BOOK of ABSTRACTS

2nd International Conference on Advanced Production and Processing

20th-22nd October 2022

Novi Sad, Serbia

Title:
Book of Abstracts of the 2nd International Conference on Advanced Production and Processing publishes abstracts from the following fields: Innovative Food Science and Bioprocesses, Nutraceuticals and Pharmaceuticals, Sustainable Development, Chemical and Environmental Engineering, Materials Design and Applications, Petroleum Refining and Production.

Publisher:
University of Novi Sad, Faculty of Technology Novi Sad,
Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

For publisher:
prof. Biljana Pajin, PhD, Dean

Editorial board:
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Editor-in-Chief:
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Design and Printing Layout:
Saša Vulić

CIP - Каталогизација у публикацији
Библиотеке Матице српске, Нови Сад

658.5(048.3)

INTERNATIONAL Conference on Advanced Production and Processing (2 ; 2022 ; Novi Sad)
Book of abstracts [Elektronski izvor] / 2nd International Conference on Advanced Production and Processing, 20th-22nd October 2022, Novi Sad ; [editor-in-chief Zita Šereš]. - Novi Sad : Faculty of Technology, 2022

Način pristupa (URL): https://www.tf.uns.ac.rs/download/icap-2022/book-of-abstracts.pdf. - Opis zasnovan na stanju na dan 14. 10. 2022. - Nasl. s naslovnog ekrana.

ISBN 978-86-6253-160-5

a) Технологија - Производња - Апстракти

COBISS.SR-ID 77341961

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3D PRINTABLE FOODS USING PLANT-BASED JAMMED EMULSIONS

Simha Sridharan, Costas Nikiforidis

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3D printed materials are of great relevance for the production of therapeutic and specialized foods. An approach to forming 3D printable materials is to use jammed oil droplets. Jammed oil droplets are highly viscous and can be extruded through the nozzle of a 3D printer, while after chemical crosslinking they acquire a self-standing ability. However, the molecules currently used to stabilize and cross-link the oil droplets have questionable biocompatibility. Therefore, our research aims to produce 3D printable jammed emulsion using pea proteins. Pea protein stabilized emulsions were extremely stable with monomodal droplet size distribution. The jammed emulsions showed gel-like visco-elastic rheological properties. However, upon 3D printing, the material was not self-standing owing to the lack of plasticity. In order to induce plasticity, we used self-associating properties of pea proteins by pH trigger. Our previous work has shown that pea proteins self-associate into adhesive protein particles at pH 3. The adhesive nature of pea proteins could aid in creating additional droplet-droplet interaction to create plasticity in the jammed emulsions. Therefore, jammed emulsions in the presence of pea protein particles were formed by simple pH adjustment. These emulsions with protein particles formed a material with elastoplastic rheological properties. The emulsion flowed smoothly above a critical stress, and after extrusion exhibited the required self-standing properties for 3D printing. The adhesive protein particles act as physical cross-links between the jammed oil droplets, creating the plasticity necessary for 3D printing. In this research, we show that by understanding the associating properties of pea proteins and their behavior in bulk and on interfaces, pea protein-based 3D printable material was created for the first-time.

Keywords: 3D printing, Plant-based foods, Proteins, Rheology

Acknowledgements: The work is featured at the cover of the Journal Advanced Functional Materials: https://doi.org/10.1002/adfm.202101749

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THE CHALLENGE OF ASSESSING CONTAMINANTS OF EMERGING CONCERN IN THE ENVIRONMENT

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The list of contaminants of emerging concern (CECs) has steadily increased during the last decades including a broad spectrum of organic and inorganic compounds showing different physicochemical and toxicological properties. Some of the more prominent groups include persistent and mobile organic compounds (PMOCs), polar pesticides, pharmaceuticals, flame retardants, a significant number of chemical groups employed as plastic additives, personal care products, anthropogenic particles including nanomaterials and micro-and nano plastics, among others. For the analysis of organic chemicals liquid chromatography separations coupled with mass spectrometry analysers (LC-MS), have been the techniques of choice for environmental analysis. Nowadays, thanks to its unique ability to measure analytes based on accurate mass, full-spectrum high-resolution mass spectrometry (HRMS) can simultaneously gain qualitative and quantitative information on a virtually unlimited number of analytes.
In this presentation, several examples of the analysis of CECs in the different environmental compartments using targeted, non-targeted and suspected screening approaches will be presented.

Keywords: *CECs, environmental samples, LC-MS, PFASs, Nanomaterials, Natural toxins, Micro-nano plastic*

Acknowledgements: Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or EU executive agency. Neither the European Union nor the granting authority can be held responsible for them.

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MEMBRANES IN BIOPROCESSING

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This lecture discusses how different membrane functionalities impact on their performance. Similarly to biological membranes, synthetic membranes may be designed in order to organize the physical space, allowing for defining different "compartments" and regulating the transport of diverse (bio)chemical species between them. This permselectivity behaviour results from specific functionalities of membranes, which may be achieved by designing their morphology, chemical character and topography. Transport regulation may be achieved by making use of different mechanisms – size exclusion, electrostatic interactions, affinity interactions -, that determine the rate of selective transport of different species. This lecture discusses the structure-function relation in synthetic membranes and how this relation can be used in favour of specific membrane processes. Different case-studies will be discussed, namely for membrane bioreactors targeting specific solutes and for membrane contactors regulating the transport of ionic species. The use of membrane contactors in order to induce protein crystallization will be discussed. The relevance of membrane monitoring using molecular probes will be also addressed for the monitoring of local oxygen concentration and temperature, making possible to acquire concentration and temperature profiles inside membranes and/or at their surface. Finally, the need for membranes that after being used and disposed can be easily biodegraded, avoiding the accumulation of synthetic polymers in landfills, with potential contamination of soils and water bodies, will be addressed. The development of biopolymeric membranes, that should be sufficiently stable to assure a prolonged operating lifetime but, simultaneously, easy to degrade when disposed will be discussed.

Keywords: Membrane, Transport regulation, Monitoring, Biopolymeric.

Acknowledgements: "Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or EU executive agency. Neither the European Union nor the granting authority can be held responsible for them. This study is conducted under the project TwiNSol-CECs that has received funding from Horizon Europe programme under grant agreement no.101059867."

Innovative Food Science and Bioprocesses

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YEAST EXTRACT – FOOD OF THE FUTURE

Eleonora Zabos

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Yeast extract is a common name for various forms of processed yeast products used as food additives and flavour enhancers to improve sensory properties and nutritional value (protein, carbohydrates, amino acids, nucleotides, vitamins, and minerals). The production of yeast extract begins with the propagation of yeast cells in laboratory conditions, and continues through pure culture in large scale, first to mother yeast cream, then to the commercial fermentation. Recently, they have been used as a substitute for sodium glutamate, as an adjustment to a healthy diet. Certain types of products may contain free glutamic acid. Glutamic acid in yeast extract is produced in the fermentation cycle of certain yeast strains. It is often used in the production of soups, sauces, seasoning, sausages and pate, ready to go foods, prepared meals, and snack products. Nowadays, it is increasingly used in the pharmaceutical and cosmetic industries. Autolysis is a process in which cellular components are broken down by enzymes present in yeast cells, and then converted into a liquid form. Washing of the cell wall by separation process gave a liquid crude extract, which contains all the components of cell juice. The consistency of the finished product varies from liquid form to paste-like consistency. It is also commercially available in a powder form. In terms of flavour, yeast extracts often have a very strong and salty flavour. Yeast extract is also safe in terms of allergenicity. Yeast extract is not only a natural but also a safe ingredient with a long history of safe use.

Keywords: Yeast exract, Fermentation, Autolyses

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INNOVATIVE TECHNIQUES FOR THE EXTRACTION AND EXPLOITATION OF BIOACTIVE INGREDIENTS IN FOODS

Ioannis Mourtzinos*, *Stamatia Christaki*, *Anastasia Kyriakoudi

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The conventional recovery methods of bioactive compounds from various plant materials tend to be replaced through the last years, in light of the environmental crisis and the search for more sustainable green processes. In terms of Green Chemistry principles, innovative extraction methods have been developed and proposed aiming to replace the conventional ones, regarding the reduction of time, energy, and solvent consumption. In addition, further technological development has enabled the use of novel exploitation techniques for the optimal application of the obtained bioactive ingredients in different food systems.

Among innovative extraction techniques, ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid/subcritical water extraction, hot pressurized liquid extraction and enzyme-assisted extraction are commonly used for the recovery of bioactive compounds (e.g., terpenes, polyphenols, and carotenoids) from various materials. Their advantages include limited extraction time and subsequently low energy consumption, high efficiency, simplicity of use, protection from degradation of thermosensitive bioactive compounds and low solvent consumption. Recently, novel extraction techniques based on electrotechnologies have been proposed, as they are entirely compatible with the Green Chemistry statements, offering a variety of benefits regarding extraction yield, thermal stability, and operational costs. These nonthermal technologies are based on the application of electric field and include pulsed electric field (PEF) and cold plasma-assisted extraction. In addition to extraction techniques, innovative, eco-friendly, natural solvents, such as aqueous cyclodextrin solutions, glycerol and deep eutectic solvents (DES), are being preferred over conventional, organic solvents, in terms of sustainability and compatibility regarding food applications.

The obtained bioactive compounds, following their recovery using green extraction technologies, are being further exploited in the food industry as natural antioxidant and/or antimicrobial agents and pigments. Novel encapsulation techniques and various carriers have been proposed for the protection and easier application of these bioactive compounds in different food systems, overcoming limitations such as thermal instability and intense organoleptic characteristics. Nanotechnology-based systems, such as nanoparticles and nanofibers, are widely preferred as delivery systems, employing ionic-gelation technique and electrospinning, respectively for their production.

Keywords: Bioactive compounds, Green Chemistry, Novel extraction methodologies, Encapsulation, Sustainability

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PROCESS OF SETTING UP QUALITY SYSTEMS DURING A GREENFIELD FOOD PRODUCTION PROJECT

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During a Greenfield project of a food production factory it's important to include all the elements of Food safety and Quality system requirements during a project planning phase. Key elements are presentenced in the form of a timelines. Main streams are explained in details: construction & layout, food safety, laboratories setup, supplier management, quality organization, certification, hold & release, customer support and training plan. Inputs are based on literature and experience, industry expertise, intercompany support, cooperation with science and educational institutions and government institutions.
Planning in advance and on time, with multidisciplinary approach can assure that food production facility is prepared to operate in accordance with all quality and food safety prerequisites, with maximal utilization from beginning and no need for additional investments cost.

Keywords: Food safety, Quality, Greenfield project

Acknowledgements: Barry Cellebaut.

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MILK PEPTIDES AS POTENTIAL INHIBITORS OF *E. COLI* RNA POLYMERASE (RNAP) AND DNA GYRASE

***Jovana Degenek*[1], *Vladimir Vukić*[1], *Katrina Kanurić*[1], *Mirela Iličić*[1], *Dajana Vukić*[1], *Salo-Ahen Outi*[2]**

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Peptides of milk origin serve not only as nutrients, but can also affect certain physiological functions. Recently, antimicrobial peptides have been in the middle of scientific interest due to the fact that they can find use as novel drug candidates. It was proved that fresh cheese peptides showed antimicrobial effects against *Escherichia coli*, so the aim of this investigation was to define which peptides, formed during milk fermentation, can have that activity. For that purpose, two possible antimicrobial target molecules have been identified - RNA polymerase (RNAP) and DNA gyrase (DNAGYR) of *E. coli.*

The milk derived peptides database was constructed by building 3D models, based on their amino acid sequences (100 peptides). Peptides were prepared by use of LigPrep, Schrödinger package. Structures of target molecules were retrieved from the PDB database – RNAP with PBD ID: 6XLL and DNAGYR with PDB IDs: 6RKS and 4DUH. Target molecules were prepared by use of Protein Preparation Workflow. Receptor Grid Generation tool was used for targeting binding sites. Molecular docking simulations were performed by Glide (with extra precision), after which binding energy calculations were done by the Prime/MMGBSA method. After the molecular docking process with extra precision was completed, visual inspection of peptides in different poses and their selection for the calculation of binding energies followed. On that occasion, groups of peptides were selected for each binding site. In total, 287 peptide poses were selected. Values of calculated binding energies ranged from -75.02 to -59.90 kcal/mol. Subsequently, based on the highest predicted binding energies and visual inspection of binding poses, 8 peptides were selected to form protein-peptide complexes with RNAP and 12 with DNAGYR.

According to the obtained results of binding energies, the selected milk peptides show a high affinity for the formation of the protein/peptide complexes, and thus for the tendency to inhibit the action of RNAP and DNAGYR.

Keywords: Fresh cheese, Peptides, Antimicrobial activity

Acknowledgements: This work was supported by program of Ministry of Education, Science and Technological Development of the Republic of Serbia (grant number 451-03-68/2022-14/200134)

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TESTING ANTIMICROBIAL 3D PRINTING FILAMENTS FROM FOOD SAFETY ASPECT

***Igor Gáspár*[1], *Ivana Pajčin*[2], *Flóra Vitális*[1], *Vanja Vlajkov*[2], *Aleksandar Jokić*[2], *Dragoljub Cvetković*[2], *András Koris*[1], *Jovana Grahovac*[2]**

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3D printing is among the fastest growing additive manufacturing technologies with wide range of possible applications in food industry. The biggest safety risk is imposed by microbial contamination in gaps between the 3D printed layers in applications where direct food contact is required for 3D printed objects. The first proposed method to prevent or reduce the emerging microbial contamination is coating the 3D printed object with epoxy resin, resulting in smooth, cleanable surface like any other injection moulded object. The main limitation of this method is that every point of the object has to be coated, while sometimes is hard to cover the inner object surfaces. The second method is heat treatment of the 3D printed object, but this method requires usage of special heat-resistant materials, which can withstand boiling temperature.
The method presented in this study is based on application of antimicrobial filaments for 3D printing of milk cups. The microbial contamination was investigated by the surface swab method after 10 days of simulated kitchen usage, with every day 15 min-contact with milk, followed by washing with tap water and dish soap. Regular polylactic acid cups were used as a reference material. Three different filaments based on metal ions, which reduce microbial reproduction, were tested (Copper 3D: PLActive, Purement antimicrobial filament and Philament antibacterial PLA). The results of this study have shown a significant reduction of microbial presence (6-fold reduction of CFU/cm^2) on the 3D printed cups made from antimicrobial filaments compared to standard PLA cups.

Keywords: Food safety, 3D printing, Antimicrobial filaments, Food industry, Microbial contamination

Acknowledgements: The research was supported by Hungarian and Serbian bilateral scientific and technological cooperation project funded by the Hungarian National Office for Research, Development and Innovation (NKFI, 2019-2.1.11-TÉT-2020-00249) and Ministry of Education, Science and Technological Development of the Republic of Serbia.

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SINGLE-STEP CLARIFICATION AND RECOVERY OF ALGAL PROTEIN HYDROLYSATES BY TANGENTIAL FLOW ULTRAFILTRATION

Zivko Nikolov, Laura Soto-Sierra

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Current lifestyle trends and increasing demand for high-quality vegetarian food products and health-promoting nutraceuticals have brought both leafy plants and microalgae at the forefront of alternative food sources. Protein hydrolysates are one of the most valuable products that can be obtained from delipidated microalgae. The advantages of protein hydrolysates over other extracted protein products from microalgae include enhanced solubility, digestibility, and potential bioactivity. The previous process simulation work (SuperPro Designer, v12) revealed that direct hydrolysis of delipidated algal biomass (LEA) was the most economical route to obtain algal protein hydrolysates at a cost of less than $5/kg. In this study, we compared tangential flow filtration (TFF) as a single-step process alternative to the traditional centrifugation, precipitate wash, and depth filtration process.

The direct hydrolysis process of lysed LEA consisted of acidic precipitation of insoluble impurities followed by centrifugation, and depth filtration. To maximize the yield of hydrolyzed protein, the precipitated material was centrifuged, washed with water to release trapped peptides, and then again centrifuged. Combined supernatants were clarified by depth filtration to yield a hydrolysate that was free of chlorophyll pigments. TFF was performed using Spectrum KrosFlo KR2i system with 1mm i.d. hollow fibers. LEA hydrolysate slurry was concentrated by TFF ultrafiltration-until the solids concentration in the retentate reached ~ 200 g-DW/L. Following the concentration of the slurry, the Spectrum hollow fiber system was operated under continuous diafiltration mode for 2 diafiltration volumes (DV).

The results demonstrated that tangential flow ultrafiltration process was superior to the traditional protein fractionation method in several ways. First, the membrane diafiltration process uses a single and easily scalable unit operation (tangential flow filtration) to separate and “wash out” hydrolyzed protein from the algal residue. Second, the protein recovery yield achieved with the tangential flow process was >70 % compared to 64% previously achieved by centrifugation and dead-end filtration method. Finally, *membrane filtration-processed protein hydrolysates* were more heat and pH stable than those produced by the traditional centrifugation – depth filtration process.

