorption efficiency of fibrous textile waste due to the changes in structural and surface properties, or conversion to efficient carbon adsorbents. Obtained results have shown that fibrous textile waste can be utilized for the preparation of highly efficient adsorbents for the fast removal of different pollutants from water.

Keywords: fibrous textile waste; biosorbents; carbon adsorbents; characterization; pollutants

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CHEMOMETRIC MODELLING OF THE ADSORPTION PARAMETERS OF DRUG RESIDUES FROM WATER USING MODIFIED FLY ASH AS ADSORBENT

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Rapid technological development has led to an increased amount of industrial waste. One form of industrial waste, fly ash, is a product of coal combustion in thermal power plants. Thus, the basic idea of this work is the use of this type of waste, as an adsorption agent, in order to remove drug residues from water. The adsorption properties of ten pharmaceutically active components on three different modifications of fly ash have been examined, as well as the impact of the operating parameters on the adsorption process. The parameters that affect the adsorption process are as follows: the period of contact between the adsorbent and the drug residues, the pH value of the pharmaceutical solution, the mass of the adsorbent, and the volume of the adsorbent solution. The results of the adsorption experiments were processed by chemometric methods of multivariate analysis, namely, artificial neural networks and cluster analysis. The results obtained by chemometric analysis have shown that the best adsorption characteristics were achieved by hydrothermally activated fly ash modified by chitosan, while the parameter that impacts the adsorption process mostly was the contact time. Drugs with the highest level of significance in terms of adsorption (more than 70%) were doxycycline, cilazapril, clopidogrel and bromazepam. The neural network predicted which drugs would be mostly adsorbed on modified fly ash and could be used as a starting point for future research.

Keywords: fly ash; pharmaceuticals; chemometrics; artificial neural networks

Acknowledgements: This research was supported by the Science Fund of the Republic of Serbia, through the project Serbian Industrial Waste Towards Sustainable Environment: Resource of Strategic Elements and Removal Agent for Pollutants - SIW4SE within program IDEAS (GRANT No 7743343).

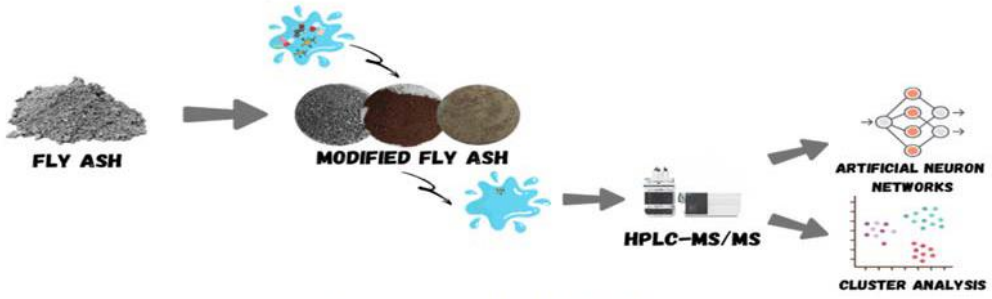

Figure 1. Graphical abstract

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Session 5

Biotechnology for Biobased Products

Chair: Mirjana Rajilić-Stojanović

ANTIOXIDANT POTENTIAL AND PHENOLICS CONTENT OF HORSERADISH ROOT JUICE ENCAPSULATED WITHIN DIFFERENT CARBOHYDRATE MATRICES

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Horseradish is a plant grown for its succulent and spicy root, which presents a rich source of antioxidants such as phenolic compounds, vitamin C, and isothiocyanates. Due to its antioxidant properties, cold-pressed horseradish root juice could be an active ingredient in functional foods. However, fresh juices undergo enzymatic and microbiological changes, so it is necessary to preserve them. One of the approaches commonly used for this purpose is encapsulation by spray-drying, which involves the entrapping of bioactive components within carrier agents. This ensures the protection of the bioactive component from undesirable external factors and its controlled release. Many studies have shown that the use of a combination of maltodextrin and hydrocolloids as encapsulation carriers results in high-quality encapsulates. So, the aim of this study was the encapsulation of horseradish root juice and the characterization of the obtained encapsulates as potential antioxidants in food production.

Maltodextrin/guar gum (MD/GG) and maltodextrin/gum Arabic (MD/GA) were used as carriers to encapsulate horseradish root juice by spray-drying. Total phenolic, flavonoid, and phenolic acid contents (TPC, TFC, and TPAC, respectively) and antioxidant activity (DPPH, ABTS, and FRAP methods) were determined by standard spectrophotometric methods.

MD/GG and MD/GA with encapsulated horseradish root juice contained 1628 and 1568 mg gallic acid equivalents/100 g, respectively, of TPC; 264 and 253 mg catechin equivalents/100 g, respectively, of TFC; and 3272 and 3397 mg caffeic acid equivalents/100 g, respectively, of TPAC. Results for the antioxidant activity (expressed as mmol Trolox equivalents/100 g) of MD/GG and MD/GA were 0.8 and 0.7, respectively (DPPH); 6.9 and 7.6, respectively (ABTS); and 8.8 and 8.6, respectively (FRAP).

In conclusion, horseradish root juice encapsulated within carbohydrate biopolymers contained significant amounts of phenolic compounds and high antioxidant activity, which makes it a potential replacer for synthetic antioxidants in the food industry and opens the possibility for further research on this topic.

Key words: horseradish root juice; encapsulation; carbohydrate biopolymers; phenolics; antioxidant activity

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PHYSICOCHEMICAL CHARACTERIZATION OF SPRAY-DRIED HORSERADISH ROOT JUICE ENCAPSULATED WITHIN MALTODEXTRIN/ALGINATE

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Spray-drying is one of the widely used techniques to extend the shelf-life and easier handling of vegetable juices. However, the high temperatures in the spray-drying chamber may cause the degradation of the bioactive components of the juices. Also, the enzyme activity and sugar content of fresh juices can lead to difficulties in drying and resulting in powders with unfavorable physicochemical properties. To overcome these problems, juices can be encapsulated within various biopolymers. Carbohydrates, maltodextrin, and alginate were used as carriers for the spray-drying encapsulation of bioactive components of various plant juices and extracts. To our knowledge, there are no reported studies on the encapsulation of horseradish root juice within these carriers. Therefore, this study aimed to investigate the influence of the maltodextrin/alginate carrier mixture on the physicochemical properties of the horseradish root juice preserved by the spray-drying encapsulation technique.

Root juice powder without a carrier (C, control sample) and maltodextrin/alginate encapsulates of root juice (MD/AL) were prepared by spray-drying. The powders were analyzed using standard analytical methods to determine the moisture content, water activity, hygroscopicity, oil holding capacity, bulk, and tapped density.

Moisture content, water activity, and hygroscopicity were lower in MD/AL (7.8%, 0.28, 22.9 g/100 g) than in C (10.2%, 0.32, 24.4 g/100 g). MD/AL had a higher oil holding capacity (1.4 g oil/g) compared to C (1.1 g oil/g). The values for bulk and tapped density were for MD/AL 0.5 and 0.7 g/cm^3 and C 0.6 and 0.7 g/cm^3, respectively.

Finally, the encapsulation of horseradish root juice in maltodextrin/alginate resulted in powders with significantly better physicochemical properties than spray-dried horseradish root juice without carrier. Based on this study, it can be concluded that the encapsulation process has great potential for the preservation of vegetable juices and provides many perspectives for further research and application in food products.

Keywords: spray-drying; encapsulation; maltodextrin/alginate; physicochemical characterization; horseradish root juice

Acknowledgments: This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (grant number 451-03-47/2023-01/200116).

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THE EMPLOYMENT OF PULLULAN AND COLLAGEN IN THE PREPARATION OF ELECTROSPUN NANOFIBERS LOADED WITH *TEUCRIUM MONTANUM* L. EXTRACT

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Consumer demands for various quality characteristics have never been as pronounced as they are today, prompting the food industry to integrate nutritional, sustainable and health-promoting aspects when developing or improving food products. Food by-products and traditional plant species have come into the spotlight as valuable sources for the extraction and characterization of bioactive compounds, *e.g.* phenolic compounds. Despite their recognized biological potential, such as antioxidant, anti-inflammatory, antibacterial, antimutagenic, antidiabetic, etc., the relative sensitivity to technological processing and poor bioavailability under gastrointestinal conditions seem to be the major challenges in the valorization of phenolic compounds. To overcome these problems, various encapsulation techniques have been developed. In this study, the electrospinning technique was proposed as one of the solutions to encapsulate and protect the phenylethanoid glycosides- rich extract of understudied Mediterranean plant species Mountain Germander (*Teucrium montanum*) in pullulan/collagen-based nanofibers. For this purpose, different combinations of biopolymers (80:20, 60:40, 40:60 and 20:80 pullulan/collagen, *w/w* in a total mass concentration of 12%) were dissolved in the concentrated plant water extract. Since the physical properties of the solution have a significant influence on the morphology of the nanofibers, the viscosity, surface tension and conductivity of the prepared solutions were determined. The prepared nanofibers were characterized in terms of encapsulation efficiency by HPLC-UV-DAD, morphology by SEM and *in vitro* release kinetics of polyphenols. The results showed excellent encapsulation efficiency of echinacoside, poliumoside, stachysoside A and teupolioside (> 80%), regardless of the combination of polymers used. However, the increase in collagen content affected the morphology of the nanofiber mats, which was disrupted by the presence of beads. This study provides insight into the potential of blending pullulan and hydrolyzed collagen into polyphenol-rich nanofibers by water-based electrospinning.

Keywords: collagen; electrospinning; phenylethanoid glycoside; pullulan; *Teucrium montanum*

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NANOCOMPOSITES MADE FROM NANOCELLULOSE AND BIOBASED UNSATURATED POLYESTER RESINS: RHEOLOGICAL AND MECHANICAL PROPERTIES

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Novel formulations of polymer matrices and fillers, as well as the optimization of curing parameters, have been established as an attractive area of research in the field of biocomposite materials. The present study investigated the curing parameters of a biobased unsaturated polyester resin reinforced with nanocellulose (1 wt.%). Being a remarkable nanomaterial derived from renewable resources, which exhibits exceptional properties (high tensile strengths, low density, biodegradability), nanocellulose has gained significant attention as a sustainable filler in developing nanocomposite materials. Several biobased monomers (itaconic acid, succinic acid, propylene glycol and dimethyl itaconate) were used to obtain a biobased unsaturated polyester resin. The optimization of curing parameters consisted of using two different initiators (dibenzoyl peroxide and methyl ethyl ketone peroxide) and varying them with different concentrations of cobalt octoate as an activator (0.5, 1.0, 1.5 wt.%). Gel content analysis was performed to estimate the crosslink density, giving the researchers a tool to examine how the proposed curing parameters affected the polymerization reaction. Dynamical mechanical analysis revealed that the presence of nanocellulose slightly lowered the glass transition temperature. The results of the uniaxial tensile test showed a notable increase in Young's modulus when compared to neat biobased unsaturated polyester resin, indicating that the obtained nanocomposite material could have application in the contexts requiring high elasticity.

Keywords: biobased materials; nanocellulose; itaconic acid

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ALGINATE-BASED MICROFIBERS FOR 3D CULTURES OF OSTEOSARCOMA CELLS

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Cancer research and anticancer drug development are shifting from studies of conventional two-dimensional (2D) cell cultures towards more biomimetic three-dimensional (3D) *in vitro* cancer models. One of the approaches in this direction is based on the application of biomaterials as cancer cell carriers, providing cells with a 3D structure and allowing cell-cell interactions. The aim of this study was to investigate alginate hydrogel microfibers with or without hydroxyapatite (HAP) particles for immobilization and culture of osteosarcoma cells and to validate this 3D *in vitro* model for short-term anticancer drug testing.

Murine osteosarcoma K7M2 wt cells were suspended (4×10^6 cells/ml) either in the neat 2 wt.% Na-alginate solution or the solution containing 2 wt.% commercial HAP powder. Next, the suspension was manually extruded through a 26-gauge needle into the gelling bath containing 0.18 M Ca^{2+}. After 15 min of gelation, the obtained microfibers were washed and transferred into the T-25 culture flasks (1.5 g of microfibers in 15 ml medium) and cultured up to 21 days. Histological analysis showed that the microfibers supported cell viability and the formation of cellular aggregates in both cases. Also, both 3D cultures were validated in anticancer drug testing by using 0.25-20 μM doxorubicin. Although the results in the two types of microfibers were not statistically different, both 3D cultures showed higher resistance to the applied drug as compared to the control 2D culture (the IC_{50} values were ~3 and ~0.5 μM, respectively). The obtained results indicated that the proposed model could be used for anticancer drug screening, while the potential beneficial effect of the HAP presence in the cell microenvironment should be further investigated.

Keywords: microfibers; hydroxyapatite; doxorubicin; 3D culture model

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THE PROCESSING, BIOACTIVITY AND BIOCOMPATIBILITY OF SCAFFOLDS BASED ON MULTI-ION DOPED CALCIUM-PHOSPHATES COATED WITH CHITOSAN

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Biocomposite scaffolds featuring a ceramic base strengthened by a polymer phase represent a class of biomaterials that is increasingly being developed with the aim of replacing biological tissues and releasing active substances in a controlled manner. Essential attributes, such as bioactivity and biocompatibility, are imperative for these biomaterials to actively foster new tissue formation while maintaining a harmonious interaction with the biological environment. The aim of this study was to examine the possibility of processing scaffolds based on calcium-hydroxyapatite (HAp) doped with magnesium, strontium, and fluorine ions, coated with polymer chitosan, and to investigate their bioactivity and biocompatibility. In this study, the hydrothermal method was employed to synthesize doped Hap powder, which was subsequently utilized in creating scaffolds through the sponge replica technique, followed by sintering and chitosan coating. The microstructure, mechanical properties, bioactivity, biocompatibility, and drug release characteristics of scaffolds influenced by dopant ions and chitosan were evaluated. An improvement in mechanical properties by coating the scaffold with polymer was observed.

Microstructure and the bioactivity of the scaffolds were determined by scanning electron microscopy (SEM). The SEM revealed a consistent macroporous scaffold structure characterized by interconnected pores. Bioactivity was investigated by keeping the scaffolds in simulated body fluid for 28 days. The uncoated scaffolds showed satisfactory bioactivity, while lower bioactivity was observed in the coated scaffolds due to the slow degradation of chitosan. The MTT test was employed to investigate the in vitro cytotoxicity of the synthesized scaffolds on MRC-5 human lung fibroblast cells. The scaffolds demonstrated to have a positive impact on cell viability, even slightly stimulating the cell proliferation. Additionally, scaffolds were shown to successfully release the drug hydrazone. In conclusion, the properties of the obtained scaffolds were significantly improved by the addition of ions and chitosan coating, which indicates their potential application in tissue engineering and controlled drug release.

Keywords: bioactivity; biocompatibility; scaffold; tissue engineering; controlled drug release

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FREEZE-DRYING TECHNIQUE FOR IMMOBILIZATION OF YEAST CELLS IN THE MALTODEXTRIN-PROTEIN SYSTEM

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Recently, the beer production process has been raised to a higher level by the application of new yeast cell immobilization techniques. Along with standard materials for cell immobilization, polysaccharide-protein systems are emerging to increase the viability and protection of cells throughout the fermentation process. The main objective of this work was the immobilization of brewer's yeast cells (*Saccharomyces pastorianus* strain W34/70) by the freeze-drying technique (lyophilization). For this purpose, a 1.5% solution of maltodextrin (Mal) was used, as well as a mixture of maltodextrin and whey protein isolate (WPI) in a ratio of 1:1. After the freeze-drying, the obtained powders with immobilized culture were analysed in terms of cell viability, moisture content, sample solubility, mean particle diameter, and surface charge. Besides, the morphological characteristics were determined by scanning electron and optical microscopy. Cell viability was maintained at a satisfactory level even after 4 weeks (Mal $4.5x10^7$ CFU/ml, Mal-WPI $2.5x10^7$ CFU/ml). The results also showed that the addition of protein reduced the moisture content (Mal 7.43±0.21%; Mal-WPI 4.00±0.07%) in the samples, while the solubility did not change drastically with the addition of the isolate. The low values for the moisture content indicate that the maltodextrin-WPI systems are microbiologically stable and suitable for further application in the food industry. The surface charge of all samples was determined to confirm the physico-chemical stability of the cell-based system. It was found that all samples had a negative surface charge, but their low values indicate a tendency to aggregate, which was also confirmed by optical microscopy. The results indicate that the freeze-drying technique is suitable for cell immobilization in polysaccharide-protein systems, with the possibility of application at the industrial level where high productivity of the process with maximum cell protection is desired. Protein-polysaccharide systems provide suitable cell protection and have potential for application in the beer industry.