Keywords: Microalgal protein, Protein hydrolysates, Membrane processing, Diafiltration

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ENRICHMENT OF WHITE ĆUPTER WITH FATTY ACIDS: EFFECTS OF ADDITION OF BREWERS' SPENT GRAIN

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The possibility of the incorporation of brewers' spent grains into the human diet has already been investigated and published. Ćupter is Herzegovinian candy made of must and flour/semolina. The incorporation of brewers' spent grains into the ćupters production in terms of nutritive, chemical, and sensory analysis of the product have only been subject of research until now in a small number of studies. Polyunsaturated fatty acids, such as linoleic and oleic acids, were found in significant amounts in brewers' spent grains which was confirmed in this study. The aim of this research was to observe changes in fatty acids composition of white ćupters produced with brewers' spent grains addition. Semolina (Samples 1 and 2) and flour (Samples 3 and 4) were partially replaced with brewers' spent grains. Brewers' spent grains was originating from industrial (Samples 1 and 4) and craft breweries (Samples 2 and 3). Values for fat were higher in brewers' spent grains originating from the industrial brewery but a more significant increase in fatty acids composition was detected in samples produced with brewers' spent grains originating from a craft brewery.

Keywords: Brewers' spent grain, Fatty acids; Ćupter, Traditional product, Grape must

Acknowledgements: The authors wish to thank Trojanska and Hercegovačka brewery for the donation of brewers' spent grain and Agroodak for the donation of grape must.

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INSECTS AS NEW GENERATION PROTEIN SOURCES: NOVEL PRODUCTION AND CHARACTERIZATION APPROACHES

***Hami Alpas*, Mecit Halil Oztop, Berkay Bolat, Ahmet Erdem Uğur**

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This study aimed to investigate the novel production and characterization approaches of two edible insect species with high protein and nutritional value; *Acheta domesticus* and *Tenebrio molitor*. The two insect species include 36 and 60% of crude protein in granulated form respectively. In this study, emulsion properties (stability and capacity), fat and water holding capacity of the proteins were analyzed. Glycating protein with sugars is known to improve the physical properties of proteins. Simultaneous glycation of monosaccharides (glucose and fructose) with the proteins extracted from these two insect species were performed using high hydrostatic pressure (HHP). Glycation was also performed conventionally just by using incubator at 50 ºC. HHP was performed at 2 different pressure and temperature levels. Following HHP treatment, proteins were incubated at 50 ºC for 6, 12 and 24 hrs. Solubility, foaming, emulsifying ability of the glycated proteins were examined. Hydration and gelling behavior were explored through Nuclear Magnetic Resonance (NMR) Relaxometry experiments (T_2 relaxation times). These results possibly provide a comprehensive solution for more nutritious foods for the mankind due to possible shortage of food source, raw material and reducing the Food Loss and Waste (FLW).

Keywords: High hydrostatic pressure (HHP), Acheta domesticus, Tenebrio molitor

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EXTRACTION OF POLYPHENOLS FROM OLIVE POMACE OF ŽUTICA VARIETY

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On a global scale, waste valorization is receiving more and more attention. In the plant processing industry, the technologies that generate waste can be minimized or utilized are increasingly emphasized. Olive oil industry generates more than 30 m^3 of wastewater and 20 million tons of pomace. Wet pomace contains polyphenols as bioactive compounds. Nevertheless, several studies have shown the importance of phenolic compounds in the prevention or treatment of human diseases due to their potential antioxidant activity. In this regard, this study focuses on the extraction of polyphenols from olive pomace obtained from the two-phase process of the endemic Montenegrin variety Žutica.
The effect of drying temperature was evaluated. Central Composite Design of experiment was used for the evaluation of three factors during the conventional solid-liquid extraction of polyphenols from the olive pomace, this is temperature (40 – 80 °C), ethanol concentration (10 – 90 v/v%) and solids concentration (2 – 12 g/100 mL). Continuous stirring (215 rpm) was used during the two-hour leachings, the solvent evaporation loss was obviated by a cover. Spectrophotometric Folin-Ciocalteu Method and FRAP method were applied to determine the Total Phenolic Content (TPC), and the Antioxidant Activity (AA) of extracts.
As results of the preliminary experiments, decreasing the drying temperature from 60 to 40 °C, increased the TPC value more than 300 %. The highest experimental values of TPC and AA were achieved at 50 v/v% ethanol, 60 °C and 12 g pomace /100 mL solvent. These maximum values were 503.62 mg GAE/L and 815.96 mg ASE/L. Furthermore, model fitting through ANOVA analysis suggested quadratic model for the prediction of both, TPC and AA responses, with correlation coefficients (R^2) of 0.9801 and 0.9569, respectively. Solids ratio variable was identified as the most relevant factor, while temperature had higher effect on antioxidant activity than polyphenol content.

Keywords: Polyphenol, Antioxidant activity, Extraction, Olive pomace, Waste management

Acknowledgements: The authors acknowledged the European Union and the European Social Fund (grant agreement no. EFOP-3.6.3- VEKOP-16-2017-00005), the Tempus Public Foundation under the Stipendium Hungaricum Scholarship Program for their financial support and the company Uljara Metovic for the donation of the olive pomace for this research.

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EFFECT OF NITROGEN FERTILIZATION ON SOYBEAN FLOUR QUALITY

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Legumes, in particular soybean is considered as an important source of protein. In order to increase their production yield, nitrogen (N) is an essential fertilizer, the major limiting nutrient. Additionally, nitrogen is an important entity in proteins. Hence, the evaluation of the effect of nitrogen fertilization on soybean flour and its protein is of considerable interest.
Field experiments were designed to determine the effect of three different levels of N fertilization (0, 70, and 140 kg N ha^{-1}) on soybean grain and the physicochemical, functional and rheological properties of soybean flour were evaluated. The results indicated that the N fertilization did not affect its ash content but increased the moisture content. The water and oil absorption capacity exhibited insignificant effect of N fertilization at all the levels. The color measurement revealed that the supplementation with 70 kg and 140 kg of N significantly decreased the L* (lightness) value of soybean grain flour, while it increased the *a** (redness) value. However, the 140 Kg of N dose significantly increased the *b** (yellowness) value when compared with the control sample.
Although the results showed that the N fertilization did not have a strong impact on the soybean flour quality, especially on the functional proprieties, it is interesting to study their effect on soybean protein properties. Therefore, the study of the functional proprieties of soybean protein such as emulsion capacity, stability and foaming capacity and stability are still being assessed so that its effective application as food ingredient can be established.

Keywords: Soybean, Protein, Nitrogen fertilization, Physicochemical properties, Functional properties

Acknowledgements: The authors are grateful to the Stipendium Hungaricum for their financial support.

ICAPP 2022
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EGG – SUPERCHARGED FOOD PRODUCT

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Table egg is one of the best supercharged food product. Egg is an excellent source of quality proteins, vitamins (A, B, D, E, K), folates, choline, lecithin, minerals (phosphorus, zinc, iron, selenium, iodine) antioxidants, zeaxantin and lutein carotenoids, important for reduction of age related macular degeneration and also have many health promoting, imunostimulating, therapeutic and functional properties. This mini review provides novel information for the promotion of nutritive value of eggs, enriched with bioactive components and with improved quality. Supplementation of layer diets with increased amount of the bioactive components resulted with produced eggs as functional food with potential benefits on the human health.

Key words: Eggs, Bioactive components, Functional food

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PLASMA ACTIVATED WATER AS A NOVEL DECONTAMINATION METHOD FOR WATER AND FOOD CONTACT SURFACES

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Cold atmospheric plasma has been attracting attention as a green technology for the inactivation of microorganisms in recent years. This study evaluated the efficiency of a novel method based on the use of plasma-activated water (PAW) in inhibiting *Pseudomonas aeruginosa* as an opportunistic foodborne pathogen that can cause severe human infections. Specifically, the study evaluated the effectiveness of PAW, produced by a plasma bubble reactor, in reducing *P. aeruginosa* planktonic cells in tap water and biofilms grown onto stainless steel (SS) coupons. Planktonic cells and biofilms were treated with PAW at different discharge frequencies (500–1500 Hz) and exposure times (0–20 min). Both planktonic cells and biofilms were significantly reduced after PAW treatment, achieving higher reductions with higher exposure times and discharge frequencies. Furthermore, PAW treatment led to a gradual reduction, higher than 4-Log, for young (24 h old) and mature (48 and 72 h old) biofilms, and planktonic cells of *P. aeruginosa* after 20 min. The experimental data were used to develop two predictive inactivation models indicating that bacterial reduction depends on discharge frequency and exposure time. This work illustrates the first conclusive evidence supporting PAW's potential to inactivate both planktonic cells and biofilms of *P. aeruginosa* in water and on stainless steel, respectively. These results can pave the way toward the development of novel green decontamination options based on PAW, to control *P. aeruginosa* and other pathogens in food processing environments, and thus improve food safety.

Keywords: Pseudomonas aeruginosa, Biofilm, Planktonic cells, Foodborne pathogen, Plasma-activated water

Acknowledgements: We would like to thank the University of the West of England Bristol for funding this study.

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QUALITY OF POULTRY MEAT - DEFECTS AND MYOPATHIES OF BROILER BREAST MEAT

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The aim of the study was to analyze physicochemical and histopathological changes and to evaluate selected microbiological risks of the chicken breasts with myopathies or defects. The tests included visual determination of the occurrence of quality defects, physicochemical analysis of the breast muscles, microbiological evaluation and determination of antibiotic resistance of selected bacteria isolated from the intestinal contents of broiler chickens.

Changes in color parameters and pH were observed in the breast muscles of chickens with meat quality defects. In defective meat, especially with bloody bruises, on the stained polyacrylamide gel, after electrophoretic separation, clear bands are observed corresponding to protein substances with a molecular weight above 60 kDa, which are not visible in normal meat. In the case of meat with white stripes, additional bands of proteins with a mass of approx. 220 kDa can be observed. Statistical analysis of the results of the assessment of the intestinal microflora of broiler chickens proves that the presence of petechiae has an influence on the reduction of the amount of beneficial bacteria of the genus *Lactobacillus*.

There was no correlation between the MICs of colistin, cefotaximone or meropenem responsible for antibiotic resistance in *Escherichia coli* isolates due to defects in chicken breast muscles.

Keywords: Broilers, Pectoral muscle, Meat defects

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ARSENIC IN FEMALE CATTLE LIVERS AND KIDNEYS FROM VOJVODINA, NORTHERN SERBIA

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Arsenic (As) occurs naturally in the environment and is present in soil, ground water and plants. As occurs in a broad variety of As compounds, of which inorganic As is the most toxic form. Inorganic As has been classified by the International Agency for Research on Cancer in group 1 as carcinogenic to humans. The European and Serbian legislation has established maximum permissible levels for inorganic As only in rice and rice products. EFSA panel on contaminants in the food chain established a tolerable weekly intake for As of 15 µg/kg body weight.
Samples (liver and kidney) were collected from 26 cattle slaughtered at the slaughterhouse in Novi Sad (Vojvodina, northern Serbia), during 26 consecutive weeks (i.e. samples from one animal per week were collected). All animals were slaughtered for human consumption. Slaughtered heifers and cows (female cattle) came from 26 different farms for milk production (i.e. one animal per farm was sampled) in Vojvodina (northern Serbia), so it can be stated that samples of liver and kidney were collected from the whole region. Information about animals (date of birth, sex and type of the animal) were received from farms with copy of the passport. The investigated cattle aged from 412 to 2502 days. Samples (liver and kidney) were collected from the same cattle and minced in a stainless-steel cutter. After homogenization, approximately 250 g of samples were taken for analysis. Samples were vacuum packed in plastic bags and stored at constant temperature (-80°C) until determination of As. As content was determined using ICP-OES (inductively coupled plasma-optical emission spectrometry) method, after digestion by microwave. A strict analytical quality control programme was employed during the study. The As concentrations in the livers and kidneys ranged from below detection limits (LOD < 0.0030 mg/kg) to 0.0246 mg/kg wet weight and from below detection limits (LOD < 0.0030 mg/kg) to 0.0432 mg/kg wet weight, respectively.
Thus, content of As in edible offal (liver and kidney) can be used as relevant indicator of environmental contamination by As. Generally, monitoring and control of As in living organisms, i.e. red meat and edible offal, is necessary.

Keywords: Arsenic, Liver, Kidney, Cattle, Vojvodina

Acknowledgements: This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia, under Grant 451-03-68/2022-14/200134.

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AUTO-ML GC/MS FINGERPRINTING STRATEGY FOR CEREAL FLOUR AUTHENTICATION

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Despite food authentication being a global challenge since decades, not much work has been done in developing authentication methodologies of cereal flours and bakery products. This research represents an innovative and rapid method for classifying types of non-gluten and gluten-containing cereal flours: 10 corn, 5 wheat, and 5 barley samples. To achieve this aim, a gas chromatography – mass spectrometry (GC/MS) instrument was coupled to an automated machine learning algorithm (AutoML). Grains were sampled from the experimental fields of the Institute of Field and Vegetable Crops in Novi Sad, Serbia. Cereals were milled into flour, after which liposoluble matter was extracted with *n*-hexane, and derivatized into corresponding volatile compounds using a 0.2 M trimethylsulfonium hydroxide solution. Total ion current chromatograms consisting of 1666 datapoints/scans were used as raw signals, each of them representing a unique fingerprint of a cereal class. However, the aim of this work was to apply the Weka open-source software in automated mode, as a single, highly parametric machine learning framework for classifying types of flour into classes defined by botanical origin and gluten content. This was achieved using an Auto-Weka package with a state-of-the-art Bayesian optimization method, thus solving the combined algorithm selection and hyperparameter optimization (CASH) problem. The Weka's learning algorithm took into account all classifiers provided by the software: 27 base learners, 10 meta-methods, and 2 ensemble methods. Both 60 and 120 min time-budgets were carried out by the computer unattended. In each case, a Support Vector classifier (SMO) using normalized polynomial kernel was recommended as the most optimal, using a 10-fold cross-validation to exploit the performance gains on a given dataset. Cereal flour samples were adequately classified in 3 groups: non-gluten corn, and gluten wheat and barley. The presented approach directly supports the application of artificial intelligence on processing chemical information, in order to develop methods for food authentication.

Keywords: Authentication, Automated machine learning, Gas chromatography – mass spectrometry, Cereal flour, Classification

Acknowledgements: The presented research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Programs no. 451-03-68/2022-14/200134, 451-03-68/2022-14/200222).

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THE EFFECT OF PRETREATMENT AND MATRIX ON THE *IN VITRO* DIGESTIBILITY, NUTRIENT BIOAVAILABILITY, AND ANTIOXIDANT PROPERTIES OF CEREAL GRAIN-BASED FOOD INGREDIENTS

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Regardless of the continent, nation, or dietary preferences, cereals have, for centuries, been among the indispensable staple food ingredients. The value of these grains is immeasurable for humankind, given that they provide energy, carbohydrates, proteins, fibers, minerals, and vitamins to the majority of the world's population. However, the digestibility and bioavailability of the nutrients, as well as bioactive compounds are some of the factors that may influence the utilization of these grains by large. The main goal of this study was to investigate in what manner the pretreatment and food matrix may influence the *in vitro* digestibility, nutrient bioavailability, and antioxidant properties of cereal grain-based ingredients intended for human consumption. Samples of six wheat varieties with different amylose content: untreated, ultrasonicated, and hydrothermally treated wholegrain flour; samples of differently colored maize grain (yellow, white and red dent, blue and yellow popcorn and one sugary genotype): wholegrain maize flour - untreated and toasted at different temperatures (100˚C, 125˚C and 150˚C), maize kernel fractions - pericarp, endosperm and germ; blue popcorn powder – a by-product of the grain milling process remaining on the sieve, as well as the anthocyanins extracted from the blue maize powder and microencapsulated by spray drying on different polysaccharide carriers, were used in this study. The *in vitro* multi-step digestion protocol consisting of oral, gastric, duodenal, and colon phases was applied. The amylose content negatively influenced the digestibility of the hydrothermally treated flour, while the total starch content had a negative impact on the digestibility after the ultrasound treatment. The untreated and toasted maize flour samples showed differences depending on the genotype, kernel color and the applied temperature, with digestibility ranging from 26.91% to 35.34%. Furthermore, wholegrain flour of sugary maize hybrid showed the highest digestibility (57.36%). Regarding digestibility of different kernel fractions, germ manifested the highest degradation level, while fiber-rich pericarp was the least affected by digestion processes. The anthocyanin extract microencapsulated on the carrier consisting of the combination of maltodextrin and hydroxypropyl-β-cyclodextrin showed the highest *in vitro* digestibility (73.63%) in comparison with pure extract (68.45%) and the blue popcorn powder (54.51%). The findings of this study can provide some useful answers for further research on this subject.

Keywords: Cereals, Digestibility, Pretreatment, Nutrient bioavailability, Antioxidant properties

Acknowledgements: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-9/2021-14/200040).

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CHEMICAL COMPOSITION OF HAZELNUT COCOA SPREADS WITH ADDED COCOA SHELL AND CASEIN

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Confectionery products are widespread products that are consumed by all generations. One of them is hazelnut cocoa spread, which is used as a spread on bread and filling in various products. These spreads mainly contain fats and dry ingredients such as sugar, cocoa powder, milk powder and different nuts (hazelnuts, peanuts, pistachios). Since spreads are rich in sugar and are often consumed by children, scientists and industry are trying to replace sugar in these products. The cocoa shell is a by-product of the food industry that is rich in fibers and bioactive components but also presents a global problem for the environment. Because of all that many researchers are trying to implement this by-product in foods production. The aim of this study was to produce hazelnut cocoa spreads without sugar and to use the cocoa shell as a partial replacer. Spreads were produced with a ball mill and xylitol and stevia were used as additional replacers for sugar. Sugar replacers were used in following proportions: cocoa shell and stevia:xylitol 50:50, 40:60, 30:70, 20:80, 10:90, 0:100. In addition, 1 and 2% of casein were used as a replacer for milk powder. After the production of hazelnut cocoa spreads, analyses were performed. The content of insoluble, soluble and total fibers was determined by the enzymatic-gravimetric method. The protein content was determined by the Kjeldahl method and dry ashing at 550 °C was used for ash content determination. Results showed that the addition of cocoa shell caused a significant increase in insoluble, soluble and total dietary fibers while the addition of casein did not affect the content of fibers. Also, the cocoa shell addition caused a more pronounced increase of proteins compared to the addition of casein. Ash content showed the same trend because the addition of cocoa shell caused a more significant increase of ash compared to the addition of casein.

Keywords: Hazelnut cocoa spread, Cocoa shell, Casein, Fibers, Sugar

Acknowledgements: The research was supported in part by Croatian Science Foundation under the project UIP 2017-05-8709.

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THE CONCENTRATION OF COPPER IN FRUIT PRODUCTS INTENDED FOR HUMAN CONSUMPTION IN THE REPUBLIC OF SERBIA

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Copper is an essential micronutrient for humans and animals. In the plant, copper plays an important role in respiration and photosynthesis. It is a component of several enzyme systems involved in carbohydrate, nitrogen and cell metabolism. Copper compounds belong to the group of inorganic compounds which are used as a fungicide and bactericide. For this reason, European Union, as well as Serbia, has established legislation for copper in certain foodstuffs in order to protect public health. From January 2015 to December 2017, we determined concentrations of copper in 17 different fruit products (apple juice, banana powder, dried apple, dried chickpeas, dried figs, dried grapes, dried papaya, dried pineapple, dried plum, frozen apple puree, frozen apricot puree, frozen plum, frozen raspberries, frozen sour cherry, frozen sour cherry puree, frozen strawberry, tropical dried fruit mix). Fruit products originate from 8 countries, including Serbia. Fruit products samples were analysed by using inductively coupled plasma – atomic emission spectrometry (ICP-AES). A strict analytical quality control programme was employed during the study. Measurable copper was found in all analysed samples. The copper levels ranged from 0.065 (apple juice) to 7.555 (dried apple) mg/kg. Average concentration of copper in the analysed fruit products was 1.381 mg/kg. The maximum copper concentration was below maximum level (5.0–10.0 mg/kg) set by Serbian legislation, which were valid until the end of 2019.

Keywords: Fruit products, Copper, Maximum levels

Acknowledgements: This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia, under the Grant 451-03-68/2022-14/200134, and by the Provincial Secretariat for Higher Education and Scientific Research, Autonomous Province of Vojvodina, under the Grant 142-451-2039/2022. Also, this research was done in liaison with the activities defined by the grant for the establishment and implementation of the research-innovation-scientific program "Centre of Excellence (CoE) for digitalization of microbial food safety risk assessment and quality parameters for accurate food authenticity certification (FoodHub)", financed by the Ministry of Science of Montenegro, under the Grant No. 01-3660/2.

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EFFECT OF STORAGE PERIOD ON COLOUR AND TEXTURAL PROPERTIES OF COOKIES WITH WHEAT MALT FLOUR

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The aim of this research is to investigate the changes in external and internal color of cookies influenced by the incorporated wheat malt flour (WMF) as a partial and full substitute for wheat flour (WF) in different quantities (20, 40, 60, 80 and 100%) during storage period of 60 days. The reference cookies were made only with WF. Moreover, the texture in terms of hardness and fracturability as well as water activity of cookies were examined on 0^{th}, 7^{th}, 14^{th}, 30^{th} and 60^{th} day of storage. The color was expressed with the parameters L*, a*, b*, BI* and ΔE. The surface color of the cookies becomes darker with the gradual increase of the addition of the WMF (L* value decreases from 72.79 to 52.00), the red color becomes more intensive (a* value increases from 4.20 to 10.39), at the expenses of the yellow color whose intensity is decreasing (b* value is changing from 29.30 to 14.58). Similar tendency can be observed in the internal color. The overall change of color ΔE significantly changes with the incorporation of WMF in the cookies formulation. The storage period does not deteriorate color, since ΔE for internal and external color decreases in all types of cookies compared to the reference. BI* differs statistically significant ($p < 0.05$) after baking and over the storage period (0^{th}, 7^{th}, 14^{th} and 30^{th} day). The water activity also changes statistically significant ($p < 0.05$) over this period. The hardness of the cookies is comparable when the WF was substituted with WMF until 60%, however higher concentrations of WMF gave harder cookies. As a consequence, fracturability also decreased over time.

Keywords: Cookies, Color, Malt wheat flour, Storage period, Texture

Acknowledgements: This work was supported by the Bulgarian Ministry of Education and Science, National Research Fund under the contract number KII-O6-M56/3-2021.

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ADDITION OF PULLULAN DURING ENCAPSULATION OF VANILLIN IN ALGINATE HYDROGEL BEADS

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Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a dominant phenolic component present in *Vanilla planifolia* pods. It is widely used in food, beverages, pharmaceutical and cosmetic industries as a flavor and could be used to mask undesirable off-flavors developed during storage. Except for its flavoring properties, it also acts as an antioxidant. Since food additives such as flavors and antioxidants are often sensitive to external factors, it is necessary to prevent their degradation by encapsulation process. In the present study, vanillin was encapsulated into alginate hydrogel beads using B-390 BUCHI encapsulator under constant conditions (1000 µm nozzle, 130 mbar, 100 Hz, 1000 V). As an encapsulation mixture, 3.75% alginate and 1% vanillin were used and 5% $CaCl_2$ was used as a cross-linking solution. Pullulan (0.5%, 1%, 1.5% and 2%) was added to the encapsulation mixture to examine its effect on the concentration of vanillin in hydrogel beads. Vanillin concentration was determined using HPLC and antioxidant activity of the beads was evaluated by DPPH, ABTS, FRAP and CUPRAC assays. It was observed that the addition of 0.5% pullulan caused an increase in vanillin concentration and consequently the same sample had the highest antioxidant activities. Results of this study showed that the wall material composition affects the concentration of the active agent in hydrogel beads.

Keywords: Vanillin, Pullulan, Encapsulation, Alginate, Beads

Acknowledgements: This work was supported by the Croatian Science Foundation under project (IP-2019-04-5749) ''Design, fabrication and testing of biopolymer gels as delivery systems for bioactive and volatile compounds in innovative functional foods (bioACTIVEgels)'', Young Researchers's Career Development Project – Training of New Doctoral Students (DOK-2020-01-4205).

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TESTING OF VITAMIN C CONTENT AND MICROBIOLOGICAL QUALITY CONTROL IN FRESH AND DRIED ROSE HIP (*Rosa canina* L.) FRUIT

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Rose hip (*Rosa canina* L.), wild rose or dog rose is widespread in almost all of Europe, Africa, western and northern Asia. Rose hip fruit is a rich source of vitamin C and contains a large number of different chemical compounds including pectins, tannins, organic acids. The fruit is usually used to make jam, syrup or dried. The aim of this scientific work is to examine the activity of water (a_w), pH value, acid content, vitamin C content and microbiological quality control in fresh and dried rose hips.Water activity was measured with an a_w-meter (Pawkit Decagon, Germany). pH value was measured with a pH meter (InoLab WTW, Germany). The acid content was determined by volumetric method, titration with NaOH. Vitamin C content was determined spectrophotometrically (Jenwey spectrophotometer 6305, United Kingdom). Microbiological quality control included food spoilage bacteria *Salmonella* spp. ISO 6579-1:2017, *Listeria monocytogenes* ISO 11290-1:2017, *E. coli* ISO 16649-1:2018 and *Enterobacteriaceae* ISO 21528-2:2017. The results of these tests indicated that both fresh and dried rose hips are a good source of vitamin C. The results of microbiological analysis showed the absence of *Salmonella* spp., *Listeria monocytogenes* and *E. coli*. The presence of *Enterobacteriaceae* was less than 10 cfu / ml.

Keywords: Vitamin C, Rose hip fruit, Quality control

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MICROBIOTA AND EFFECT OF DIFFERENT FREEZING REGIMES ON VOLATILE AROMA COMPOUNDS OF BLACK TRUFFLE *TUBER AESTIVUM*

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The use of truffles in food is based mainly on artificial flavors addition, aiming to achieve an intense aroma in the products. As truffle is a natural product with nutritional and functional properties, it is important to find optimal way for truffle storage. As the microbiota contribute to truffles aroma, the bacterial and yeast composition in rhizosphere and fruiting body of the Serbian truffle, as well as the impact of at different freezing methods on volatile profile of the truffle *Tuber aestivum* during 90 days of the storage were determined.
Bacterial and yeast isolation from fresh truffle was conducted and isolates were identified using 16s rRNK and 18s rRNK. Effect of truffles freezing at -20°C, -80°C with and without previous dipping in liquid N_2 on the volatile compounds was observed using GC/MS.
Results demonstrated that isolated bacteria belonged to the phylum *Proteobacteria*, *Firmicutes* and *Actinobacteria*, where identified species mainly belonged to *Firmicutes,* genus *Bacillus* sp. Isolated yeasts were identified as *Cryptococcus* sp., *Debaromyces hanseinii*, *Candida fermentati* and *Rhodotorula mucilaginosa*.
The GC/MS analysis revealed differences in volatile profile of fresh and frozen truffles. Frozen samples were richer with compounds 2-butanone, 2-methyl-butanal, methanethiol and 2-butanol after freezing or during storage. Content of DMS, acetaldehyde, 3-octanone, ethanol, 2-methyl-1-propanol significantly decrease immediately after freezing.
Overall, gained results indicated that freezing of truffles as preservation method had profound effect on volatile compounds, where previous dipping in liquid N_2 showed no significant impact on volatile profile of truffle *Tuber aestivum*.

Keywords: Microbiota, Black truffle, Aroma compounds, Freezing

Acknowledgements: Ministry for Education, Science and Technological Development of Republic of Serbia Agreement 451-03-68/2022-14/200116

ICAPP 2022
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ANTIBACTERIAL ACTIVITY OF MANUKA HONEYS WITH DIFFERENT UMF VALUES

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Manuka honey is a dark monofloral honey, rich in phenol compounds and attracts a lot of attention because of its antimicrobial action. The antibacterial power of Manuka honey is linked with the Unique Manuka Factor (UMF) which correlates with the contents of methylglyoxal and total phenols. Different types of Manuka honey have different effects on bacteria, so gram-negative bacteria are more resistant than gram-positive bacteria.
The aim of this research was to compare the effects of Manuka honey UMF 15+, Manuka honey UMF 5+ and their growth inhibition of five different species of bacteria: Streptococcus group D, β-hemolytic streptococcus, *Pasteurella multocida*, *Streptococcus uberis* and *Truepqera pyogenes* from the collection isolate of the Laboratory of clinical microbiology.
Two samples of Manuka honey of different UMF were used in the experiment. Honey was used in undiluted and diluted state (honey: distilled water 50:50% and 75:25% (v/v)). Disc diffusion method was used to evalaute antibacterial activity.
Manuka honey UMF 5+ had an inhibitory effect on all tested strains in all combinations with a range of action from 10.67 (Streptococcus D group) to 43.33 mm (*Trueperella pyogenes*), except for *Trueperella pyogenes* in combination with honey: distilled water 75:25% (v / c) (0.00 mm).
Manuka honey UMF15 + in all combinations had an inhibitory effect on the tested bacteria with a range of action from 12.33 mm to 42.00 mm.

Keywords: Manuka Honey, UMF 5 +, UMF15 +, Inhibition, Antibacterial effects

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HERBAL ESSENTIAL OILS - COMPARISON OF THE EFFECTS ON *Salmonella* Typhimurium AND *Listeria monocytogenes* GROWTH

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Despite the development of modern medicine diseases caused by microorganisms are one of the leading causes of death in the world. Although today there are many antimicrobial drugs with wich modern medicine fights infectious diseases, the incereasin resistance of microorganisms to existing therapeutics is the reason for continuous investment in the development of new drugs with antimicrobial properties. In addition to healthcare this challenge is present in the food and cosmetics industry where there is a need for innovative, effective and safe preservatives. In doing so many researchers are focusing their work on traditional medicinal plant species as a source of new molecules with antimicrobial activity. Also, due to the growing interest of healthcare professionals and patients in complementary treatments the use of essential oils is regaining its affirmation. Therefore, it is important to know the level of pharmacological action and effectiveness of essential oils.

The aim of this study was to examine the effect of rosemary, mint, fennel, eucalyptus, anise, cinnamon, orange, thyme, cloves, and lemon essential oils on the growth of pathogenic bacteria *Salmonella* Typhimurium and *Listeria monocytogenes* from the collection of Laboratories for food, feed and water microbiology.

A difference in the antimicrobial activity of essential oils depending on the pathogen, plant type and concentration of essential oil was observed. The greatest inhibitory effects where shown by essential oils of cinnamon, thyme, cloves and rosemary. The essential oils of anise and orange did not inhibit the growth of *S.* Thypimurium and *L. monocytogenes* in any combination. Also, fennel and eucalyptus oil did not show an inhibitory effect on the growth of *L. monocytogene*s. The zone of inhibition of essential oils for *S.* Typhimurium ranged from 8.66 mm to 38.00 mm depending on the type of essential oil and the combination with alcohol, and the zones of inhibition of essential oils for *L. monocyogenes* ranged from 19.00 mm to 38.33 mm.

Keywords: Essential oils, Antimicrobial activity, Bactericidal, Bacteriostatic action

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INFLUENCE OF DIFFERENT SWEETENERS ON SENSORY ATTRIBUTES OF RASPBERRY JAMS

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Jams are alternative for fruit preservation, and usually, they are prepared with a high amount of sugars, mainly sucrose. There is a great interest for jam production with lower amount of sucrose, or with some type of sweetener replacement because modern consumers are concerned about the nutritional and caloric value of food they consume. They are interested in healthier food with the sensory characteristics of the sucrose-based product.
Thus, this study investigates the influence of different sweeteners on the sensory attributes of raspberry jams (Willamette raspberry jams and Wild raspberry jams). The jams were prepared with different sweeteners (fructose, sorbitol, agave syrup) and low amount of sucrose. The sensory analysis was performed by scoring method for jelly products assessment. Sensory attributes were assessed by 10 highly experienced testers, using a different number of points: color 0-4, smell 0-2, taste 0-8, and consistency 0-6.
The results of sensory analysis of processed raspberry jams showed good acceptability. However, both types of raspberry jams with sorbitol were assessed with the highest average total grade, showing better sensory attributes as compared with jams prepared with other sweeteners. The Willamette raspberry jams and Wild raspberry jams prepared with agave syrup had the lowest average grade for all sensory attributes.
Our results suggest that sorbitol, used as sweetener in both types of jams, was the best option for sucrose replacement.

Keywords: Sensory attributes, Raspberry jams, Sweeteners

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THE IMPACT OF COMBINED EMULSIFIER ON CRYSTALLIZATION PROPERTIES OF TRANS FREE FAT INTENDED FOR FAT FILLING PRODUCTION

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This paper investigated the influence of two separate emulsifiers (E1 and E2) and emulsifier 2 in 1 (E2in1) on crystallization characteristics of fat with no trans fatty acids intended for production of confectionary fat fillings. The emulsifiers were added to fat samples in amounts 0.3, 0.45 and 0.75 wt%.
The crystallization properties of fat samples were determined by rheometer, measuring the viscosity during cooling process. Also, NMR technique was used for measuring the solid fat content (SFC) at different temperatures, as well as for measuring the change of SFC in a function of time at 20 ºC.
The results showed that all amounts of both types of emulsifiers affected the acceleration of crystallization in accordance with the added amounts during the crystallization of molten fats from 50 to 10 ºC, where the same amount of E2in1 had significantly ($p<0.05$) higher impact on viscosity increasing. Also, the fat samples with E2in1 had significantly ($p<0.05$) higher values of SFC at temperatures 20, 25, 30, and 35 ºC in comparison to fats with the same amount of two separate emulsifiers E1 and E2. However, observing the crystallization kinetics, it was shown that emulsifier E2in1 caused higher crystallization rates compared to control fat sample and samples with E1 and E2 and lower amount of crystals formed. The least amount of formed crystals was present in the fat sample with the addition of 0.75% of E2in1 which may negatively or positively affect the hardness of fat filling during cookie production, as future research will show.