Keywords: maltodextrin; immobilization; yeast cells; whey protein isolate; freeze-drying

Acknowledgements: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (SCIENCE AND TECHNOLOGY DEVELOPMENT PROGRAMME–RESEARCH PROJECTS OF THE REPUBLIC OF SERBIA AND THE PEOPLE'S REPUBLIC OF CHINA, Contract No. 451-02-1236/2023-05)

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NANOSTRUCTURES BASED ON PULLULAN AND PUMPKIN GREEN LEAF PROTEINS AS CARRIERS FOR VITAMIN B12

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Leaves available as by-products from some crops could be used as a major protein source for food applications. Here, pumpkin leaves from field crop side streams were used for the preparation of a protein-rich extract. Namely, pumpkin leaves were mechanically processed with additional steps of acidic treatment and lyophilization to produce the extract. The extract (1 mass%) was mixed with a spinnable biopolymer-pullulan (5 mass%) with the addition of vitamin B12 (vitB12) as a model vitamin. This homogeneous mixture was electrospuned through a stainless-steel needle (18G) at a steady flow rate of 25.2 mL/h using a syringe pump and an electric field (17 kV) between the positively charged needle and grounded metallic collector plate. The starting solutions (plain pullulan-PUL, pullulan with extract-PUL+E, and pullulan with extract and vitB12-PUL+E+B12) as well as the resulting structures were analyzed regarding physicochemical and morphological properties.

The addition of the extract increases the surface tension of the PUL solution, but vitB12 decreases it. On the other hand, the viscosity measurements showed an increase in the value with the addition of extract and vitB12. PUL solution had a conductivity of 0.163 mS/cm, and the value increased upon adding the extract and vitB12 (1.420 mS/cm and 0.978 mS/cm, respectively). According to SEM images, electrospinning resulted in the formation of beaded fibers. The lower the viscosity of the solution, the greater the proportion of particles observed, which is in accordance with literature data. The mean value of the particle size decreases from 388.89 nm (PUL) to 176.68 nm (PUL+E). However, the addition of vitB12 leads to an increase in the mean particle size to 255.94 nm. The addition of extract didn't have a strong effect on average fiber diameter, while a slight increase was observed in PUL+E+B12. FTIR analysis suggests intermolecular interactions between the constituents of the pullulan-extract-vitamin B12 fibers. As a conclusion, the combination of pullulan and protein-rich extract has the properties of a stable carrier for the encapsulation of vitamin B12.

Keywords: electrospinning; pullulan; pumpkin leaves protein; nanostructures; vitamin B12

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THE USE OF STARCH AND β-LACTOGLOBULIN COMPOSITE HYDROGELS AS FRAMEWORKS FOR PRESERVING C-PHYCOCYANIN

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Our study aimed to preserve the natural blue dye of C-phycocyanin (C-PC) phycobiliprotein from *Spirulina* microalgae due to its importance in the food industry. We incorporated C-PC into hydrogels formed by combining starch and β-lactoglobulin (BLG) using high-pressure (HP) processing to achieve this objective. Notably, thermal treatment resulted in the complete loss of colour derived from C-PC.

We performed a comprehensive characterization of the resulting HP gels by rheology measurements, texture profile analysis (TPA), small-angle X-ray scattering (SAXS), and scanning electron microscopy (SEM).

Different compositions of binary (BLG/C-PC) and ternary (starch/BLG/C-PC) systems were processed under high-pressure (HP) conditions reaching up to 4,500 bar. The C-PC pigment was effectively preserved by mixing BLG and starch with C-PC at pH 7, maintaining concentrations of 180, 5, and 10 mg/mL, respectively. The same concentrations of components were retained in the binary systems.

Rheological properties of the gels were determined using a rheometer with plane/plane geometry, and texture analysis was conducted through TPA. These findings enabled the assessment of food gel's properties, such as hardness, springiness, chewiness, and cohesiveness. The structural characteristics of the gels were determined by SAXS, offering insights into the interactions between C-PC, BLG, and starch after HP processing. Adding C-PC and starch formed solid gels with a larger mesh than the pure BLG gels. SEM scans of the gel surface revealed that all components influenced the overall morphology of gels. Even at low concentrations, the addition of starch notably influenced the gels' visual appearance and mechanical properties. Our investigation highlights the superior effectiveness of HP treatment in the preservation of C-PC compared to high-temperature treatment, evident in the sustained colour integrity of the C-PC blue dye.

Keywords: C-phycocyanin, high-pressure processing, high temperature, food gels, rheology, texture profile analysis, small angle neutron scattering, scanning electron microscopy

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EFFECT OF THE LIGNIN FUNCTIONALIZATION ON THE MORPHOLOGY AND ADSORPTION POTENTIAL OF THE LIGNIN-BASED MICROSPHERES

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The morphological properties and adsorption potential of lignin-based porous microspheres (LgMS) for heavy metals ions were investigated. LgMS were produced via suspension copolymerization of unmodified or acryloyl modified kraft lignin with amine (polyethylene imine - PEI) or acrylate (trimethylolpropanetriacrylate - TMPTA) functional polymers. Structural and morphological characterization of LgMS was performed using FTIR, BET, and SEM techniques. Copolymerization of the unmodified lignin and PEI, using epoxy chloropropane cross-linker, provided highly porous LgMS, with 800 ± 80 μm diameter, 7.68 m2g−1 surface area and 7.7 mmol g−1 of terminal amino groups. The LgMS produced by copolymerization of the acryloyl functionalized lignin and TMPTA provided a decrease in LgMS diameter (253 ± 42 μm), while surface area and porosity increase, 69.4 m2 g-1and 59%, respectively. The effect of acryloyl functionalization of kraft lignin on the LgMS adsorption capacity for Ni2+ ions was also studied in a comparative study. The results showed that final adsorption performances of LgMS were affected by the key factors including lignin functionality and the LgMS synthesis condition. Lower adsorption capacity of 22.6 mg g-1 for Ni2+ was observed for acryloyl LgMS due to lower affinity for formation electrostatic interactions of Ni2+ ions with phenol/hydroxyl groups responsible for adsorption. Opposite was found for amino LgMS where 49.4 mg g-1was achieved. However, this study indicated that removal of heavy metal ions from wastewater can be realized through the application of eco-friendly lignin-based porous microspheres.

Keywords: lignin-based microspheres; kraft lignin; removal of heavy metals; eco-friendly materials

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DIFFERENT TREATMENTS OF LIGNOCELLULOSIC BIOMASS FOR ENHANCED DELIGNIFICATION AND ENZYMATIC HYDROLYSIS

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Lignocellulosic biomass (LCB) valorization is a fundamental step toward circular bioeconomy. The complex structure of lignocellulose must be disrupted to conduct thorough valorization. Conventional methods often lack sustainability, by having high energy requirements, or a harmful impact on the environment. To overcome these impediments, novel tenable methods must be developed. This work compares different green solvent-based treatments of LCB, regarding the delignification rate and the enzymatic hydrolysis efficiency. Due to abundance and high availability, corn residues were used as model substrates. Deep eutectic solvent (DES), microwave-assisted alkaline treatment, combined non-thermal plasma/Fenton, and non-thermal plasma/alkaline treatment were selected as green methods for LCB treatment, having higher selectivity favoring lignin degradation and milder reaction conditions. Treated biomass was analyzed for acetyl bromide soluble lignin content and subjected to enzymatic hydrolysis with Cellic® CTec2, followed by hexose and pentose content determination. The best results regarding lignin content were obtained with DES, when the choline chloride to lactic acid ratio was 1:10, achieving a delignification rate of 86%. Combined plasma/alkaline treatment for 10 minutes decreased lignin content by 77%, while microwave-assisted alkaline treatment decreased it by 74% for only one minute. When combined with the Fenton reagent, a 30-minute-long plasma treatment reduced lignin content by 53%. Moreover, FTIR spectroscopy confirmed partial depolymerization of lignin, making it more suitable for further application. Each treatment intensified LCB decomposition and facilitated enzyme penetration, increasing hexose yield by 2.3-5.6 times, and pentose yield by 1.7-8.0 times compared to the untreated biomass. Methods implemented in this work contributed to feasible biomass delignification. Coupling alkaline hydrogen peroxide with microwave irradiation or non-thermal plasma or applying reusable DES could significantly promote proficient biotechnological exploitation of lignin and carbohydrate fractions in a time-saving and cost-effective way. The possibility of biotechnological production of natural antioxidants and natural aromatic compounds should be particularly underlined.