Keywords: Trans free fat, Emulsifiers, Solid fat content, Viscosity, Crystallization kinetics

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia program (451-03-68/2022-14/200134).

ICAPP 2022
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WASTE PLANT MATERIAL – A NEW SUBSTRATE FOR KOMBUCHA BEVERAGE WITH EXPRESSIVE ANTIBACTERIAL ACTIVITY

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Kombucha is a fermented tea beverage which is traditionally prepared by fermenting sweetened black tea (*Camellia sinensis* L.) with consortium of acetic acid bacteria and yeasts (also known as a tea fungus). Large quantities of medicinal and spicy plants are grown on the territory of Serbia. After distillation of medicinal plants during the production of essential oils a significant amount of solid (solid plant waste mass) and liquid (liquid plant waste and hydrolates) by-products remains. The potential of these waste can be directed towards obtaining kombucha beverage with functional and health benefits. This paper introduces obtaining kombuha beverages from waste materials remain after distillation of chamomile (*Chamomilla matricaria* L.) (solid plant waste, in amount 9g/l and liquid plant waste, diluted in a ratio of 1:10) and determination of its antibacterial activity. During five days of fermentation, chemical (pH and titratable acidity) and microbiological (number of yeasts and acetic acid bacteria) parameters were determined. Antibacterial activity was determined by agar-well diffusion method. Tested strains were: Gram-negative (*Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028, *Pseudomonas aeruginosa* ATCC27853) and Gram-positive bacteria (*Listeria monocytogenes* ATCC 35152, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923). Tested samples were: Kombucha beverages, acetic acid solutions, uninoculated plant waste substrates and neutralized Kombucha beverages. Kombucha beverages and acetic acid solutions had expressive antibacterial activity against all tested strains. Beverage from liquid waste had higher activity due to higher acetic acid concentration. Uninoculated substrates and neutralized Kombucha beverages did not show any activity. The results indicate a high potential of chamomile waste material as a substrate for kombucha fermentation. Commercialization of beverages obtained from waste material, could contribute to solving the problem of accumulated waste material, which would have a significant environmental contribution.

Keywords: Kombucha, Waste material, Chamomile, Antibacterial activity

Acknowledgements: The financial support of the Ministry of Education, Science, and Technological Development of the Republic of Serbia (contract no. 451-03-68/2022-14/200134) is gratefully acknowledged.

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ANTIOXIDATIVE PROPERTIES OF GLUTEN-FREE CRACKERS BASED ON CHICKPEA FLOUR AND PUMPKIN SEED OIL PROCESSING BY-PRODUCTS

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During the digestion process antioxidant compounds could be released from food matrix, as well as transformed into other compounds with lower bioaccessibility due to interaction with other constituents such as fibers, proteins, and polysaccharides. To produce beneficial effects bioactive compounds should be available for absorption once the whole digestive process has occurred. Since the utilization of chickpea flour and pumpkin seed oil byproducts results with high protein and fiber products, this examination was conducted to determine antioxidant activity and potential benefit effects to human health. Five different formulations were produced where Control sample contained 100% chickpea flour, while in other formulations pumpkin seed press-cake flour (virgin (VF) and cold pressed (CF)) were used at two substitution levels (20 and 35%, w/w). After preparation of gluten-free crackers, in vitro digestion was carried out (cephalic, gastric, and intestinal phase simulated with enzymes, temperature and pH control). Before and after digestion protein content, electrophoresis, antioxidative tests and total phenolic content was determined. Obtained results showed that antioxidant activity, as well as total phenolic content increased after conducted in vitro digestion in all samples. Compared to Control sample, higher values of mentioned parameters are noticed. Furthermore, electrophoresis and protein examination show the breakdown of proteins into smaller molecular weights. From obtained results it could be concluded that in vitro digestion of prepared cookies resulted with higher antioxidant activity, which implies on potential benefits of consuming this type of product.

Keywords: In vitro digestion, Antioxidants, Crackers, Chickpea, Pumpkin seed oil by-products

Acknowledgements: This work was financially supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Contract No. 451-03-68/2022-14/200222).

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THE COMPOSITION OF PHENOLIC COMPOUNDS OF COOKIES ENRICHED BY NETTLE (*Urtica dioica* L.) SEEDS AND EXTRACT

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By replacing part of the wheat flour with ground nettle seeds (CWS) and applying an aqueous extract instead water to mix the dough (CWE) were obtain the cookies with improved phenolic composition compared to the control cookie without nettle seeds or extract (CC). HPLC analysis with isocratic elution was performed to determine the effect of the addition of nettle seed and extract on the free and bound phenolic composition. Among the identified and quantified compounds in free and bound phenolic extracts of cookies are mainly phenolic acids, while of the flavonoid only naringin (glycosidic form of naringenin).

Of the phenolic acids, in the free phenolic extracts of all cookies, the most abundant was chlorogenic acid, whose content decreased statistically significantly ($p < 0.05$) in CWS > CWE > CC order. Compared to CC, the content of chlorogenic acid in CWS and CWE was 10.3 and 2.02 times higher, respectively. Gallic acid is also present in certain amounts, as follows: the content of gallic acid in CWS was approximately 8 times higher, while in CWE the content was 1.57 times higher compared to CC. The results of bound phenolic extracts indicate that in CWS the protocatechuic acid was the most abundant, whose content was not statistically significant higher ($p > 0.05$) compared to CC. The content of chlorogenic acid was the 2 times higher in CWE compared to CC, but this difference was not statistically significant. Unlike free phenolic extracts, *trans*-ferulic and protocatechuic acids were detected in bound phenolic extracts. The results also show that the caffeic, ellagic and coumaric acids detected only in extracts of CWS (in free and bound form), indicating that these phenolic acids originate from nettle seeds. The content of all phenolic compounds in free form differed statistically significant ($p < 0.05$) compared to their content in bound form.

It can be concluded by replacing part of the wheat flour with ground nettle seeds and applying an aqueous extract to mix dough, the cookies with improved and increased content of phenolic compounds (especially chlorogenic, gallic, caffeic, ellagic, coumaric and syringic acids) were obtained.

Keywords: Nettle seeds, Extracts, Phenolic compounds, Composition, Cookies

Acknowledgements: Republic of Serbia - Ministry of Education, Science and Technological Development, Program for financing scientific research work, ev. no 451-03-68/2022-14/ 200133.

ICAPP 2022
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DESIGN AND CHARACTERIZATION OF SPRAY-DRIED MICROCARRIERS FOR PREPARATION OF FUNCTIONAL WHEY-BASED BEVERAGES

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Starter cultures are essential components of fermented foods with the potential to enhance preservation, improve nutritional quality, and modify the sensory qualities of the products. The study characterized the microcarriers with the probiotic starter culture which could be used for the preparation of functional whey-based products. The culture was encapsulated using the spray-drying technique and implemented in the whey-based medium. The protective effect of alginate-whey carriers on the microencapsulated dairy starter culture (Lactoferm ABY 6) during the spray-drying, fermentation, and storage time were analyzed. The production yield, encapsulation efficiency, solubility, particle size, and moisture content were determined after the microencapsulation process. The SEM analysis was used for the characterization of powder and carrier surface morphology. The encapsulation efficiency and powder solubility were 75,8±0,09 % and 86,0±1,62 %, respectively. The cell viability in the powders and beverages was monitored for 28 days. The results showed that the microencapsulation process improved cell viability (> 9,08 $\log_{10}$CFU/mL) during the fermentation process. Moreover, microencapsulated probiotic cells have a higher survival rate in beverages in comparison with free cells in storage time. The application of this type of encapsulation technique showed potential regarding cell preservation in the production and storage of functional beverages.

Keywords: Functional foods, Microencapsulation, Starter culture, Spray-drying technique, Whey-based beverages

Acknowledgments: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. Contract No. 451-03-68/2022-14/200287).

ICAPP 2022
2nd International Conference
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THE PRESENCE OF NITRITE IN MEAT PRODUCTS FROM THE MARKET OF REPUBLIC OF SRPSKA

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Nitrites are preservatives that are added to meat products to improve the quality, durability and safety of products. Due to carcinogenic nitrosamines in meat products, there is a potential danger to human health.
The aim of the research is to determine the content of residual nitrites in 576 meat products from the market of Republika Srpska, to identify products that contain higher and which contain lower amounts, and to look back to the requirements of the Ordinance on Food Additives. (SG RS, number 96/20), which defines amount of nitrites which is allowed to add in meat products. In the period May 2015 - May 2022, 576 meat products were tested using the *BAS ISO 2918* method.
Nitrites were not quantified in 20% of products (< LOQ). The highest average value of nitrite is in heat-treated meat sausages in pieces (45.73 ± 15.854 mg / kg), finely chopped (42.84 ± 15.245 mg / kg) and coarsely chopped boiled sausages (37.40 ± 21.396 mg / kg). In durable dry meat products, the lowest average amounts of nitrite are 6.17 ± 2.229 mg / kg, and higher in semi-durable cured meat 36.30 ± 17.331 mg / kg. In cans, the average value of nitrite is 13.29 ± 10.288 mg / kg, and in pate 15.71 ± 10.473 mg / kg.
Based on the obtained results, the amount of residual nitrite below the amount of nitrite allowed to be added to meat products by the Rulebook that can be added to meat products was determined. Larger amounts of nitrites were recorded in heat-treated products, and less in non-heat-treated permanent products. From the point of view of the potential danger of nitrites for human health, and thus the safety of meat products, the most acceptable are permanent meat products.

Keywords: Meat product, Nitrites, Carcinogenic

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POLYPHENOL COMPOSITION AND ANTIOXIDANT ACTIVITY OF PROBUS AND PROKUPAC RED WINES FROM SERBIA

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Thanks to their chemical structure and functional properties, polyphenolic compounds not only contribute to the final complexity of the sensory characteristics of wine, but also show a positive, beneficial effect on human health. Their biological activity and the appropriate level of bioavailability have a protective effect on the reduction of oxidative stress.
In this paper, HPLC was used for establishing the polyphenol profile of wines from indigenous grape varieties Probus and Prokupac (samples Pb1-Pb5 and Pk1-Pk5, respectively, collected from different local wineries). Their antioxidant activity was determined using spectrophotometric DPPH (2,2-diphenyl-1-picrylhydrazyl) (AA_{DPPH}) and reducing power (RP) assays. HPLC analysis revealed that among phenolic acids, *p*-hydroxybenzoic acid had the highest concentration in all tested wine samples (42.32-319.46 mg/L), making it the most dominant phenolic compound. When it comes to flavonoids, analyses showed that rutin was the most dominant one in all wine samples (4.08-15.94 mg/L). Based on the results of HPLC analyses, it is noticeable that Probus wines had significantly higher content of total anthocyanins and total polyphenols than Prokupac wine samples. The highest antioxidant activity, tested by DPPH and RP assays, was determined for sample Pb1 (917.51 mmol TE/100 mL and 1530.14 mmol TE/100 mL, respectively). Principal Component Analysis (PCA) was used to examine the relationship between the observed wine samples and their polyphenolic compounds and antioxidant activity, as well as to group the samples according to similarity based on the observed parameters. Qualitative results for this analysis show that the first three main components together represent 72.99% of the total variance which can be considered sufficient to present the whole set of experimental data. The most significant positive influences on the calculation of the second (PC2) main coordinate were achieved through the content of chlorogenic acid (10.5% of the total variance), AA_{DPPH} (7.4%) and RP (10.8%), while for calculation of the third coordinate (PC3) the content of protocatechuic acid (22.4%), rutin (10.9%), quercetin (9.5%), total flavonoids (13.3%) and AA_{DPPH} (5.2%) were the most influential.

Keywords: Indigenous, Wine, Polyphenols, Antioxidant activity, PCA

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STABILIZATION OF SUNFLOWER OIL BY ADDING GARDEN SAVORY (*SATUREJA HORTENSIS*)

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Sunflower oil is one of the most widely used oil in the food industry and it is categorized into two groups: cold pressed and refined sunflower oil. Cold pressed sunflower oil has better nutritional properties than refined sunflower oil, but it oxidizes more easily that decreases its stability. In this study, refined sunflower oil (brand „Briliant“) and cold pressed sunflower oil (brand „Fila“) were analyzed. The bottles with volume of 1L were used for storing the examined oils; however part of the oil was removed in order to investigate oil volume of ¼, ½ and ¾ of the bottle volume. Dry garden savory (*Satureja hortensis*) was added to one part of the oils in order to examine their stability. The oil samples were stored for period of 4 weeks, after which their peroxide value, which are a measure of the oil oxidation, were determined. The highest peroxide value is observed in the oil sample with a volume of ¼; namely, for refined oil this value is 5.88 mmol O_2/kg, while for cold pressed oil is 11.76 mmol O_2/kg. The oils with the same volume ratio that contain savory had significantly lower peroxide value. In general, the added garden savory reduced the oil oxidation, especially in cold pressed sunflower oil, resulting in much more stable oils.

Keywords: Sunflower oil, Garden savory, Stabilizing, Oxidation

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THE CONTENT OF SOME PHTALATES IN MUSHY PEACH AND APPLE JUICE DURING STORAGE TIME

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The esters of phthalic acid - phthalates are used to improve properties of packaging material for food products, such as flexibility, softness and transparency. In this research the presence and the content of four phthalates (dibutyl phthalate-DBF, benzyl butyl phthalate-BBF, di-n-octyl phthalate-DNOF and di-2-ethylhexyl phthalate-DEHP) in mushy juice of peach and apple with different storage times (50-330 days) were examined. Phthalates were extracted from the juice by using ethyl acetate (1:1 v/v), at room temperature, during 2h and with stirring every 15 minutes for 1 min. For determination of the content of each phthalates separately, the HPLC method was used (Agilent 1100 High Performance Liquid Chromatograph, a Zorbax Eclipse XDB-C18 column-4.4 m x 150 mm x 5 µm-Agilent Technologies, Wilmington, USA, and an UV/ViS detector). The column temperature was 30 ºC, and the solvent mixture of (A) acetonitrile/water (85:15 v/v) and (B) acetonitrile was used with gradient program. The sample volume of 20 µl was injected, and phthalates were detected at 225 nm. The series of standard solutions in the concentration range of 3-20 µg/mL of each phthalates separately (all purchased from Sigma Aldrich, Germany) was made by methanol and the calibration curves constructed based on the chromatogram peak area (mAU*s) and four different concentrations. The degrees of linearity was r^2>0.93. In the examined juices, during investigated self life, two of the four tested phthalates were found: dibutyl phthalate in a concentration range 31.08-94.78 µg/L and di-2-ethylhexyl phthalate in range 46.80-76.72 µg/L, i.e. in a content of 29.54-90.09 µg/kg and 44.47-72.93 µg/kg of juice, respectively. Their content increased during storage, and the most phthalates migrated for the first 50 days of storage. The obtained results showed the content of dibutyl phthalate and di-2-ethylhexyl phthalate, as well as the total content of phthalates during self life was within the allowed limits (by the Regulation (EC) 10/2011 for plastic food contact materials) of 0.3, 1.5 and 60 mg per kg of food, respectively.

Keywords: Migration, Phthalates, Content, Storage time, Juice

Acknowledgements: Republic of Serbia - Ministry of Education, Science and Technological Development, Program for financing scientific research work, Ev. No. 451-03-68/2022-14/200133

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INFLUENCE OF SPIRULINA ON PHYSICAL PROPERTIES OF DOUGH FOR CRACKERS

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Spirulina, blue–green alga (*Cyanobacteria*), contains proteins (60–70%), carbohydrates, vitamins C, D, E, and minerals such as Fe, Ca, Cr, Mg, Na, Zn, Mn, P, K and Cu. Spirulina biomass is a commercial source of various bioactive metabolites, including γ–linolenic acid, pigments such as chlorophyll, phycocyanin and β–carotene. Several food products are formulated with the addition of spirulina, and the number of new food products with this valuable component is increasing on the market. The aim of this work was to observe the influence of spirulina powder on physical properties of gluten free dough for crackers. A part of integral rice flour, in appropriate recipe for crackers, was replaced with 5, 10 and 15% of spirulina powder. After mixing the dough, the rheological and textural characteristics of obtained dough samples were analyzed. Change in the color of the dough was also observed, due to specific green–blue color of the spirulina powder. The addition of spirulina contributed to viscoelastic properties of dough and increased the resistance of dough to applied strain within non–destructive limits, thus it can be more easily manipulated. With increase in amount of spirulina addition the compliance of dough increased, thus these samples had softer consistency and lower hardness. The dough extensibility was slightly increased compared to control dough, but increase in spirulina content did not further contribute to this effect. The addition of spirulina certainly affected the color change of the dough from dark white to intense green, what means that it will have a great impact on the sensory quality of baked crackers. Thanks to the favorable properties of the obtained dough with rice flour and spirulina powder, a high sensory quality of baked cracker is expected, what is an excellent basis for the further development of gluten–free fine bakery product with high nutritional value.