Keywords: gas plasma; deep eutectic solvents; microwave irradiation; corn stalk; waste valorization

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SYNTHESIS AND PROPERTIES OF COMPOSITE HYDROGELS BASED ON INTERPENETRATING NETWORK OF GELATIN AND POLY(METHACRYLIC ACID), AND PARTICLES OF BIOACTIVE GLASS AND HYDROXYAPATITE

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Critical-size bone defects resulting from trauma, tumor resection, or other pathological conditions, do not undergo spontaneous self-healing. They require the application of artificial implants to replace the lost volume and provide support for tissue growth and regeneration. Due to their porous and hydrated structure, as well as the ability to carry and release cells and active substances in a controlled manner, composite hydrogels are being extensively investigated as materials for bone tissue repair. In this study, composite hydrogels based on interpenetrating network of gelatin and poly(methacrylic acid), and nanoparticles of bioactive glass and multi-ion doped calcium hydroxyapatite (HAP), were synthesized and evaluated. HAP nanoparticles were synthesized by hydrothermal method, while bioactive glass nanoparticles (BAG) were obtained through a microemulsion process assisted by ultrasonic waves. The composite hydrogels were synthesized through a thermally induced free-radical polymerization. Scanning electron microscopy revealed that the hydrogel's structure was macro-porous with pores larger than 200 μm, while HAP and BAG particles were evenly distributed in the matrix. The increase in the concentration of HAP and BAG particles resulted in enhanced mechanical properties and a reduced swelling degree. To enhance the functionality of the composite hydrogels, the ability to bind and release the orange peel extract, known for its antimicrobial, anti-inflammatory, and anticancer properties, was investigated. The obtained composite hydrogels demonstrated the ability to load and release active substances, contributing to greater functionality of composite hydrogels and the expansion of their potential for application in real systems.

Keywords: poly(methacrylic acid), gelatin, IPN hydrogel, bioglass, bone tissue engineering

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Session 6

Functional Food and feed

Chair: Oswaldo Hernández-Hernández

EDIBLE FLOWERS OF MARIGOLD (*CALENDULA OFFICINALIS* L.) AS FUNCTIONAL FOOD

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Marigold (*Calendula officinalis* L.) from the Asteraceae family is annual or perennial plant with many purposes: pot or garden ornamental plant, an ingredient in cosmetic products, medicinal plant and edible food decoration. Marigold flowers are source of bioactive compounds beneficial to human health. About that, in this study 80% methanol and 80% acetone extracts were used for spectrophotometric determination of photosynthetic pigments chlorophyll a and b, and total carotenoid content (TCC), total phenolic (TPC), flavonoid (TFC), and hydroxycinnamic acid derivative (HCAs) content (Tab. 1) and antioxidant activity by TAC (in vitro phosphomolybdenum), FRP (ferric reducing power), CUPRAC (cupric reducing antioxidant capacity), DPPH• (2,2-diphenyl-1-picrylhydrazyl radical scavenging) assays (Tab. 2). The obtained results were analyzed by one-way analysis of variance (ANOVA) and post hoc Tukey`s HSD test and the results were expressed as mean ± standard deviation (SD).

Table 1. Phytochemical composition of marigold flowers

Solvent	Chlorophyll *a* (μg g^{-1})	Chlorophyll *b* (μg g^{-1})	TCC (μg g^{-1})	TPC (mg g^{-1} FAE*)	TFC (mg g^{-1} QE)	HCAs (mg g^{-1} CGAE)
Acetone (80%)	16.90±1.53	0.00	21.92±0.45	3.89±0.18 **a**	1.88±0.15 **a**	1.94±0.02 **a**
Methanol (80%)	/	/	/	3.08±0.14 **b**	0.67±0.09 **b**	0.93±0.08 **b**

*FAE-ferulic acid, QE-quercetin, NE-naringenin and CGAE-chlorogenic acid equivalents.

Table 2. Antioxidant activity of marigold flowers

Solvent	TAC μg g^{-1} AAE**	CUPRAC μg g^{-1} AAE	FRP mg g^{-1} AAE	DPPH• μmol g^{-1} TE
Acetone (80%)	5.96±0.43 **a**	7.76±0.82 **a**	5.34±0.10 **a**	15.88±0.17 **a**
Methanol (80%)	4.27±0.08 **b**	8.31±0.17 **a**	4.40±0.23 **b**	16.04±0.44 **a**

** AAE-ascorbic acid and TE-Trolox equivalents.

The obtained indicated statistically significantly higher content of all tested parameters in acetone extracts compared to methanol extracts. The content of photosynthetic pigments was lower compared to other edible flowers pigment content from the Asteraceae family. Regarding the antioxidant activity of marigold flowers, in TAC and FRP assays acetone extract also had higher antioxidant activity, while in CUPRAC and DPPH• assays there is no statistically significant difference between the tested extracts. Based on their phytochemical properties, marigold flowers can be classified as functional food.

Keywords: antioxidant activity, *Calendula officinalis* L., marigold, phytochemical characterization.

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THE EFFECT OF USING ALTERNATIVE SOURCES OF PROTEIN FROM ANIMAL SPECIES ON THE PRODUCTION PARAMETERS OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Fishmeal is dominantly used as a protein source in commercial aquafeed for rearing rainbow trout, due to its favorable amino acid content. However, the use of this component has become unsustainable because of the small stocks of wild fish species used for its production, which directly affects the increase in price. Previous studies have shown that animal protein sources represent a potentially good choice for use in the production of feed for fish due to their high protein and fat content. Worms are used for feeding fish and support the principles of the circular economy. The aim of this research was to compare the possibility of completely replacing fishmeal with alternative protein sources and to investigate their effect on the production parameters of rainbow trout. In this study, four experimental diets were formulated: a diet control with fishmeal (FM) and three diets where fishmeal was replaced with mealworm (MW), earthworm (EW), and zooplankton (ZO). The production parameters were calculated: body weight gain (BWG), feed conversion rate (FCR) and biometric index, i.e. hepatosomatic index (HSI). The results for BWG were from 45.61 g for MW to 6.23 g for ZO. The values for the FCR parameter ranged from 1.48 for MW to 1.59 for FM. As can be seen, rainbow trout fed the diet with MW had the best growth, as well as the best digestibility of the feed, which can be explained by the affinity of rainbow trout to different protein sources, while the HSI value showed that the diets do not negatively affect fish health. In conclusion, mealworms and earthworms used in rainbow trout diets showed high values in production parameters and can be defined as sustainable alternatives for the replacement of fishmeal. Finally, they could be used as a potential functional ingredient in aquafeeds.

Keywords: rainbow trout; fishmeal replacement; mealworm; earthworm; zooplankton

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SENSORY EVALUATION OF BAKED RAINBOW TROUT (*Oncorhynchus mykiss*) FED DIFFERENT NON-CONVENTIONAL PROTEIN SOURCES

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Rainbow trout is one of the most used cold-water fish species in the human diet all over the world, thanks to its rich source of protein, minerals, and omega-3 fatty acids. Fishmeal is used in commercial formulation diets for trout, due to its appropriate nutritional composition. However, due to the high price of fishmeal, there is a tendency to replace it with different plant or animal protein sources. The use of plant protein sources in fish diets is limited because of the presence of anti-nutrients and the lack of essential amino acids, whereas this is not the case with animal protein sources. In addition to a good chemical composition, mealworms (*Tenebrio molitor*), earthworms (*Eisenia fetida*), and zooplankton have a low environmental impact with well-known functional components that have a positive effect on fish health. There is no comparative study that investigated the sensory quality of rainbow trout fed with a diet based on entirely non-conventional protein sources until now. The aim of this study was to evaluate the sensory quality of baked rainbow trout, three diets were formulated, in which mealworm, earthworm, and zooplankton substituted 100% of fishmeal, and their effect on the individual sensory properties. The descriptive sensory analysis and the quality rating method, using a linear and categorical scale, were used. The mean rating scores were within the range of "very good" quality for baked rainbow trout fed the diet based on mealworm and earthworm with noticeable positive properties, except diet based on zooplankton. Negative changes in that sample are associated with a dark appearance and a hard and sticky texture. According to the sensory evaluation performed, the application of non-conventional protein sources as a potential functional feed showed promising results in terms of the overall quality of the baked rainbow trout.

Keywords: rainbow trout; non-conventional protein sources; worms; quality rating method; descriptive sensory analysis

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BROCCOLI MICROGREENS-APPLE JUICE AS NOVEL BEVERAGES: TOTAL PHENOLIC, FLAVONOIDS AND ANTIOXIDANT ACTIVITY

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Recently, microgreens have been recognized as a potential food of the future, and their application in the formulation of new products has been scarcely investigated. Only a few studies promote the use of microgreens and sprouts in the formulation of novel products, primarily beverages. The most often cultivated, analyzed and used microgreens are from the *Brassicaceae* family, because they present a good source of bioactive compounds, primarily glucosinolates, isothiocyanates and phenolic compounds. However, products from these microgreens species have a typical flavor, with herbaceous, grassy and sulphurous notes, often repulsive to consumers. Therefore, this study aims to examine total phenolic (TPC) and flavonoids (TFC) content, as well as antioxidant properties ($ABTS^{\bullet+}$ and FRAP) of novel sensorially acceptable broccoli microgreens-apple juice (BC-AJ). Previously produced cold-pressed broccoli microgreens and apple juices were mixed in the ratios 51% and 49% respectively, and further analyzed by well-known spectrophotometric methods such as Folin-Ciocalteu's (TPC) and aluminum chloride (TFC) methods, as well as methods based on radical scavenging ($ABTS^{\bullet+}$) and ion reducing (FRAP) activities for evaluation of antioxidant properties. Results for the TPC and TFC of BC-AJ were 109.78 ± 1.08 mg GAE/100 mL and 64.68 ± 2.25 mg QE/100 mL, respectively. Furthermore, novel BC-AJ had a good ability to scavenge $ABTS^{\bullet+}$ radicals (162.90 ± 4.42 mg TE/100 mL) and a tendency to reduce $[Fe^{3+}\text{-}(TPTZ)_2]^{3+}$ complexes (258.50 ± 3.26 mg TE/100 mL), probably due to the most diverse of phenolic compounds originated from broccoli and apple. Finally, broccoli microgreens-apple juice has a high content of phenolic compounds and good antioxidant properties, so it can be considered as a potentially functional beverage, but future research that includes additional *in vitro* and *in vivo* studies is necessary.

Keywords: broccoli microgreens-apple juice; cold-pressing; total phenolic content; total flavonoid content; antioxidant activity

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CHARACTERIZATION OF AMARANTH (*AMARANTHUS TRICOLOR L.*) MICROGREENS JUICE ENCAPSULATED WITHIN INULIN AND MALTODEXTRIN

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Amaranth microgreens represent a rich source of betalains and phenolic compounds, which show a broad range of positive effects on human health. However, these biocompounds are very sensitive and easily degraded, which often limits their application and bioaccessibility. For the above reasons, natural extracts and juices are most often encapsulated using different carriers, which protect and control the release of bioactive compounds. The aim of this study was to encapsulate cold-pressed amaranth (*Amaranthus tricolor* L.) microgreens juice using maltodextrin (AMD) and inulin (AIN) as carriers and to investigate total phenolic content (TPC), total flavonoid content (TFC) and antioxidant properties ($ABTS^{\bullet+}$ and FRAP) of obtained spray-dried powders. To the best of our knowledge, this is the first report on the encapsulation of amaranth microgreens juice. Before analysis, both powders were reconstituted in Milli-Q water (5% solutions) and analyzed using well-known spectrophotometric methods. The results are expressed in mg equivalents (gallic acid, quercetin, Trolox) per 100 g encapsulates. The obtained values for TPC and TFC for AMD were 291.7 ± 3.0 mg GAE/100 g and 291.3 ± 2.5 mg QE/100 g, while values for AIN were 356.9 ± 1.0 mg GAE/100 g and 289.6 ± 3.8 mg QE/100 g. The results of antioxidant activity were as follows: 546.3 ± 12.6 mg TE/100 g (AMD) and 745.2 ± 3.1 mg TE/100 g (AIN) for $ABTS^{\bullet+}$ and 713.3 ± 8.4 mg TE/100 g (AMD) and 905.1 ± 4.5 mg TE/100 g (AIN) for FRAP. Finally, maltodextrin and inulin can be successfully used for the encapsulation of bioactive compounds of amaranth microgreens. In addition, both powders show good antioxidant properties and can be used in the food industry as potentially novel additives or supplements.

Keywords: amaranth microgreens juice; encapsulation; inulin; maltodextrin; antioxidant activity

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EXPLORING THE MICROBIAL DEGRADATION PROFILE OF 3 DIFFERENT DIETARY FIBERS VIA BACTERIAL MONOCULTURE AND AN IN VITRO FERMENTATION MODEL OF THE COLON (TIM-2)

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The gut microbiota, which is a diverse, dynamic, and complex environment consisting of trillions of microorganisms in the gut, has a great impact on host health. This community is greatly affected by the host diet, with dietary fibers (DFs) as key substrates. DFs are carbohydrates with ≥10 monomeric units, which cannot be hydrolyzed by the endogenous enzymes of humans. Instead, gut microbiota can produce different glycoside hydrolases to degrade these carbohydrates to fuel their own needs, in return the host receives substrates in the form of short-chain fatty acids (SCFAs). In order to define a structure-function relationship for DFs, this study aimed to investigate the mechanistic connections between the gut microbiota with its ability to use DFs as substrates. Therefore, the focus of this study was to investigate the effects of inulin (SYN), pectin (RG-I), and soluble corn fiber (SCF) on the growth and enzymatic degradation capacity of 10 specific strains of bacteria. Furthermore, the study was extended to investigate the broader implications of the above DFs on the gut microbiota using a standardized pool of human feces in a validated, dynamic, in vitro model of the colon (TIM-2). The findings from this study will not only contribute to the knowledge of the complex relationship between DFs and gut microbiota but also provide valuable insights for the development of personalized dietary strategies.

This study first revealed that strain-specific growth stimulation occurs in response to DFs, as observed in monoculture experiments. *Clostridium sporogenes* and *Coproccocus* exhibited growth on SCF and SYN but not on RG-I, which is the most structurally complex DF among the three. On the other hand, the extensively studied *Bacteroides thetaiotaomicron* showed the highest growth across all three DFs, consistent with its role as a proficient DF-degrading bacterium. Of particular interest is the less studied *Hafnia paralvei*, which showed similar growth patterns as *B. thetaiotaomicron*, suggesting the potential for further research. Moreover, *Bifidobacterium adolescentis* and *Ruminococcus bromii* exhibited higher total growth on RG-I compared to SCF and SYN. Degradation patterns aligned with the growth rates of bacterial strains. Additionally, experiments with gut microbiota in the in vitro TIM-2 system revealed rapid utilization of DFs within 2 hours and the generation of DF-specific metabolites, highlighting the significance of synergistic interactions and cooperative degradation in the context of complex DFs.

In conclusion, our results illustrate that the structural complexity of DFs influences bacterial growth, degradation patterns, and degradation time, thus providing evidence that structurally diverse DFs can selectively modulate gut microbiota members. The lack of knowledge on the very basic structures of DFs still precludes the detailed interpretation of the results of the research. The deep mechanisms underlying a structure-function relationship require further research in the future, which we intend to do by studying many more fibers and deep microbiota analysis, which are currently ongoing.

Keywords: dietary fibers; human microbiota; structure-function relationship; TIM-2 model

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ACCELERATED SHELF LIFE TESTING AND COMPARISON OF WALNUT AND HAZELNUT PASTE WITH NATURAL AND SYNTHETIC ANTIOXIDANTS

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Serbia is one of the leading producers of nuts, especially the Province of Vojvodina. This research will examine the nutritive characteristics and shelf life of the walnut (WP) and hazelnut paste (HP). Accelerated shelf life tests have been conducted using OXITEST (Velp, USA), whereas fatty acid content was determined using GC-FID (Agilent 7890A system, Agilent Technologies, USA). In order to improve oxidative stability and sensory acceptability, natural and synthetic antioxidants have been added to the nuts paste. When present in food, antioxidants prevent the occurrence of rancidity and enhance shelf life. The most present macro-component in pastes and at the same time the least stable are lipids, present in the amount of 57.27% in the HP and 61.33% in the WP. The susceptibility of lipids to oxidation and the subsequent emergence of rancidity depends on the degree of unsaturation. Polyunsaturated fatty acids (PUFA) are present in the WP in the amount of 39.68%, instead of 11.51% in HP. The estimated shelf life of the hazelnut paste (HP) at the temperature of 20 ^{o}C is 678 days, whereas WP is stable at mentioned temperature for 219 days. The two most common synthetic antioxidants used in the food industry are butylated hydroxyanisole (BHA) and tert-butyl hydroquinone (TBHQ). The addition of BHA increased the shelf life of HP to 777 days and WP to 256 days. TBHQ improved oxidation stability of HP to 1291 days, but on the other side does not have any impact on WP (218 days). Natural antioxidant used in this research is residual espresso coffee grounds which improved shelf life of the WP the most (297 days) and HP to 795 days. Qualitative, nutritive characteristics and shelf life of the nuts pastes were significantly improved by using synthetic and natural antioxidants. The higher amount of PUFA in WP than in HP contributed to less oxidative stability.