Keywords: Spirulina, Rice flour, Dough rheology, Texture, Color

Acknowledgements: This research was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Program (451-03-68/2022-14/200134) and Program (451-03-68/2022-14/200222)

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OXIDATIVE STABILITY OF HERB INFUSED SUNFLOWER OILS

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Oxidative stability is one of the most important oil quality parameters. It determines their usefulness in technological processes, as well as shelf life. The fatty acids composition is of special importance from the oxidative stability aspect. The higher unsaturated and lower saturated fatty acids content is, the faster the oxidation reaction proceeds. Linolenic acid oxidizes the fastest, followed by linoleic and oleic fatty acids. Improvement of the oxidative stability of the oil can be achieved by adding various components with antioxidant properties i.e. synthetic antioxidants, plant extracts or plants. In this paper, the influence of the addition of rosemary and garlic to linoleic and high-oleic sunflower oil on the oxidative stability was investigated, using Oven and Rancimat test. The peroxide value (PV) of initial linoleic and high-oleic sunflower oil samples was 0.40 and 2.06 mmol/kg, respectively. Linoleic sunflower oil after 14 days of Oven test had a PV of 335.47 mmol/kg, while the same oil with the addition of 3% rosemary had a PV of 35.75 mmol/kg, and with the addition of 3% garlic 341.89 mmol/kg. After 14 days of testing, PV determined in high-oleic sunflower oil amounted 21.44 mmol/kg, while in oil with 3% rosemary added 4.68 mmol/kg was obtained, and in oil with 3% garlic added, 10.23 mmol/kg. The induction period of linoleic sunflower oil, obtained by Rancimat test, increased 2.33 times with the addition of 3% rosemary and 1.17 times with the addition of 3% garlic. The increase in the induction period in high-oleic sunflower oil is 3 times with the addition of 3% rosemary and 1.85 times with the addition of 3% garlic. The addition of both, rosemary and garlic, improved the oxidative stability of the oils, whiles rosemary showed better antioxidant effect.

Keywords: Sunflower oil, Oxidative stability, Rosemary, Garlic

Acknowledgements: This research is financed by Ministry of Education, Science and Technology Development of the Republic of Serbia, Project Number 451-03-68/2022-14/200134.

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CHEMICAL COMPOSITION, ANTIOXIDANT POTENTIAL AND TEXTURAL CHARACTERISTICS OF FRESH CHEESE SAMPLES PRODUCED WITH ADDITION OF SAGE

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The aim of this study was to investigate the influence of sage (*Salvia officinalis*) on chemical composition, antioxidant capacity and textural characteristics of fresh cheese produced with traditional starter culture XPL-1 (Chr.Hansen A/S, Denmark). Sage was used in several forms: sage herbal dust, its essential oil and supercritical fluid extract. There is a significant difference in chemical composition between fresh cheese (control sample) and fresh cheese samples fortified with sage. Textural characteristics shows that fresh cheese sample with addition of sage has three to four times lower value of firmness and work of shear compared to the control sample. The sample with addition of sage herbal dust has the highest DPPH radical scavenging activity (3.94 μM TE/g). Based on the obtained results, it can be concluded that application of sage herbal dust and its preparation in the production of fresh cheese contributes to improvement of product´s antioxidative capacity and decreases stickiness of the sample.

Keywords: Fresh cheese, Sage, Composition, Antioxidant potential, Texture

Acknowledgements: Authors want to thank Ministry of Education, Science and Technological Development of the Republic of Serbia for the financial support of the research presented in this article, Programme no. 451-03-68/2022-14/200134.

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SENSORY EVALUATION OF "VISOČKA PEČENICA" FROM TRADITIONAL AND INDUSTRIAL PRODUCTION

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"Visočka pečenica" is a traditional cured beef meat product of Bosnia and Herzegovina that has been produced in the municipality of Visoko for many years. It was protected by an Indication of geographical origin in June 2020. It is produced from the highest quality parts of beef carcass, dry salted only with kitchen salt and cold smoked and dried. For "Visočka pečenica" production, fresh beef chilled at temperatures from -1 to 4°C is used. After selecting a piece of meat, it is shaped and cut at a temperature of 4°C. The shaped pieces of meat are subjected to the salting process for about ten days at a temperature of 7°C. After salting, the pieces of meat are washed under a stream of cold water and drained for 24 hours. Drained pieces of meat are then transferred to the smokehouse for smoking. The last stage of production involves ripening to form an overall sensory impression. The industrial producers of "Visočka pečenica" have kept almost all the features of traditional production. In the comparison of traditional and industrial production, the differences found were related to the amount of added salt, the length of smoking, and the parameters of the smoking process (temperature and relative humidity inside the smokehouse, outside, and the temperature of the stokehole). This research aims to determine the impact of production technology on the sensory quality of the traditional "Visočka pečenica". Experimental production of samples of "Visočka pečenica" was performed in tradicional and industrial conditions. 40 samples were sampled twice for research needs, halfway through and at the end of the smoking process. Accordingly, the total number of analyzed samples was 80. Samples were taken from different cuts on carcass (round - *Muscullus gluteobiceps, Muscullus gluteus medius, Muscullus semitendinosus* and back musculature - *Muscullus longissimus dorsi*). A nonlinear scoring system was used for sensory evaluation. Each sensory characteristic is expressed by the corresponding number of points as follows: external appearance (3), cross-sectional appearance (3), color on the external appearance (2), color on the cross-section (2), odor (3), taste (4) and consistency (3). The evaluation committee consisted of five experts from this field. After the sensory evaluation, the samples of the "Visočka pečenica" were classified into five quality classes based on the total number of points. Sensory evaluation brings us to the conclusion that the sensory quality of samples of the "Visočka pečenica", in both ways of production, is quite uniform with some differences due to the unevenness of the smoking process. Compared to the data in the literature, the sensory quality of the "Visočka pečenica" samples in this research was of better quality. The fact that all the examined samples were classified in the first and extra class based on the total number of points of the conducted sensory evaluation, speaks in favor of the above. In the comparison of both types of production, the highest value of the total sensory evaluation was seen in the samples of thigh muscles smoked at a higher height at the end of the smoking process in the traditional production (19,37 points or 96,85% of the maximum), and the lowest samples of the

same anatomical region smoked at a lower height half of the smoking process (16,65 points or 83,25% of the maximum) in the same production. The analyzed samples at the half of the smoking process, although sampled for experimental purposes and not considered a finished product, had a very good sensory quality. PCA analysis of sensory properties showed that the examined sensory properties and the overall sensory evaluation were characteristic of samples of both productions sampled at the end of the smoking process.

Keywords: Sensory properties, Traditional meat product, Production technology, Quality

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THE EFFECT OF ANTIOXIDANTS ON QUALITY AND OXIDATIVE STABILITY OF ANIMAL FATS

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The beef, sheep and goat tallow are obtained by the process of dry melting of adipose tissue or tallow. Fats are found in the majority of foods that are consumed daily, especially beef tallow. The composition and consistency of fats depends on the breed, the nutrition of the animals and the region of the body from which the fat was taken. The stability of fat depends on the type of fat, fat composition and fatty acids. The addition of antioxidants can improve resistance according to the oxidative stability and quality of fats. On the melted fat, before the addition of antioxidants, the content of saturated and unsaturated fatty acids, water content, peroxide value and free fatty acids were all examinated. On the same samples, with and without the addition of natural antioxidants (extract sage, rosemary and ginger) added in the fats at a concentraction of 0.2% and synthetic antioxidans (PG-propyl galate, BHA-butylhydroxyanisole and TBHQ-butylhidroksitoulol) added in the fats at a concentraction 0.01%. The oxidative stability of fats determined by the shelf life test at 98°C during 7 days. The every 24 hours, the peroxide value and free fatty acids was examined. The results of the research show that the applied antioxidants successfully stabilized all the animal fats. Of the natural antioxidants, sage and ginger extract provided greater antioxidant stability in beef and sheep fat for up to 96 hours of storage, and all three extracts in goat fat. In the period from 96 to 168 hours after test of the samples at a temperature 98°C, in all samples there was an increase in the peroxide value and free fatty acids. Of the synthetic antioxidants propyl galate in all samples had very good antioxidative properties from the beginning to the end of the test. The synthetic antioxidants butylhydroxyanisole and butylhidroksitoulol sucesfully increased the stability of beef and sheep fats, and the most in goat fat.

Keywords: Animal fats, Quality, Fatty acids, Antioxidants, Shelf life test

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THE INFLUENCE OF TECHNOLOGICAL PROCESS PARAMETERS ON THE OXIDATIVE STABILITY OF BLACK CUMIN OIL (*Nigella sativa* L.)

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The influence of technological process parameters on utilization and oxidative stability of black cumin oil (*Nigella sativa* L.) has been investigated in this study. Screw press was used for cold pressing of black cumin seeds. Three products were obtained by pressing: crude oil, oil sludge and oil cake. The process parameters were changing during the pressing: the temperature of the press head (60, 75 and 81°C), the frequency of the electric motor (15, 18 and 22 Hz) and the size of the outlet for oil cake (5, 6 and 8 mm). Crude oil was naturally precipitated for 7 days at room temperature in a dark place and then filtered. The obtained oil was studied and the following quality parameters were determined using standard methods: peroxide value, free fatty acids content, unsaponifiable matter and moisture content. In oil cake, the content of oil and moisture was determined. The same parameters were determined in black cumin seeds. Based on these parameters, the degree of pressing efficiency was calculated. By increasing temperature of the press head, decreasing the size of the outlet for oil cake and frequency of the electric motor, higher production of black cumin oil was achieved. Furthermore, the oxidative parameters i.e. peroxide value and free fatty acids content were best in this case. Based on the research results, it can be concluded that the process parameters are a key factor in the technological process of pressing black cumin seeds and greatly affect oil yield and quality.

Keywords: Black cumin oil, Cold pressing, Process parameters, Oxidative stability

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DETERMINATION OF BENZYL BUTYL AND DI-N-OCTYL PHTHALATE FROM MATRIX OF MUSHY PEACH AND APPLE JUICE BY USING LIQUID-LIQUID EXTRACTION

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The phthalates are di-alkyl or alkyl/aryl esters of 1,2-benzendicarboxylic acid which are widely used in polymeric food packing materials to improve their properties. As they are classified as toxic substances, determination of phthalates content in various food matrices are of great interest. In this research the extraction the benzyl butyl and di-n-octyl phthalate from matrix of mushy peach and apple juice by ethyl acetate, hexane and mixtures of ethyl acetate and hexane in ratio of 2:1 and 1:2 (v/v), were examined. The juice was spiked by the solution of phthalates mixture concentration of 200 µg/ml to made concentrations of each phthalate separately in juice matrix of 1, 5 and 10 µg/ml. To determine the content of phthalates, the high performance liquid chromatograph (HPLC) method with a C_{18} column was used. For phthalate detection, the solvent mixture of (A) acetonitrile/water (85:15, v/v) and (B) acetonitrile by gradient program and an UV/ViS detector at 225 nm were used. A solution of mixtures of standard of phthalates in the concentration range 3-20µg/mL, was made by methanol and the calibration curves constructed based on the chromatogram peak area (r^2>0.98). Results showed that the extraction recovery depended on extragens, phthalate and its concentration. The extraction recoveries for benzyl butyl phthalate were higher than for di-n-octyl phthalate: the best achieved for benzyl butyl phthalate was 68.11% at concentration of 5 µg/ml by mixture of ethyl acetate and hexane in ratio of 1:2 (v/v), and for di-n-octyl phthalate was 15.75% at concentration of 1 µg/ml by mixture of ethyl acetate and hexane in ratio of 1:2 (v/v).

Keywords: Phthalates, Juice, Extraction, HPLC analysis

Acknowledgements: The project was financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia (ev.No.451-03-68/2022-14/200133).

ICAPP 2022
2nd International Conference
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CHANGES IN TECHNO-FUNCTIONAL PROPERTIES OF MAIZE FLOURS INDUCED BY DRY-HEAT TREATMENT

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Thermal treatments have numerous applications as a valuable tool for extending the shelf-life of the flours by reducing the enzyme activity and moisture content. Besides the biological effects, the dry-heat process has a significant impact on the techno-functional and nutritional properties of flours. Hence, the effects of the dry heat treatment at different temperatures: 100, 125, 135, 150, and 165°C, on the technological and functional properties, as well as antioxidant capacity of maize flours were investigated. The experimental material consisted of two maize hybrids with different colors and kernel types (white-standard and blue-popping). Maize samples were ground on a lab mill to a fine powder (<500µm) and flour samples evenly spread on a glass plate and thermally treated for 1 h in a ventilation oven Memmert UF55. Results showed that dry heat treatment increased the insoluble dietary fibre and free phenolic compounds of the investigated maize flours, while the bound phenolic compounds, anthocyanins, and pasting properties decreased with the rising of the applied temperature. The antioxidant capacity ranged from 10.05 to 13.32 mmol Trolox Eq/kg and 20.51 to 24.03 mmol Trolox Eq/kg in white and blue maize flour, respectively. The water absorption index showed an increase to a temperature of 135°C after which it began to decrease and reached a minimum at a temperature of 165°C. Meanwhile, the solubility index increased with an increase of temperature, and doubled its value after a temperature of 135°C. The dry-heat treatments largely modulated pasting properties of maize flours. The untreated maize flour samples showed a higher peak viscosity than the treated samples. Especially, white and blue maize flour samples at 165ºC reached the lowest viscosity value of all samples and the result was attributed to the structural changes in flour. Dry-heat treatment had a significant effect on the maize flour color parameters and the results of the parameters L^*, a* and b* indicated that the flour showed darkening and browning effect as the dry heat treatment temperature increased. Browning also indicates the formation of melanoidins as the end-product of the Maillard reaction, which confirms the maximum value of antioxidant capacity at a temperature of 165°C. All results showed that a temperature of 135°C had favorable impact on the techno-functionality of maize flours. However, due to the different kernel structures of the used maize genotypes and inter-relations between chemical compounds within the food matrix, the overall impact of dry-heat treatments was not completely elucidated.

Keywords: Dry-heat treatment, Maize flour, Technological properties, Functional properties, Antioxidant capacity.

Acknowledgements: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200040).

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SUPERCRITICAL CO_2 INFLUENCE ON α-AMYLASE ACTIVITY IN GRAHAM FLOUR

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Wheat is one of the most important cereals, which besides starch, contains different enzymes with specific functions from germination to breadmaking. Enzyme α-amylase present in wheat flour is responsible for the mobilization of starch reserves in the germination of cereals and many legumes, and it is a crucial quality parameter in the commercial utilization of most grains. Usually, wheat flour may require supplementation of α-amylase to consider an excellent way to improve the diet of consumers. In the baking process, the α-amylase level must be adequate to hydrolyze starch from damaged starch granules to fermentable sugars, making it easier for yeast to act. Graham flour is a variety of whole wheat flour. Both types of flour are made from the whole wheat grain. However, graham flour is more coarsely ground, and it is not sifted during the milling process. In general, graham flour is considered healthier as it is more nutrient-rich and has a higher protein content than white wheat flour.The present study focused on α-amylase activity in graham flour, which has been incubated in supercritical carbon dioxide ($SCCO_2$). To determine the α-amylase activity, the protein extract from $SCCO_2$-treated and untreated graham flour was prepared. One enzyme unit (U) is the amount of enzyme required to produce 1 µmol of glucose per minute under the defined assay conditions. During the study, it was found that $SCCO_2$ increased the activity of α-amylase without altering the quality of graham flour. Such a flour pre-treatment process could reduce the use of enzyme additives needed to improve bread production.

Keywords: Enzyme activity, α-amylase, Graham flour, Supercritical carbon dioxide

Acknowledgements: This work has been fully supported by Slovenian Research Agency Program P2-0046 "Separation Processes and Product Design" and Research Project J2-3037 "Bionanotechnology as a tool for stabilization and applications of bioactive substances from natural sources", as well as by the Ministry of Education, Science and Sport, Slovenia, Research project Contract No. C3330-19-952031.