Keywords: walnut paste; hazelnut paste; antioxidants; accelerated shelf life testing (ASLT)

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OLYPHENOLICS AND NUTRITIONAL PROFILE OF APPLE CULTIVARS (*Malus domestica*) FROM THE SERBIAN MARKET

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Apple (*Malus domestica*) is a temperate zone fruit, but on a global level, it is economically and culturally one of the most important fruit species. Regarding fruit production worldwide, apples (86 million tons) are ranked second after bananas (120 million tons). Apples are one of the most extensively produced and consumed fruits worldwide. Available on the market for the whole year, they represent a significant part of the diets and are an excellent source of nutrients. The health-protecting properties of apples have been mainly attributed to the presence of polyphenols. It is important to determine the polyphenolic profile, nutritional composition, and sugar content of apples whether they are for consumption or further processing into various products. Each apple sample was divided into at least 4 pieces and freeze-dried for at least 72 hours at 0.05 mbar. The homogenized powder was leached three times with 10% C_2H_5OH (2*3ml,1*2ml) each time for 30min on the tube rotator, centrifuged, and the supernatant transferred to the 10ml flask and made up to 10 ml with dist. H2O, diluted 1:25 filtered through RP18 columns and injected. By HPLC analysis 15 polyphenols, 10 organic acids, and sugars and sugar alcohols were determined in 7 different apple cultivars from the local market. The highest sugar content is contained in the Golden Delicious cultivar of 10.3%, while the lowest sugar content is found in the Granny Smith cultivar (7.6%). Granny Smith also contains the highest level of malic acid (6.96±0.48 g/kg FW). The cultivar Gala has the highest level of chlorogenic acid (156.37±41 g/kg FW), while the Granny Smith cultivar contains the highest level of catechin, epicatechin, and procyanidin B2 of 21.16±1.48, 59.53±7.65 and 92.96±7.65 g/kg FW, respectively. Obtained results provide detailed information on nutritional potential of tested apple cultivars and thereby could encourage their wider cultivation and consumption.

Keywords: apple, cultivar, polyphenols, sugars, organic acids

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IMPACT OF PLUM POMACE ADDITION ON ANTHOCYANIN CONTENT AND COLOUR OF PLUM SPREAD

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Plum spreads were prepared from purple-blue plum (*Prunus domestica*) cultivar 'Čačanska Rodna' in semi-industrial vacuum cooker at 50 °C and pasteurized at 85 °C (1h). This study aimed to evaluate the influence of incorporated lyophilized plum pomace powder on anthocyanin content as well as chromatic properties of functional plum spread, versus control. Pomace, a by-product of juice plum processing, is considered as a valuable source of polyphenols, particularly anthocyanins, which are antioxidants and natural pigments responsible for colour of purple-blue and red-skinned plum varieties. Total soluble solids (TSS) were measured with hand-held digital refractometer until they reached around 40 °Brix. Colour parameters (a*, b*, L*, C*, h*) were determined using MINOLTA Chroma Meter CR-400. Total monomeric anthocyanin content (TMA) was measured spectrophotometrically using the pH-differential method. In the control sample, all the monitored color attributes exhibited elevated levels. Consequently, the functional plum spread was more reddish and darker in comparison to spread without functional powder. Anthocyanins concentration was threefold higher in functional than in control sample (28.35 and 10.49 mg CRE/100 g d. w., respectively). Obtained results suggest that plum pomace lyophilisate represents a significant source of natural pigments as well as antioxidants, thus it could be used as colour modificator in plum-based food products.

Key words: plum pomace; plum spread; anthocyanins; colour; functional food

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ASSESSING FIBER LEVELS IN PLUM POMACE TO IMPROVE THE NUTRITIONAL VALUE OF PLUM-BASED PRODUCTS

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Plum pomace, primarily consisting of fruit skin, refers to the by-product obtained after plum juice processing. It has been recognized as a source of diverse functional ingredients, such as dietary fibers and phenolic phytochemicals. Dietary fibers are known for their various health benefits, including their positive effects on the digestive system, regulation of blood sugar levels, as well as weight issues. This study aimed to evaluate the fiber concentration in plum pomace after one and two processing cycles using a manual processor, in order to investigate its functional potential as a raw material for plum-based products. Dietary fibers were determined in both the plum fruit used for juice production and the obtained plum pomaces. The domestic Serbian cultivar 'Čačanska Rodna' was used in this research. The enzymatic-gravimetric method was employed to determine total dietary fiber (TDF). The TDF content in functional plum fruit was found to be 2.20 g/100 g, while the TDF content in pomace was higher. One juice processing cycle of plums led to a TDF concentration of 3.59 g/100 g (19.16 g/100 g d. m.) in plum pomace. After additional processing of the obtained pomace, TDF concentration increased to 6.33 g/100 g (29.46 g/100 g d. m.) in the material. This suggests that plum pomace can be effectively utilized for the development of a diverse range of plum-based products, enhancing the nutritional value by increasing their fiber content. Thus, after undergoing additional processing, the plum pomace may exhibit an increased concentration of fibers, making it a better material for the development of fiber-rich food.

Keywords: juice industry by-products; plum cultivar 'Čačanska Rodna'; plum pomace; dietary fibers; functional food

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IMPACT OF ASCORBIC ACID AND CITRIC ACID ADDITION ON CABBAGE FERMENTATION IN INDUSTRIAL CONDITIONS

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This study investigates the effects of adding ascorbic acid and citric acid on the cabbage fermentation process under industrial conditions. The experiment involves two sets of samples, where ascorbic acid and citric acid are added at the beginning of fermentation in one set of samples, and in the other set, they are added before fermented samples packaging. Basic physical, chemical, and biological characteristics of the fermented samples are analyzed at the end of fermentation, i.e., before packaging, and after three months of storage of packaged product. Significant differences are observed between samples obtained through the traditional method and those where ascorbic acid and citric acid are added during fermentation and packaging. The findings highlight the influence of these additives on the overall quality of fermented cabbage, providing valuable insights for industrial cabbage fermentation processes.

Keywords: ascorbic acid; citric acid; cabbage fermentation

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SENSORY ANALYSIS OF NUTRITIONALLY IMPROVED CORN-BASED SNACK PRODUCT WITH ADDITION OF PROTEIN- AND FIBER-RICH INGREDIENTS

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Three different samples of snack products, based on corn meal (80.7%), brewer's spent grain (BSG) (14.8%) and salt (0.5%) with different ratios (4-0%, 3-1%, 2-2%) of mechanically deboned poultry meat (MDPM) and chicken liver (CL) were produced in order to obtain nutritionally improved snack product and valorise by-product of brewing industry - BSG. Snacks were produced using co-rotating twin extruder at feed rate of 50 kg/h, screw speed 900 rpm, with temperature profile in zones 3/6 of 100/120 ºC, while moisture level of feeding mixtures was 15.5%. Palm oil in an amount of 15%, together with clean label seasoning with pizza, cheese and pesto flavour, were added and mixed using tumbler. Descriptive sensory analysis was performed with ten semi-trained panellist who used 100 mm linear scale in order to evaluate intensities of different taste, odour, flavour, physical and textural properties of produced snacks. Additionally, panellists used 7 point hedonic scale to evaluate overall liking of samples.

The obtained results indicated that difference in MDPM/CL ration did not have significant ($p>0.05$) influence on physical and textural properties between samples. The produced samples showed uniform colour, while strange odour did not detected in any of samples. The control samples without seasonings showed significantly lower intensity of overall odour and flavour, while the sample containing 3% MDPM and 1% CL with pesto seasoning was the one with the highest intensity of overall odour and flavour. The addition of BSG and CL did not deteriorate snacks taste particularly in terms of bitterness since the perceived bitterness in all samples was barely noticeable. Samples containing 3%MDPM and 1%CL with pizza and cheese flavour were the most liked. The used protein and fibre rich ingredients seem as promising strategy for production of nutritionally improved snacks since all samples showed high overall liking.

Keywords: sensory analysis; functional food; snack products; brewer's spent grain

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NUTRITIONAL PROFILE OF CRICKET POWDER AS AN ALTERNATIVE FEED INGREDIENT

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Over the past decade, the cost of protein sources used in the production of animal feed has doubled and now represents 60-70% of the overall expenses. Crickets, specifically the species *Acheta domesticus* within the Gryllidae family of Orthoptera, have their origins in Southeast Asia. They stand out as one of the most commonly cultivated insects, extensively employed as a source of food and feed among all known insect species. Considering the short breeding period and low energy requirements for rearing, these insects have significant potential for widespread use in both animal and human nutrition. This study aimed to explore the nutritional profile of full-fat cricket powder as a potential animal feed. Crickets were raised in well-ventilated plastic boxes with a water source. Each box had a base diet of wheat bran, supplemented every third day with either carrots (diet 1), cabbage (diet 2), or a mixture of carrot, cabbage, and flaxseed (diet 3). After being raised for 60, 67, and 74 days, the crickets were inactivated, dried, and milled. Results revealed that cricket powder is rich in protein, ranging from 64.10% to 73.81%, and low in lipids, ranging from 17.05% to 24.44%. Protein digestibility varied from 78.29% to 82.84%. Over the 74-day cultivation period, diets 1 and 3 positively influenced the protein content in cricket powder. Proximate chemical analyses suggest that cricket powder has the potential to fully replace soybean meal and partially substitute fish meal in animal feed formulations.

Keywords: insects; animal feed; *Acheta domesticus*; cricket powder

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CAMELINA SEED CAKE AS SOURCE OF PROTEINS

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The use of oilseed cake by products in feed and food industry has increased steadily over the past decade. According to its end uses, it can be classified as valuable source of protein that could be used for human and animal nutrition. The limited utilisation of these by products is either partly caused by, or further restricted by, the presence of anti-nutrients such as trypsin (protease) inhibitors, tannins, phytate, glucosinolates, saponins, and phenolic compounds. These compounds affect protein and mineral utilization by decreasing palatability, digestibility, or metabolism, and may even exert a toxic effect resulting in liver damage.

The aim of this study was the fractionation of camelina oil cake in order to obtain fractions with a reduced content of anti-nutrients and increased availability of nutrients such as proteins. Camelina oil cake has a high nutritional value with protein content of 36.3%. After fractionation it was obtained the following fractions: >250 μm; 250 180 μm and <180 μm. Protein content was changed through fractions and in the last fraction was 40.3%, which is a consequence of the removal of cellulose materials. Also, from camelina oil cake by alkali extraction was obtained protein isolate with protein content of 83%. These results suggest that camelina oil cake and camelina protein isolate may serve as natural functional ingredients in the food and feed industry.

Keywords: camelina oil cake; food and feed nutrition; proteins; fractionations; antinutrients

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ENHANCEMENT OF THE BIOACTIVE AND NUTRITIONAL PROPERTIES OF SOY PROTEIN CONCENTRATE THROUGH THE USE OF ENZYME TECHNOLOGY

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Soybean, a protein-rich leguminous oilseed, is often unacceptable due to its taste, appearance, and smell. Enzymatic technologies offer an alternative to conventional chemical procedures for protein modification, allowing for accurate oversight and adjustment of reaction flow to desired nutritional and bioactive properties due to the high sensitivity of protease under mild reaction conditions.

Herein, the feasibility of implementing a one- or two-step biotechnological process, triggered by commercial endo- and exo-peptidases, to enhance the nutritional and bioactive characteristics of soy protein concentrates (SPC), was investigated. Two nutritionally valuable fractions, the hydrolysate (liquid stream-fraction) and the ocara (solid stream-fraction), have been separated. The hydrolysates were characterized by examining the crude protein content, protein recovery, and free amino group content. The antioxidant activity was quantified by measuring superoxide radical inhibition, and metal-ion chelation.

Commercial peptidases led to different reaction kinetics and protein recovery, resulting in different peptide profiles determined by *dead-end* ultrafiltration (3, 10, and 30 kDa) and subsequent gel-filtration chromatography using the Toyopearl HW40F resin. Enzymatic hydrolysis seemed to enhance the hydrolyzate's protein content while decreasing the ocara's protein content. The percentage of soluble protein recovered from SPC ranged between 68 and 82%, revealing that Flavourzyme was most suitable to solubilize SPC. The highest yield of hydrolyzed peptide bonds, correlated with higher antioxidant activity, was shown by Alcalase-Flavourzyme (~20%) as well as Neutrase-Flavourzyme (~21.5%), with a tendency to favor Neutrase due to more sensory-acceptable product. Each produced hydrolysate's amino acid content, sulfhydryl groups, and surface hydrophobicity have been examined, and substantial correlations with antioxidant activity have been found. Because the quantity of phytic acid and trypsin inhibitor, the principal anti-nutritional components of SPC, was greatly reduced by enzymatic hydrolysis, the ocara were classified as value-added byproducts. Neutrase-Flavourzyme hydrolyzate's antioxidant activity was attributed to its large proportion of peptide fractions below 3 kDa.

Keywords: Soy protein concentrate; Enzymatic hydrolysis; Antioxidant activity; Anti-nutritional factors; Peptide profiles; Ultrafiltration; Gel-filtration chromatography

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ULTRASOUND INDUCED FUNCTIONALIZATION OF SOY PROTEIN CONCENTRATE

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Processing conditions for the fabrication of soy protein concentrates (SPCs) have a profound impact on the tightly packed, globular structure of soy proteins, which is reflected in the weakening of structural and functional properties, limiting their use in food systems. Many scholars have investigated the modification of soy protein, but this is the first time that high-intensity ultrasound technology has been used to address its limitations through improvement of the physicochemical properties of SPC. Therefore, the aim of this study was to develop an ultrasound-based method capable of producing SPC with improved functional properties making it a multifunctional ingredient for food systems intended for human consumption. The effects of high-intensity ultrasonication (20 kHz; 30% for 0.5; 2; 5 or 10 min) on the solubility, emulsifying properties, hydrophobicity, oil and water binding capacities and color of commercially available SPC were investigated.

Ultrasonic cavitation induced the restructuring of SPC, which was confirmed by significant changes in functional and structural properties. After ultrasonic treatment (30% amplitude for 5 min), the most significant shifts in solubility were observed. The emulsion fabricated with this restructured SPC was firm, stable, without perceptible phase separation, with emulsifying activity and emulsion stability of 1024.4 ± 10.6 m^2/g and 836.3 ± 12.2 h, correspondingly. Ultrasonic treatment of 30% amplitude for 2 min enabled SPC with best oil (3.26 ± 0.4 $g_{oil}/g_{protein)}$ and water binding capacity (5.04 ± 0.9 $g_{water}/g_{protein}$). Furthermore, the results additionally revealed that with the increase in sonication time the surface hydrophobicity of SPC increased first and then decreased. The value of a^* and b^* decreased significantly with the ultrasonic treatment time increment, while lengthened ultrasonic cavitation increased the L^* value. In conclusion, the functional and structural improvement of SPC endorsed the adequacy of ultrasonic cavitation in SPC modification.

Keywords: soy protein concentrate; functional properties; ultrasound; green technologies

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EFFECT OF OILSEED INDUSTRY BYPRODUCTS INCLUSION IN DAIRY RUMINANTS' NUTRITION ON RUMENIC AND VACCENIC ACID IN MILK

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Numerous studies have focused on enhancing the value of animal products, particularly by increasing the concentration of fatty acids (FA) in milk and dairy products, which have favorable effects on human health. The importance of vaccenic acid (VA) lies in its role as a precursor of rumenic acid (RA), one of the most relevant bioactive compounds present in milk fat. RA exhibits potential as an anticarcinogenic, anti-diabetic, anti-inflammatory, anti-obesity, and anti-atherogenic compound. Manipulating animal nutrition proves most effective in achieving the desired milk FA composition, with the inclusion of unconventional feed ingredients like oilseed industry byproducts (BP) being a successful strategy for modifying milk FA profiles. This review summarizes 41 available research studies on the utilization of hemp, pumpkin, sunflower, camelina, and linseed byproducts (meal, cake, and expeller) in dairy cows, sheep, and goats nutrition, and their potential to improve milk FA composition. The increase in both milk RA and VA, when compared to the control diet without BP, ranged from approximately 70% to over 600% for camelina BP and from 20 up to 100% for linseed BP. On the other hand, pumpkin seed BP had no influence on milk RA and VA. Sunflower BP demonstrated noteworthy potential in increasing desirable milk FA. Although less investigated, hempseed BP also has the potential to enhance the milk FA profile, but further research on its utilization in dairy ruminants is needed. Altering the FA composition of milk through the use of oilseed industry BP offers dietary advantages for humans without necessitating changes in consumer preferences and maintains the nutritional benefits associated with the macronutrients and micronutrients found in milk.

Keywords: hempseed; pumpkin seed; sunflower; camelina; linseed; byproducts; dairy ruminants; rumenic acid; vaccenic acid

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OBTAINING THE FUNCTIONAL EGGS BY CHOOSING SPECIFIC FEED INGREDIENTS IN DIET OF LAYING HENS

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Functional eggs, enriched with n-3 fatty acids (FA), natural pigments, vitamins, and possessing a good sensory profile, are a subject of ongoing research. The recommendation is for eggs to be rich in n-3 FA, aiming to decrease the n-6/n-3 ratio from the current 15-20:1 to 1-4:1. The FA composition in the diet of laying hens directly influences the FA composition in the eggs. Therefore, the aim of this study was to investigate the impact of incorporating flaxseed, camelina seed and hempseed into the hen's diet on the n-6/n-3 ratio. The experiment was set up in a production facility with 120 consuming laying hens divided into four treatment: control (C); treatment E1 with flax-corn meal co-extrudate (22,5%); treatment E2 with camelina-corn meal co-extrudate (27,6%); and treatment E3 with hemp-corn meal co-extrudate (30,7%). Each treatment had 6 cages with 5 laying hens, a total of 30 laying hens per group. The content of α-linolenic acid (n-3) in treatments E1, E2, and E3 (8.87%, 4.29%, and 2.78%, respectively) was significantly higher ($p<0.001$) than in control treatment (0.98%).The content of linoleic acid (n-6) was significantly lower ($p<0.001$) in treatments E1 (11.25%) and E2 (9.89%), while in treatment E3 (20.78%) it was significantly higher ($p<0.001$) than in the control treatment (14.90%). Additionally, the content of EPA and DHA in eggs from all experimental treatments were significantly higher ($p<0.001$): 0.21% and 2.08% for E1, 0.18% and 1.31% for E2, and 0.08% and 1.34% for E3, respectively, compared to eggs from hens fed with the control treatment (0.04% and 0.69%). The n-6/n-3 ratio of 1.01, 1.73, and 5.00 in treatments E1, E2, and E3, respectively, were significantly lower ($p<0.001$) than in the control treatment (8.88). The results obtained indicate that, by incorporating specific feed, it is possible to achieve a notable enhancement in the egg's FA profile, characterized by higher n-3 FA content and an improved n-6/n-3 FA ratio.