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GANODERMA LUCIDUM AS A SOURCE OF INDUSTRIALLY IMPORTANT ENZYMES

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One of the most important medicinal mushroom is the Reishi mushroom (*Ganoderma lucidum*), which has universal healing properties as it is a rich source of biologically active compounds and enzymes. Enzymes show many benefits for the human body, for example, superoxide dismutase (SOD) is one of the most important endogenous antioxidants. They are also important in various industries; namely, laccase is widely used in the textile industry and for the effective removal of a wide range of antibiotics. Glucoamylase and α-amylase are some of the most commonly used biocatalysts in the food industry, and cellulase plays an important role by degrading insoluble cellulose into soluble sugars.
Our study aimed to determine the content of various bioactive substances and enzymes, antioxidant potential, and antibacterial activity of aqueous extract of dietary supplement capsules of medicinal mushroom *G. lucidum* (Pharmanex) obtained by ultrasound-assisted extraction technique. The content of total proteins, total phenols, proanthocyanidins, and antioxidant activity were determined by spectrophotometric analyzes. Specific enzymatic assays were used for the determination of activities for individual enzymes, such as α-amylase, glucoamylase, cellulase, laccase, and SOD. The Antibacterial activity was analyzed using the well-diffusion method on the growth of different pathogenic bacterial species. Based on the results obtained, we can confirm that *G. lucidum* is an excellent natural source of various bioactive compounds, including proteins, and enzymes, among which the highest activity of the antioxidant enzyme SOD was achieved. In addition, it also exhibits excellent inhibitory properties against the growth of tested bacterial species, among which the most susceptible was Gram-positive bacteria *Bacillus cereus*.

Keywords: Ganoderma lucidum, Enzymes activity, Antimicrobial efficacy, Antioxidant activity, Phenolic compounds

Acknowledgements: This research was supported by the Slovenian Research Agency (ARRS) within the frame of program P2-0046 (Separation Processes and Production Design), project No. J2-1725 (Smart materials for bioapplications), project No. J2-3037 (Bionanotechnology as a tool for stabilization and applications of bioactive substances from natural sources), and young researcher ARRS fellowship contract number No.1923/FKKT-2021.

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APPLICABILITY OF DIFFERENT KINETIC MODELS ON BIOSORPTION OF MOLASSIGENIC METAL IONS IN CLOSED-LOOP FIXED-BED COLUMN SYSTEM

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Valorization of by-products from the food industry is a generally accepted trend with an increasing tendency of its application in everyday life. One of the most difficult sustainability challenges is to overcome problems with an enormous amount of accumulated sugar beet pulp. Annually, around 15 million tons of sugar beet has been processed only in the EU. Novel findings have been directed towards the reutilization of the sugar beet pulp as an effective cation-exchange biomaterial. Sugar beet pulp was used as a biosorbent for molassigenic metal ions removal from the alkalized juice. Alkalized juice represents an intermediate product of the sugar juice processing stage. Dry matter (DM) of the alkalized juice represents only sucrose and macroelements (minerals). Sodium (Na) and potassium (K) are present in an alkalized juice in the amount of 9500 mg/ kg DM, whereas calcium (Ca) is present in the lower amount (700 mg/kg DM). Since all of the processes in the sugar industry are continual, biosorption was conducted in the closed-loop fixed-bed column system under the temperature 70°C, pH=12.5 and a biosorbent dose of 2.5 g/L. Metal ions content in the alkalized juice after 15, 30, 45, 60, 90, 105, 120, 180 and 240 minutes was detected according to ISO 6869:2000 using the Varian, SpectrAA—10 Atomic Absorption Spectrometer. Three non-linear kinetic models including pseudo-first, pseudo-second, and Elovich were applied to the obtained results in order to get insight into the biosorption mechanism. Equilibrium regarding all three monitored molassigenic metal ions was reached after 15 minutes. Ca ions were removed from the alkalized juice in the highest amount (51.6%). Whereas monovalent Na (14.6%) and K (7.8%) ions were less successfully removed. According to the one-dimensional correlation coefficient (R^2) the most adequate kinetic models for closed loop biosorption are pseudo-first ($R^2 \geq 0.98$) and pseudo-second ($R^2 \geq 0.98$). Therefore, the biosorption process is controlled by chemisorption and ion-exchange. Under the established conditions molassigenic metal ions removal process has rapid dynamics. Successful application of the closed-loop system potentially enables scale-up of the whole system.

Keywords: Sugar beet pulp, Kinetic study, Molassigenic metal ions, Closed-loop fixed-bed column system

Acknowledgements: This study was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract Number 451-03-68/2022-14/200222).

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2nd International Conference
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INFLUENCE OF WINTERIZATION SUPPORTED BY CELLULOSE FILTRATION AIDS ON THE REMOVAL OF UNDESIRABLE COMPONENTS OF SUNFLOWER OIL

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The refining process aims to eliminate the undesirable components from crude vegetable oil to yield refined oil with improved stability and consumer preference. The refining process consists of: degumming, neutralization, winterization, bleaching, and deodorization. Every stage of the refining process has its function to remove particular undesirable compounds (phospholipids, free fatty acids, waxes, peroxides, aldehydes, soap, trace metals and moisture) to improve the oil quality. The winterization phase aims to eliminate waxes present in crude oils, but in addition to waxes, filtration also removes other crude oil components. Traditional filtration aids (diatomite, perlite, etc.), used to support filtration, are being replaced by cellulose-based one. The aim of this paper is to examine the influence of winterization supported by cellulose-based filtration aids on the waxes, moisture, phospholipids, soap, free fatty acids, iron and copper removal in sunflower oil. The content of mentioned components was determined in 22 filtration cycles before and after horizontal frame filter. The analysis was performed in industrial conditions. Winterization supported by cellulose-based filtration aids almost completely removed waxes (average 99.22 ± 0.15%), completely eliminated remaining soap (100.00 ± 0.00%), largely removed remaining phospholipids (74.97 ± 25.08%), as well as iron (16.22 ± 16.37%) and copper (27.97 ± 28.20%). Results showed that winterization phase led to increase in moisture (15.01 ± 61.33%) and free fatty acids (9.25 ± 18.93) content. However, the moisture and free fatty acids content in oils before and after filtration was very low, below 0.28% and 0.11%, respectively, so this increase was probably caused by specifics laboratory determination methods, not the winterization process.

Keywords: Sunflower oil, Refining, Cellulose aids, Winterization, Waxes

Acknowledgements: This research is financed by Ministry of Education, Science and Technology Development of the Republic of Serbia, Project Number 451-03-68/2022-14/200134.

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

PROBIOTIC ALMOND - BASED BEVERAGE: PROMISING STEP TOWARDS A CIRCULAR BIOECONOMY

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The search for waste minimization possibilities and the valorization of by-products are key to good management and improved sustainability in the food industry. In recent years non-dairy food products have increased in popularity owing to their unique characteristics and advantages. Due to its beneficial properties, caused by the presence of various bioactive compounds, the almond parts that remain after almond milk production can be considered promising sources of ingredients for the development of non-dairy food products.
The aim of this study was to examine the potential and possibility of using a surplus product that remains after almond milk production. The antioxidant properties of the remained surplus product were characterized based on the polyphenol, flavonoid, and anthocyanin content as well as FRAP, DPPH, and ABTS antioxidant activity. The fermented beverage that combines the properties of almonds and probiotic bacteria, formulated using inulin, lyophilized fruit and aroma, was evaluated for physicochemical, microbiological, and sensory properties during cold storage (4 °C, 21 day). The surplus product that remains after the almond milk production contains 61.4 mg GAE/100g DW, 16.11 mg QE/100g DW, and 0.993 mg CYE/100g DW of polyphenols, flavonoids, and anthocyanins (respectively), and expresses DPPH, FRAP and ABTS antioxidant activity of 71.56 mg DW/mL, 50.47 mg DW/mL and 53.11 mg DW/mL (respectively).
The fermentation of surplus product that remains after almond milk production leads to the production of the beverage with satisfactory values of quality parameters as follows: pH value of 4,75, titratable acidity 28,8 °SH, syneresis 7,5%, viable cell count of 7.77 log (CFU mL^{-1}), and sensory characteristics value of 10, that is stable during 21 day of cold storage.
A new probiotic almond-based beverage that combines the properties of both almonds and probiotics can be considered as promising step toward the circular bioeconomy.

Keywords: Almond, Beverage, Probiotics, Circular bioeconomy, Antioxidants

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/200287).

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

ANTIOXIDANT ACTIVITY OF BLUEBERRY POMACE EXTRACTS OBTAINED BY ENZYMATIC-ASSISTED EXTRACTION

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Blueberries are highly consumed fruits worldwide. Apart from being rich in fibers, vitamins and minerals, blueberries are an abundant source of bioactive compounds (polyphenols, anthocyanins, phenolic acids, etc.) which possess strong antioxidant activity. Since the shelf life of these small and sensitive berries is quite short, most of the fruits are processed into frozen forms, jams, syrups, purees, or blueberry juice. The large volume of processed blueberry fruits results in significant quantities of solid waste or blueberry pomace, which contains between 25 and 50% of the phytochemicals initially present in this kind of fresh berries. Thus, there has been a great interest in reusing blueberry pomace as a resource of functionally-important bioactive molecules. The main aim of this research was to analyze the antioxidant activity (DPPH and FRAP test) of blueberry pomace extracts obtained by applying a combination of conventional solid-liquid extraction and three different commercially available enzyme preparations (cellulase- and hemicellulase-based preparation, pectolytic preparation and their combination).
The extraction of blueberry pomace samples was performed using diluted ethanol solution as solvent for 2 h at 30°C. Prior to the conventional extraction process, blueberry pomace samples were subjected to enzymatic-assisted extraction for 1 or 2 h at room temperature, using the same concentration of each enzyme preparation. In addition, in this way obtained blueberry pomace extracts were vacuum evaporated and then used for the determination of antioxidant activity. Results indicated that each applied enzyme preparation had approximately the same role during the maceration process of the blueberry pomace. In accordance with these results, obtained extracts had a similar antioxidant capacity, in the case of both DPPH and FRAP assay. Time extension of enzyme treatment from 1 to 2 h did not significantly affect the increase of antioxidant values of investigated blueberry pomace extracts.

Keywords: Blueberry pomace extracts, Enzymatic-assisted extraction, Bioactive compounds, Antioxidant activity

Acknowledgements: The research is part of the Project 451-03-68/2022-14/200134 and is financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

2nd International Conference
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DETERMINING CHANGES OF FATTY ACIDS FOR QUALITY ASSESSMENT OF EUROPEAN SQUID (*LOLIGO VULGARIS*) DURING TRADITIONAL AIR CHILLING WITH EFFECTS OF ACID WHEY IMMERSION ON STORAGE QUALITY

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The omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are found primarily in seafood. The long-chain omega-3 fatty acids, EPA and DHA can be synthesized from alpha-lipoic acid (ALA), but due to low conversion efficiency, it is recommended to consume foods rich in EPA and DHA. Loss of balance of n-6 and n-3 polyunsaturated fatty acids in the diet has been associated with the incidence of disease in modern civilization. Squids have lower contents of saturated fatty acids but higher contents of polyunsaturated fatty acids compared to ruminants. Also, it is known that the changes in fatty acids affect the product aroma. EPA and DHA reduce the nutritional value of fishery products because they are converted into compounds with lower molecular weight associated with off-flavors (e.g. aldehydes, ketones, alcohols). The aim of this study was to use fatty acid analysis as a potential method for determining quality changes and estimating shelf life of two types of squid samples (*Loligo vulgaris*) one immersed in acid whey and the other only under traditional chilling, both stored for 11 days at 4 ± 1°C. Here, n=48 individual squids that were caught in the Croatian Adriatic Sea. The fat contents of the samples were determined by the Soxlet method (HRN ISO 1443:1999). Fatty acid content and composition were determined in the samples by the gas chromatography method (HRN EN ISO 5508, 1999) using the Varian CP-3800 device, while triPlus autosampler was used for injecting. The results of DHA and EPA were compared with the results on each day of storage. Samples were analyzed post-mortem on days 2, 5, 9, and 11. The mantle length of the squid averaged to 14.5 ± 0.5 cm. On the last day of storage (day 11), the C20:5n3 content was 4% higher and the C22:6n3 content was 100% higher in the whey-treated samples. It was shown that fat content was one of the most important quality parameters. Therefore, the data suggested that immersion of squid mantles in acid whey may be beneficial storage technique that has the potential to protect polyunsaturated fatty acids.

Keywords: Squid, Loligo vulgaris, Chilled storage, Immersion, Acid whey, DHA, EPA

Acknowledgements: This research was funded by the following projects: No:KK.01.1.1.02.0005, and No:KK.01.1.1.04.0096.

2nd International Conference
on Advanced Production and Processing

THE EFFECT OF MODERN CLAIM RELATED TO PACKAGING SUSTAINABILITY ON THE SENSORY PERCEPTION OF GREEK CRACKERS

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Extrinsic product cues have been reported to affect consumer perception of food. The aim of the study was to investigate how consumer acceptability and emotional responses to traditional Greek rusks (paximathi) was affected by the information on sustainability of biodegradable and edible packaging. The experimental design comprised three sessions. First, rusks were assessed with the use of a 7-point hedonic scale and CATA questionnaire without packaging (tasting only), then in conventional (polypropylene) and biodegradable packages and finally after providing information on the sustainability of conventional, biodegradable and 'edible' packages. Results showed that information on packaging sustainability affected sensorial perception of the rusks and this was heavily driven by positive emotions. However, its influence was balanced by the negative effect of its sound. The findings provide evidence that communication on packaging sustainability, as external product cue connected to consumers' food choices, must be designed taking into consideration the impact of packaging material sensorial perception.

Keywords: Product cues, Biodegradable, Edible packaging, CATA

Acknowledgements: The authors are grateful to Poriazi Family- Lemnos for providing raw materials, expertise and equipment for the execution of this study.

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DO WE HEAR WHAT WE EAT? A COMPARISON STUDY OF INSTRUMENTAL SOUND ANALYSIS OF CRACKERS AND THEIR SENSORY PERCEPTION

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Crackers are widely consumed snacks that are made predominately from wheat flour. In order to improve nutrition and promote circular economy there have been increasing efforts to substitute wheat in crackers by alternative flours of higher nutritional value, such as local varieties of whole grains, legumes and agricultural by-products. However, both industry and consumers are strongly accustomed to the consumption of wheat in crackers and its substitution requires a multidisciplinary approach which would ensure changes of technological properties in product without compromising the sensory perception of products. The sound of crackers is known to affect the sensory perception of crispiness and crunchiness and as an extension their overall sensory perception by the consumer. The aim of the study was to investigate the effect of wheat substitution by alternative flours (10 and 30% lupin, barley, olive kernel and grape seed flour; 30 and 50% chickpea flour) on the sound profiles of crackers and the sensory perception of attributes “hardness”, “crispness”, “dryness” and “crumbliness”. Furthermore, thirty-eight emotion-based terms in the form of a check-all-that-apply (CATA) questionnaire were presented to the judges in order to provide insight into consumer perception of the samples. Statistical analysis of sound data showed differences in waveform (dB-t) values between the different samples as a result of wheat replacement by alternative flours. Results of sensory analysis for attributes “crispness” for control (100% wheat flour), chickpea and lupin differed from barley, olive stone and grape seed flours. Crackers made with lupin flour differed from all other crackers in terms of hardness, while crumbliness did not differ among samples. The majority of judges had chosen the term “relaxed” (59.6%), while half of the panel had chosen the terms “pleased” (53.2%) and “calm” (52.3%) in order to evaluate the samples with CATA. Results showed that sensory perception was affected by changes in the sound of samples in response to wheat replacement; however, consumer responses were different regarding to different types of flours. The findings provide evidence that there was a correlation between the perception of sound profiles and use of alternative flours for the substitution of wheat which needs to be considered for the development of new formulations.

Keywords: Food sound, Wheat substitution, Emotional responses

Acknowledgements: The authors are grateful to Poriazi Family- Lemnos for providing raw materials, expertise and equipment for the execution of this study.

ICAPP 2022
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TEXTURAL AND RHEOLOGICAL PROPERTIES OF COOKIE DOUGH WITH ADDITION OF CHESTNUT FLOUR

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Functional food products usually contain some specific component, in the aim to realize some health benefits and to improve diet quality. Due to nutritional quality and potential beneficial health effects, chestnut flour is suitable for formulation of functional cookies. But, that means significant changes of textural and rheological properties of dough, which must be adapted to easy manipulation during processing. In that aim, the influence of applied chestnut flour, as an ingredient, on textural and rheological properties of obtained dough was observed. Dough with 100 % of wheat flour and moisture content of 20, 22 and 24 % were control samples. Other dough formulations were prepared with the addition of chestnut flour (20, 40 and 60 %), as the partial replacement of wheat flour, calculated on the flour weight. Increase in amount of chestnut flour for 20 % caused an increase in the dough hardness and the decrease in dough extensibility and resistance to extension. Also, high amount of chestnut flour caused low flexibility and brittle consistency of dough structure with domination of linkages with hard, elastic nature. Increase in dough moisture for 2 % caused softer dough consistency, higher dough extensibility and more flexible dough structure with pronounced ability to recover. Optimal textural and rheological properties, which provide flexible manipulation with dough during processing, can be achieved with dough formulation containing 20 and 40 % of chestnut flour and 24 % of dough moisture.

Keywords: Functional food, Cookies, Chestnut flour, Rheology, Texture

Acknowledgements: Research was done within National program no. 451-03-68/2022-14/200134

2nd International Conference
on Advanced Production and Processing

SEPARATION OF PROTEIN FRACTION FROM SUGAR BEET LEAVES

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Sugar beet leaves are recognized as a potential source for various food applications based on specific nutritional profile and technological properties of leaf components. Furthermore, based on the large availability of sugar beet leaves generated as agricultural waste streams, there is a great economic interest in the valorization of such waste product.
In this study, processing approach that aims at separating/generating enriched-protein fraction is applied. The corresponding process includes pressing of sugar beet leaves, chemical treatment of the obtained leaf juice, centrifugation and further sedimentation of the protein fraction by thermal treatment and isoelectric precipitation. The obtained protein fraction was not divided in “green” and “white” protein fraction but rather collected as a total yield which could significantly impact organoleptic properties of the final protein fraction. Total sediment obtained after final centrifugation is air dried and after crushing in mortar used for further analysis. Total amino acids content was measured as well as the content of each 17 amino acids present in the sample by using ion-exchange chromatography equipped with an automatic amino acid analyzer. Protein content was measured and calculated by using standard Kjeldahl nitrogen analyzer.
Total amino acid content in the obtained dried sample was 12.48g/100g sample. Amino acids present in the largest amount were aspartic and glutamic acid, leucine, valine and arginine. Protein content measured by standard Kjeldahl method was 20.09%. Total yield of protein fraction obtained in the corresponding experiment was 5 g per 1000 g of sugar beet leaves.

Keywords: Sugar beet leaves, Protein, Sugar, Production

Acknowledgements: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia [number 451-03-68/2022-14/200134].

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

EMULSION STABILIZING CAPACITY OF THE SUNFLOWER MEAL PROTEINS

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Sunflower meal represents a by-product massively obtained after the extraction of oil from sunflower seeds. With its relatively low price, high protein content, and optimal amino acid composition it can be potentially suitable as an emulsifying agent. Thus, the aim of this study was to investigate the influence of the addition of the sunflower meal on the stability of oil-in-water emulsions. Five sunflower meal fractions, each with different protein content (16.78, 23.23, 29.67, 36.12, and 42.56%) were obtained and used in stabilization of 10% oil/water emulsions. The obtained emulsions were analyzed, where responsive variables were: creaming index, droplet size and droplet size distribution, and zeta potential. Creaming of investigated emulsions was monitored visually for seven days and the creaming index was calculated. Droplet size and droplet size distribution were measured using the laser light scattering method, while zeta potential was measured by using Zetasizer Nano ZS. Results indicated higher physical stability of the emulsions stabilized using the sunflower meal fractions with higher protein content. The creaming index decreased with an increase in protein content whereas emulsion with a protein content of 42.56%, after seven days, had the lowest creaming index of 58%. The obtained emulsion droplet D4.3 diameter ranged from 238.61 μm (sunflower meal fraction with lowest protein content) to 86.78 μm (sunflower meal fraction with highest protein content), while higher protein content contributed to the lower zeta potential values of observed emulsions. Zeta potential of emulsion obtained from sunflower meal fraction with a protein content of 16.78% was -6,80 mV, while zeta potential of emulsion obtained from sunflower meal fraction with a protein content of 42.56% was -12,20 mV.

Keywords: Sunflower meal, Protein content, Emulsion stability

Acknowledgements: This research was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia (Project No.451-03-68/2022-14/200134).

ICAPP 2022
2nd International Conference
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FATTY ACID PROFILE OF FUNCTIONAL OHRID TROUT PÂTÉ

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Fish pâté is a functional product because of its nutritive qualities. In this study, three formulations of fish pâté with different amounts of meat (60%, 50%, and 40%) of endemic specie Ohrid trout (*Salmo letnica*), and extra virgin olive oil as a fat substitute, were made. The content of unsaturated fatty acids, as well as ingredients as extra virgin olive oil and vegetable fibers make the functionality of the obtained products. The fatty acid profiles of three different formulations of Ohrid trout pâté were determined. The olive oil as ingredient in the pâté formulation affects the fatty acid profile of the pâtés and increases the content of monounsaturated fatty acids. The presence of docosahexaenoic acid in all three types of pâté is related with the quantity of meat in the pâté and varies from 0.91% to 1.16%.

Keywords: Functional pâté, Ohrid trout, Fat substitute, Fatty acids profile

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2nd International Conference
on Advanced Production and Processing

CHALLENGES IN THE APPLICATION OF UNMALTED TRITICALE IN BREWING

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Malted barley is the favoured cereal used in traditional brewing. Barley is modified during the malting process which is energy intensive. Because of that, brewing with a proportion of unmalted cereals- adjuncts- has become an attractive option for lowering costs, carbon footprint reduction and improving sustainability of the brewing chain. Adjuncts are often used as an alternative source of extract, but the main disadvantage is the decrease in enzymatic activities, which requires the addition of commercial enzymes during the brewing process. But, the first man-made cereal triticale is an exception. It shows promising brewing properties because of the high levels of enzymes activity. In combination with the low gelatinization range of triticale starch, it could be added during mashing process without additional thermal process. However, the usage of triticale in brewing could give viscosous mash, because of the solubilisation of arabinoxylans, which could lead to a slower beer filtration.
The objective of the study was to examine impacts of brewing with unmalted triticale variety NS Paun, accepted in Serbia, in a different proportions (10, 30, and 50%), with the addition of commercial enzyme for wort viscosity reduction – Shearzyme (from Novozymes) while using altered mashing regime regarding temperature, on the sensory and analytical profiles of lager beer. With an increase in triticale content in the grist, viscosity increased, which was corrected with the addition of commercial enzyme. Triticale, at every ratio, had higher wort extract when altered mashing regime was applied compared to values obtained during standard regime, with enzyme addition. The highest ethanol content was obtained for beer produced with the 10% of triticale in the grist (2.98%), when altered mashing regime was applied. The obtained results indicate that triticale variety NS Paun had good technological parameters and could be used as a partial substitute for barley malt in beer production.

Keywords: Triticale, Adjuncts, Malt, Beer

Acknowledgements: This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement No. 451-03-68/2022-14/200134).

ICAPP 2022
2nd International Conference
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APPEARANCE AND TEXTURE OF GLUTEN-FREE BREAD CONTAINING NON-GERMINATED AND GERMINATED ALFALFA SEEDS

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Visual parameters such as bread color and volume are crucial factors in consumers' decision for purchasing the product. Gluten-free breads (GFB) are usually characterized by lighter crumb and crust color, reduced specific volume and crumbling texture. When compared to wheat counterparts, apart from low nutritional value, currently available GFB do not completely fulfill the consumers' expectations in terms of external appearance (color, volume) and texture. To overcome the above mentioned quality defects many research studies have focused on investigation of novel raw materials (legumes, pseudocereals, seeds, fruits) for GFB production.

Alfalfa as extensively cultivated forage legume, and its seeds could arise as a functional ingredient in GF bread-making influencing both appearance (color, volume, texture) and nutritional value, considering high content of protein, dietary fiber, essential polyunsaturated fatty acids, minerals, vitamins and associated polyphenols. However, alfalfa seeds usage could be limited by the presence of antinutrients and beany flavor demanding seeds processing prior to application. To address these limitations germination as a natural processing technique can be applied. In this context, present study investigates the influence of non-germinated and germinated alfalfa seeds flour addition (5% on maize flour/starch basis) on GFB appearance (crust and crumb color and specific volume) and texture parameters (hardness, cohesiveness, chewiness, springiness). Corresponding properties of GFB containing alfalfa were compared with control and commercial GFB.

GFB containing non-germinated and germinated alfalfa were characterized by darker crust and crumb color (lower L^* values) with less green ($a^* < 0$) and yellow ($b^* < 0$) coloration compared to control GFB. Volume-depressing effect was observed after inclusion of alfalfa and it was more pronounced in GFB containing germinated alfalfa. Hardness values ranged between 322.6 and 2818.2 g, where GFB containing non-germinated alfalfa had the softest crumb. GFB containing alfalfa had increased cohesiveness compared to control, whilst no significant change in springiness was observed.

Keywords: Gluten-free bread; Germination; Alfalfa; Hardness; Color;

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia [number 451-03-68/2022-14/200134].

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF JUNIPER BERRY (*Juniperis communis* L.) ESSENTIAL OILS

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Juniper berry (*Juniperus communis* L.) is the most widespread type of juniper, which is characterized by nutritionally valuable components that are distributed in all parts of the plant. In this research dried juniper berries, collected on the territory of Montenegro, were used for isolation of essential oils using distillation technique in a microwave oven (MAHD). The process of isolating the essential oils was based on varying the power of the microwave oven (200 W, 400 W, 600 W, and 800 W) for 2 hours, in order to examine the influence of process parameters on the chemical composition and biological activity of the oil. Also, the essential oils release kinetics were deterimined. The obtained essential oils were analyzed by the GC-MS method. The antioxidant potential was examined using several *in vitro* antioxidant assays: ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl) and CUPRAC (cupric reducing antioxidant capacity). The inhibitory effects of the extracts against different enzymes, cholinesterases, α-amylase, and α-glucosidase, were analyzed . The results of the analysis indicate that the highest content of α-pinene and sabinene was recorded in the oil obtained by applying a power of 400 W (33.36±2.23% and 5.70±0.69%, respectively), while the highest content of β-pinene was obtained at a power of 600 W (30.872±1.10%). On the other hand, the power of 800 W was the most suitable for isolating essential oil with highest content of myrcene (18.166±1.42%). The best scavenging capacity and reduction potential were achieved with oil obtained at 200 W (27.69±0.70 and 140.00±1.63 mg TE/g, respectively). Juniper oil isolated with a power of 800 W was the most potent inhibitor of the enzymes AChE (2.79±0.01 mg GALAE/g) and α-glucosidase (0.95±0.07 mg ACAE/g). All oils were very rich in monoterpenes, especially α-pinene, β-myrcene, β-pinene, and sabinene, which are presumably responsible for pronounced biological activities.

Keywords: Juniperus communis L*, Essential oil, Chemical composition, Biological potential*

Acknowledgements: This research study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. 451-03-68/2022-14/200134).

ICAPP 2022
2nd International Conference
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FORTIFICATION OF BETAINE CONTENT IN SPELT BISCUITS ENRICHED WITH WILD GARLIC OSMODEHYDRATED IN SUGAR BEET MOLASSES

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Betaine is known as bioactive compound with important physiological functions, primarily as an osmolyte and methyl donor. Since betaine cannot be synthesized in adequate amounts in the human body, including betaine-rich ingredients or betaine-fortified foods in the daily diet is essential for the prevention and treatment of many chronic diseases. Sugar beet molasses is the main source of betaine, and wholegrain flour from spelt contains more betaine compared to common wheat flour. In our previous study, the formulation of a new product, spelt biscuit with the addition of wild garlic osmotically dehydrated in sugar beet molasses was defined. The aim of this research was to examine the betaine content in wild garlic leaves after osmotic dehydration in molasses, as well as in biscuits with its addition (5 and 10 % flour basis). The content of betaine was measured by the developed and validated HPLC-ELSD method. Results revealed that through the mass transfer by osmosis, 433.83 mg/100g of betaine penetrated into the wild garlic samples immersed in molasses (5884.92 mg/100g of betaine), for 4 hours at room temperature. Consequently, the betaine level in biscuits enriched with osmodehydrated wild garlic was rised up to 3 times in comparison to the control biscuits.

Keywords: Betaine, Molasses, Spelt biscuit, Osmotic dehydration

Acknowledgements: This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant Numbers 451-03-68/2022-14/20013.

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2nd International Conference
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SHELF LIFE OF SPELT MUFFINS SUPPLEMENTED WITH APPLE POWDER ENRICHED WITH SUGAR BEET MOLASSES

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Muffins are a popular snack meal because of their consumer-appealing features (ready-to-eat form, affordable price, soft texture, and pleasant taste). However, changing the typical batter composition, which usually includes wheat flour, oil, egg, sugar, and milk, may affect the sensory aspects of muffins, such as color, texture, and flavor, as well as consumer approval. Within our previous study, spelt muffins were supplemented with 10, 20, and 30 % apple powder obtained by freeze-drying with osmotic pre-treatment in sugar beet molasses solution, introduced as a whole spelt wheat flour replacement in muffins. Results revealed that adding 30% apple powder is the most acceptable for consumers. The obtained muffins had improved nutritional properties with decreased textural properties. As a continuation of research, this study investigated the highest-rated spelt muffins (with 30% apple powder) for microbial activity and sensory scores during storage (0-10 days). The total microbial count (TMC), yeasts, and molds load was not increased during the storage period. The results indicated that during storage at 25°C, the muffins' shelf life could be limited to 6 days without any quality deterioration.

Keywords: Spelt muffins, Molasses, Supplement apple powder, Shelf life

Acknowledgements: This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant Numbers 451-03-68/2022-14/20013.

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QUALITY PARAMETERS INVESTIGATION OF THE BISCUITS WITH OSMOTICALLY DEHYDRATED NETTLE LEAVES

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For centuries, nettle (Urtica dioica L.) leaves have had beneficial applications in treating a wide range of diseases or disorders. The specific chemical composition makes this plant valuable for different applications. The impact of nettle leaves osmotic dehydration in sugar beet molasses on biscuit quality characteristics (texture, color, and chemical composition) is explored in this study. The results revealed that adding osmotically dehydrated nettle leaves in sugar beet molasses improved biscuit texture features by reducing hardness, increasing fracturability, and positively changing biscuit color characteristics. The results of this investigation revealed that the chemical composition of biscuits with added osmotically dehydrated nettle leaves was enhanced in comparison to the biscuits with added fresh nettle leaves, where proteins, total sugars, cellulose, and ash compositions were increased in amounts of 1.56, 2.9, 14.7 and 4.98 % respectively. Compared to fresh nettle leaves, adding osmodehydrated nettle leaves to the biscuit dough formulation resulted in greater Zn, Cu, and Fe biscuit content by 2.55, 14.78, and 15.32 %, respectively. It can be concluded that the addition of osmotically treated nettle leaves in sugar beet molasses positively affected the observed quality parameters of the biscuits.

Keywords: Nettle leavs, Osmotic dehydration, Sugar beet molasses, Biscuits

Acknowledgements: This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant Numbers 451-03-68/2022-14/20013.

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BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF CABBAGE AND PEA MICROGREENS

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Significant interest in the food sector with a focus on healthier food leads to the growing popularity of microgreens, young vegetables produced from the seeds of vegetables, cereals, or herbs, harvested without roots between 10 and 14 days from seeding. As very young plants, microgreens possess a broad spectrum of phytochemicals, such as phenolic compounds and pigments (chlorophylls, betalains, etc.). Therefore, microgreens are considered as functional food, stand- alone, or as ingredients, with a great impact on the human diet. In thisstudy, the content of bioactive compounds (phenolics, chlorophylls, and carotenoids) and antioxidant activity (DPPH$^{\bullet}$ and reducing power assay) was determined in cabbage and pea microgreen samples after simulation of digestion. The content of bioactive compounds for cabbage and pea microgreens were: total phenolics 0.525 and 1.015 mg/100 DW, carotenoids 3.98 and 3.00 mg/100 DW, and chlorophylls 303.22 and 184.06 mg/100 DW, respectively. On the other hand, antioxidant assays have shown the scavenging activity against DPPH$^{\bullet}$ to be 165.54 and 248.42 μmol TE/g DW, while reducing power was 122.21 and 194.51 μmol TE/g DW. In conclusion, it can be proposed that, due to the presence of bioactive compounds, microgreens express antioxidant and health beneficial properties. This study has shown that cabbage microgreens had stronger antioxidant activity, owing to their higher content of total phenolics, while pea microgreens contain more carotenoids and chlorophylls.

Keywords: Microgreens, Antioxidant activity, Bioactive compounds, Cabbage, Peas

Acknowledgements: This research study was funded by the Ministry of Education, Science, and Technological Development of the Republic of Serbia (Grant No. 451-03-9/2022-14/200134).

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REDUCTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN TRADITIONAL DRY FERMENTED SAUSAGES

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The aim of this study was to examine the influence of modification in traditional smoking process on the content of 16 PAH, from Environmental Protection Agency list (16 US-EPA PAH). Two groups of dry fermented sausages were prepared - T and E group. The first group of sausages was smoked in traditional conditions, i.e. fireplace was in the smokehouse and the distance between fire and samples was about 2 m (T group). The second group of sausages was smoked in experimental conditions where the fireplace was separated from the smoking chamber, and smoke was transported to it through the pipes about 1 m long (E group). Sausages smoked in traditional conditions had a significantly higher ($P<0.05$) total content of 16 US-EPA PAHs (1030.75 µg/kg), compared to the content obtained for sausages smoked in the experimental smoking chamber (73.62 µg/kg). Benzo[a]pyrene was not detected in any investigated sausage sample. On the other hand, PAH4 (sum of benz[a]anthracene, chrysene, benzo[b]fluoranthene, and benzo[a]pyrene content) was significantly higher ($P<0.05$) in sausages of T group (5.10 µg/kg) compared with sausages of E group (ND) at the end of drying period.
According to the results obtained in this study both groups of examined dry fermented sausages met the criteria prescribed by Regulations Commission of the European Union No. 835/2011 (BaP ≤ 2 µg/kg; PAH4 ≤ 12 µg/kg). Also, results of this study confirmed that modifications in traditional smoking conditions lead to significant reduction of PAH content in dry fermented sausages.