Keywords: functional eggs; n-6/n-3 ratio; flaxseed; camelina seed; hempseed

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EFFECT OF DEXTRAN COATING ON STRAWBERRY QUALITY DURING REFRIGERATED STORAGE

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The use of edible films and coatings made of natural materials such as polysaccharides provides a sustainable technological alternative to traditional plastic food packaging materials as one of the contemporary methods for preserving food items and guaranteeing their quality and freshness throughout their shelf lives. This is especially important for fresh fruits as highly perishable.

The aim of the present study was to investigate the effect of dextran-based coating on strawberry (*Fragaria* × *ananassa*) quality attributes during refrigerated storage. Dextran was synthesized using dextransucrase from *Leuconostoc mesenteroides* T3. Coating solution was made by dissolving 12g of dextran in 300 mL distilled water. Glycerol (25% w/w) and Tween 80 (5% w/w) were added as plasticizer and surfactant, respectively. Strawberries were coated by dipping in coating solution or water (control) and after drying were placed in plastic trays and stored at 8 °C. The influence of coating on strawberry quality was assessed through measuring the percentage of weight loss (WL), total soluble solids (TSS) and titratable acidity (TA) of the coated fruits and compared with the control uncoated samples.

The percentage of WL increased during storage in both coated and uncoated strawberries and although it was less for the uncoated strawberries, the difference was not significant. The TSS content was lower in dextran-coated fruit during the entire storage period. Since TSS is an indicator of the ripeness of the fruit it could be concluded that dextran coating slows down the ripening, thus increasing the shelf life. Unexpectedly, coated strawberries exhibited lower TA values. However, the relative changes were significantly smaller among the coated samples group, implying that the coating slows down the respiration process in which the acids are consumed.

Overall, the results of this work show that the dextran coating has the potential to preserve quality of strawberries during cold storage.

Keywords: edible coating; dextran; strawberry; shelf life; *Leuconostoc mesenteroides*

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VALORIZATION OF SOYBEAN MEAL FOR PRODUCTION OF HIGH PROTEIN ANIMAL FEED AND VALUE-ADDED PRODUCTS USING NEW STRAIN OF *AUREOBASIDIUM PULLULANS*

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By-products of soybean oil production are high-quality protein supplements for animal feed. However, they are rich in anti-nutritive factors and indigestible components, among which the special attention is focused on galactooligosaccharides, due to lack of α-galactosidase in monogastric animals. Enzymatic treatment of soy-based products and microbial fermentation are employed for overcoming these issues.

The main goal of this study was to apply fermentation with a selected strain of black yeast-like fungus (*Aureobasidium* spp.) of the soybean products in order to obtain high-protein soy-based animal feed. First, screening for an appropriate strain of microorganism among ten natural isolates from grapes has been performed. Keeping in mind complex structure of soybean polysaccharides the first selection criterion was the ability of growth in the presence of arabinose, xylose, galactose, mannose, raffinose, or soybean oil as sole carbon source. More important, enzymatic activity of *α*-galactosidase was detected in new isolates. The highest *α*-galactosidase producer was selected for cultivation on soybean meal. The selected strain was characterized in terms of physiological and technological properties and identified as *Aureobasidium pullulans* P8. In order to obtain high protein soybean meal, it has been utilized in two types of fermentation, i.e. solid state (SSF) and submerged (SMF) under varying conditions. Maximal protein content (61.11% based on dry weight) was obtained after 5 days of SMF at 30 °C and 10% of dry matter, while SSF produced 57.78% protein after 7 days of incubation at 30 °C with substrate contained 30% of dry matter. Extracellular enzymatic activities of cellulase, pectinase, amylase, xylanase and *α*-galactosidase were detected in supernatant after SMF, indicating its potential reusability for hydrolysis of new batch of soybean substrate.

This investigation revealed the versatile extracellular enzymatic potential of newly isolated black yeast-like fungus *Aureobasidium pullulans* P8 and its potential for production of high protein soybean meal.

Keywords: soybean meal; *Aureobasidium pullulans*; *α*-galactosidase; fermentation; black yeast-like fungus

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COMPARISON STUDY OF NETTLE (URTICA DIOICA) AND ROSEMARY (SALVIA ROSMARINUS) GROWN IN CROATIA AND CHINA IN TERMS OF BIOACTIVE COMPOSITION

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Nettle (*Urtica dioica*) and rosemary (*Salvia Rosmarinus*) have a long history of use in traditional medicine both in the area of Asia and Europe. Their functional properties are due to the presence of various biologically active compounds, including polyphenols. The aim of the present study was to compare the bioactive composition of nettle and rosemary grown in Croatia and China. The bioactive characterization included the determination of total phenolic content, antioxidant capacity (ABTS and DPPH assays) and chlorophyll content by applying spectrophotometric methods, as well as determination of individual phenolic compounds using HPLC-PAD methodology in the water extracts of investigated samples. The prepared extracts were also sensory evaluated and the most appropriate ones - in terms of bioactive composition and sensory acceptance, were used for the formulation of functional chocolate pralines. Total phenolic content in rosemary samples grown in Croatia and China was 55.53 and 54.42 mg GAE/g dw, respectively, while in nettle samples it was 15.30 and 6.52 mg GAE/g dw, respectively. The most represented polyphenolic compound in rosemary samples was rosmarinic acid with higher content in Croatian sample (13.16 mg/g dw) than Chinese (5.93 mg/g dw). In the nettle originating from Croatia, the dominant phenolic compound was caffeoylmalic acid (1.96 mg/g dw), but in the nettle originating from China, this compound was not present. Rosemary (from China) and nettle (from Croatia) extracts were successfully used as the main filling ingredient of chocolate pralines, since both types of newly formulated chocolate pralines were sensory evaluated as highly acceptable and their antioxidant capacity was increased by polyphenolic compounds originating from rosemary and nettle.

Keywords: chocolate pralines; nettle; polyphenols; rosemary

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NANOFILTRATION AS A TOOL FOR HIGH-YIELD PURIFICATION OF DIETARY OLIGOSACCHARIDES

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Fructo-oligosaccharides (FOS) are linear bioactive molecules consisting of one terminal glucose and other fructose units and belong to the group of prebiotics with scientifically proven benefits for human health. Physiological active compounds like trisaccharides (FOS3), tetrasaccharides (FOS4) and pentasaccharides (FOS5) can be produced from sucrose using enzyme-fructosyltransferase. The reaction mixture obtained in this way contains, in addition to the desired prebiotics, glucose, fructose and an unreacted amount of sucrose. Direct incorporation in products of food and pharmaceutical origin is limited due to the presence of unwanted carbohydrates (mono- and disaccharide), and to increase the functional value of the mixture, the attention was focused on their removal. In this regard, membrane separation techniques present a very suitable solution for obtaining a product with a higher FOS proportion. This study aimed to determine the optimal conditions for purification process of produced FOS mixture using nanofiltration membrane modules. Therefore, the influence of membranes with different MWCO (300-500Da and 600-800Da), temperature (20-45°C) and carbohydrate concentration in feed solution (1-5%) at a constant flow rate of 22 mL/min was investigated. From the initial mixture containing 60.2% FOS, 29.7% monosaccharides and 10.1% sucrose, after purification process under determined optimal conditions (membrane 300-500Da, temperature 35°C and carbohydrate concentration 3%), the product with 88.8% FOS, 10.0% sucrose and 1.2% monosaccharides in total carbohydrates was obtained. Purification yield was greater than 95% and the rejection coefficients for glucose, fructose, sucrose, FOS3, FOS4 and FOS5 were 0.17, 0.20, 0.95, 0.99, 1.00 and 1.00, respectively. Based on the calculated purification factor, it can be concluded that the initial mixture is refined 1.47 times. This result suggests a significant increase in the purity of the final mixture, which can be used as a sugar substitute or addition to numerous products that would be suitable for all consumers, including those suffering from diabetes.

Keywords: fructo-oligosaccharides, prebiotics, enzyme synthesis, optimization of nanofiltration process, membrane separation technology

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