Keywords: Dry fermented sausage, PAH, BaP, Smoking conditions

Acknowledgements: This research was financially supported by the Provincial Secretariat for Science and Technological Development of the Autonomous Province of Vojvodina (142-451-2039/2022), as well as by the Ministry of Education, Science and Technological Development, Republic of Serbia, Program (451-03-68/2022-14/200134).

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THE POSSIBILITY OF AFLATOXINS SYNTHESIS OF DIFFERENT *A. flavus* AND *A. parasiticus* STRAINS ISOLATED FROM CEREALS AND NUTS

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Molds represent significant and frequent contaminants of a large number of food products. Cereals and cereal products, which are very susceptible to mold contamination, play a significant role in the diets of numerous animals and people around the world. Besides cereals, nuts and seeds are the most tested food products for mycotoxin content, especially aflatoxins. Aflatoxins synthesized by some species of the genus *Aspergillus*, especially *A. flavus* and *A. parasiticus,* are the strongest natural carcinogens, and aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) occur as the most common contaminants of food and feed.

In this paper, 15 different strains of *A. flavus* and 3 different strains of *A. parasiticus* isolated from products based on cereals and nuts from the territory of the Republic of Serbia were examined for the possibility of aflatoxin synthesis. Using two distinct methods, the total number of molds in the samples was isolated: direct-plating method and dilution method. Identification of isolated molds was done based on their macroscopic and microscopic characteristics. The aflatoxin content was determined in the YES broth after 14 days of storage. Aflatoxins were extracted from YES broth samples by using solid-phased extraction. Identification and quantification of aflatoxins were carried out by ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry. The dry mycelia masses after 14 days of storage were also determined.

The dry mycelia masses of different isolated *A. flavus* strains ranged from 0.9373 g to 4.3585 g, while the dry mycelia masses of isolated *A. parasiticus* strains were of similar values, around 2.6000 g. Dominant content of AFB1 and a slightly weaker content of AFB2 and AFG1 were observed, while the content of AFG2 was lower than the limit of quantification (LOQ). In three isolated *A. flavus* strains, the content of AFB1 was lower than LOQ, while in other strains it ranged from 0.0138 µg/L to 5224 µg/L. The content of AFB1 in different isolated *A. parasiticus* strains ranged from 41.6 µg/L to even 8840 µg/L, which is also the highest recorded AFB1 content in all tested samples.

Keywords: Cereals, Nuts, A. flavus, A. parasiticus, Aflatoxins

Acknowledgements: This work was supported by the Provincial Secretariat for Higher Education and Scientific Research, Republic of Serbia, Autonomous Province of Vojvodina (Project No. 142-451-2623/2021-01) and by the Project " New methods for the control of aflatoxigenic molds and aflatoxins in food - current trends and future perspectives", supported by Matica Srpska, Novi Sad, Serbia.

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ANTIMICROBIAL ACTIVITY OF CELL-FREE SUPERNATANT OF *LACTOBACILLUS* SPECIES AND BASIL ESSENTIAL OIL MIXTURE ON FOODBORNE BACTERIA

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The increase of need for naturally health-safe food has led to the increased interest in natural preservatives, such as essential oils of aromatic plants or lactic acid bacteria metabolic products, which could be an alternative to chemical preservatives.
In this work, antimicrobial activity of mixtures made from cell-free supernatant of selected *Lactobacillus* species (*L. paracasei* ssp. *paracasei*, *L. fermentum*, *L. rhamnosus*, and *L. plantarum*) and basil essential oil on pathogenic foodborne bacteria (*Escherichia coli*, *Salmonella* Enteritidis, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*) was investigated by microdillution method.
Minimal inhibitory concentration (MIC) for cell-free supernatant of *Lactobacillus* spp. was in the range of 31.2 to 125 µL/mL, while minimal bactericidal concentration (MBC) ranged from 125 to 250 µL/mL. MIC for basil essential oil ranged from 3.9 to 15.6 µL/mL, while MBC ranged from 7.8 to 62.5 µL/mL. Mixtures of cell-free supernatant of *Lactobacillus* spp. and basil essential oil showed stronger inhibitory effects on the tested bacteria in comparison to the individual supernatant of *Lactobacillus* spp. or oil. Mixtures containing higher ratio of basil essential oil showed stronger inhibitory effect on investigated bacterial species. Some of the tested mixtures exhibited synergistic effect on the inhibition of *S. aureus*, *L. monocytogenes*, and *B. cereus* growth with the FICindex from 0.74 to 0.99.
The obtained results showed that mixtures had strong antimicrobial activity and therefore could be used as natural food preservatives.

Keywords: Cell-free supernatant of Lactobacillus spp., Basil essential oil, Synergistic effect

Acknowledgements: This work was supported by the Program of the Ministry of Education, Science and Technological Development of the Republic of Serbia (No. 451-03-68/2022-14/200134) and by the Provincial Secretariat for Higher Education and Scientific Research, Republic of Serbia, Autonomous Province of Vojvodina (No. 142-451-2623/2021-01).

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QUALITY CHARACTERISTICS OF BEEF JERKY MADE IN LABORATORY CONDITIONS

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Beef jerky is a traditional meat product that is preserved by means of salting (curing) and drying, i.e. reduction of water activity (a_w). This study was carried out to investigate the quality characteristics of beef jerky made in laboratory conditions. In order to prepare the meat for drying, the beef muscle (*M. Semimembranosus*) was firstly cut into thin slices and then into strips (approx. 10 x 1.5 x 0.5cm). The strips were marinated by dipping in a solution containing salt and flavoring ingredients (three types of marinade – S_1, S_2, S_3). Heat processing of jerky was conducted using constant climate chamber, Model KBF 115 (BINDER GmbH, Germany), and it was comprised in two distinct steps: "cooking phase" (temperature of 70°C during 2h and 15min; relative humidity ranged from 80% to 93.4%, being ≥90% for at least 1h.) and "drying phase" (temperature ranged between 60°C to 65°C; relative humidity gradually decreased from 70% to 35% during 4h and 15 min). Quality and safety characteristics of obtained beef jerky were verified by microbial analyses, determination of water activity (a_w), moisture-protein-ratio (MPR), as well as nutritive value. Pathogenic bacteria (*Salmonella spp., Escherichia coli* and *Enterobacteriaceae),* yeasts and molds, were not detected in any sample. Water activity (a_w) (0.786 - 0.814) and MPR (0.61 - 0.66) in final products, were lower than recommended maximal values, being 0.85 and 0.75, respectively (FSIS Compliance Guideline, 2014). The protein content of the analyzed samples ranged from 47.61% to 49.96%, which makes jerky a rich source of protein. In the same time, the lipid content was very low, ranging from 5.67% to 6.29%. The energy value of produced beef jerky ranged between 267 (S_1) and 280 (S_3) kcal/100g, amounting approx. 11% of average daily energy requirement for normal adult person.

Keywords: Beef jerky, Microbiological safety, Nutritive value, Water activity

Acknowledgements: This research was financially supported by Ministry of Education, Science and Technological Development, Republic of Serbia, Institute of Food Technology in Novi Sad (Grant Number: 451-03-68/2022-14/200222).

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PHYSICO-CHEMICAL PROPERTIES OF FLAXSEED OIL CAKE-BASED BIOPOLYMER FILMS AFECTED BY PROCESS PARAMETERS

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In the area of edible packaging, materials obtained from food industry by-products/waste occupy an important place. After oilseeds cold pressing in order to obtain edible oils, valuable by-products are left over and could be used for animal feed enrichment, biofuel production or for the extraction of valuable components. These cakes or meals offer a suitable substrate for biopolymer materials (films) production due to the high protein and polysaccharide content.
In this work, the whole flaxseed oil cake was used as a substrate for the synthesis of biopolymer composite films. Plasticizer (glycerol) was added to the 10% flaxseed oil cake suspension in various concentrations: 10%, 20% and 30% to create the filmogenic solution. Then the solution was heated to temperatures of 60°C and 90°C during 20 minutes after which the pH was adjusted to 8, 10, and 12 and, finally, 18 samples were obtained. The produced films were visually very similar: blurred, dark brown, rigid and frail, with color gradient (the films were getting darker as the applied pH increased). Basic physico-chemical properties were identified: moisture content, solubility and swelling degree.
The moisture content was mostly affected by the amount of added glycerol. An increasing trend of moisture content was observed, which increases along with the increase in the applied plasticizer content. Moisture content values for samples with 10% glycerol were in the range 5.86%-8.14%, and for samples with 30% added glycerol in the range 22.93%-33.46%. Further, within each sample group, a decrease in the value of moisture content is noted in the samples where a higher temperature was applied. The obtained results for solubility values imply that a higher glycerol concentration influenced higher values of the solubility. On the other hand, higher applied temperatures and higher glycerol concentrations caused lower values of the swelling degree, while the influence of applied pH was insignificant. All tested parameters of the biopolymer material based on flaxseed oil cake are within the limits characteristic for biopolymer composite films. In order to apply film based on flaxseed oil cake in the food packaging sector, it is necessary to additionally examine the mechanical, barrier and biological properties of the film.

Keywords: Flaxseed oil cake, Process parameters, Physico-chemical properties, Optimization

Acknowledgements: This paper is a result of the program of the Ministry of Education, Science and Technological Development, Republic of Serbia, No. 451-03-68/2022-14/200134.

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APPLYING DIFFERENT INFRARED SPECTROPHOTOMETRY METHODS FOR DETERMINING THE DEGREE OF DEACETYLATION OF CHITOSAN

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Chitin, the most abundant biopolymer after cellulose, is a linear, highly crystalline polysaccharide, built from acetylglucosamine monomer units connected by β-1,4 glucosidic bonds. High degree of acetylation (DA) and the crystallinity of chitin molecules make it insoluble in common commercial solvents. Chitosan is the name for chitin substitutes with a low degree of acetylation. The degree of deacetylation in chitin usually ranges from 5% to 15%, and in chitosan from 70% to 95%. This contributes to the solubility of chitosan in aqueous solutions of acids and enables the use of chitosan and its bioactivity. When chitosan biopolymer film properties are considered, DA is an important input, due to the proven influence of DA on chitosan film´s mechanical, physico-chemical and bioactive properties. Developed methods for chitosan DA determination are based on different approaches: infrared spectroscopy, UV-spectrophotometry, nuclear magnetic resonance, colloidal titration, and potentiometric titration. Various procedures using infrared spectroscopy technique have been developed to determine DA of chitosan. In this article, these methods were tested for application on chitosan isolated from blue crab shell (*Callinectes sapidus*). Two different approaches were assessed: 1) determination of the absorption ratio of a probe band and of a reference band and estimation of DA of unknown samples by comparison with samples with known DA and 2) evaluation of several proposed absolute methods that are used in the literature for the DA determination.
Results showed that applied absolute methods gave results that varied between the methods applied, but were consistent among different samples within the framework of a particular method. Use of different probes and reference bands for calculation of ratios that would be compared to the reference samples of a known DA was more appropriate method. Selected ratios were A_{1655}/A_{3450} and A_{1320}/A_{1420}.
IR techniques for chitosan DA determination without reference to samples of known DA or calibration curve could be used for qualitative evaluation of DA, while quantitative determination should include mentioned references. Use of absolute methods for determination of DA by IR spectroscopy was shown not to be appropriate in this work.

Keywords: Chitosan, Acetylation, Degree, Infrared spectrophotometry

Acknowledgements: This paper is a result within the program of the Ministry of Education, Science and Technological Development of the Republic of Serbia, No. 451-03-68/2022-14/200134

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PULSED ELECTRIC FIELD PROCESSING OF PISTACHIO NUTS WITH PRESERVATION OF QUALITY PROPERTIES AND SURFACE DISINFECTION

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Pistachio nuts (*Pistacia vera L.*, Anacardiaceae family) due of their nutritional and sensory properties have been part of the human diet since prehistoric times and have been consumed by civilizations. Pistachios are a good source of vegetable protein and especially with L-arginine, rich in fiber and in Cu, Mg, Mn, vitamin A, vitamin C and B vitamins. They are rich sources of phenolic compounds such as anthocyanins, flavonols, flavonoids, flavanones, isoflavones, proanthocyanidins, stilbenes, phenolic acids and hydrolysable tannins, which are important as antioxidants. After harvesting, pistachios are usually sun dried on ground and during this period they are contaminated with food spoilage and foodborne spoilage microorganisms that resulting in quality degradation. Higher microbial load not only cause decrease in quality but also bring safety issues. Processing of pistachio nuts with heat, sanitizers such as ozone and antimicrobial agents are either cause deterioration on their quality or not allowed to be used. Thus, alternative technologies such as pulsed electric fields (PEF) are in search for surface disinfection of pistachios without adversely affecting its physical and sensory properties. Pilot scale PEF treatment system with changing energies from 2.97 to 71.23 J with monopolar square wave pulses with the maximum peak voltage of 20 kV. It has been shown that moderate electric field strengths have provided a significant increase on total phenolic substance content, total antioxidant capacity, and total chlorophyl content. While color (L*, a* and b*) values of the samples fluctuated; titratable acidity of the samples were not changed by with the applied energy. Increased energy application caused significant decrease on both total mesophilic aerobic bacteria and total mold and yeast. Sensory analyses of the PEF-treated samples had higher scores than that of the control samples in terms on appearance, color, taste, aroma and overall acceptance.

Keywords: Pistachio nuts, Pulsed electric fields, Microbial inactivation, Quality properties

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ENERGY VALUE AND MYCOLOGICAL QUALITY TESTING CONTOROL OF DOMESTIC JAMS

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Fruit is a rich source of bioactive compounds. In order to remain available out of season, while retaining bioactive compounds, the fruit is processed into a stable product, such as jam. Traditional jam preparation involves heat treatments, which can lead to changes in nutritional properties. The aim of this scientific work is to examine the energy values of jams in various domestic fruit species. Energy values of jams were calculated from the content of carbohydrates, proteins and fats made in accordance with the Ordinance on the quality of fruit and vegetable products, Official Gazette of the Republic of Serbia: 128/2020, 130/2021. The results of these tests indicated that fruit jams are low in fat but a good source of energy and carbohydrates. In order to test the frequency of yeasts and molds, microbiological methods were performed in accordance with SRPS EN ISO 21527-2:2011 standard. The results showed a complete absence of yeast and mold.

Keywords: Jam, Energy values, Yeasts, Molds

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EFFECT OF TEMPERATURE RANGE AND KILNING TIME ON THE OCCURRENCE OF POLYCYCLIC AROMATIC HYDROCARBONS IN MALT

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Kilning is an integral part of malt production. Kilning temperatures can range 80 – 220 °C, depending on what type of malt is produced. Polycyclic aromatic hydrocarbons (PAHs) are prone to appear at higher temperatures and are generally designated as undesirable in food and beverages. Sixteen PAHs are framed into the legislation, but there is a lack of scientific data related to PAHs in malt, malt-related foods and beverages. The aim of this paper was to asses and quantify the occurrence of different PAHs in malts exposed to different kilning temperatures (50 - 210 °C) over a variable time frame. The results indicate that some of the PAHs detected at lower temperatures disappear when malt is exposed to high temperatures (>100 °C). Phenanthrene was no longer detected at 100 °C, indeno[1,2,3-cd]pyrene at 130 °C, while fluorene, anthracene and benzo[a]anthracene were not quantified at 170 °C.

Keywords: Malt, PAH; Kilning temperature

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CERTIFICATION MARK TAS(Q) AS A DISTINCTIVE SIGN OF HIGH QUALITY TRADITIONAL FOOD PRODUCTS

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In order to standardize quality of traditional food products and to enhance their expansion in urban food market within Serbia and Hungary cross-border region, Interreg-IPA CBC project Traditional and Standard Quality - TASQ (HUSRB/1602/41/0146) was implemented. Quality assurance system for sensory, nutritional and processing quality evaluation was developed aiming to distinguish between traditional foods of high quality. Also, common certification mark Q was registered in Intellectual Property Office (IPO) of both neighbouring countries. For certification purposes, developed system was applied on a number of traditional food products. TASQ project team members conducted the on-site visits to traditional food producers providing them the consultancy regarding the improvement of their production process, traceability documentation, packaging and labeling. Simultaneously, the samples of nominated traditional products were taken for analyses. In order to determine food products' nutrient profile and to identify possible safety hazards, as well as to evaluate food sensory profile, the state-of-the-art laboratory equipment, methods and researchers were involved in the project. Overall, 158 traditional products (meat products, dairy products, honey, vegetable oils, processed fruits and vegetables, juices/beverages, pasta and baker's wares, confectionery, spices and teas) were certified with gold, silver or green Q mark, representing distinct quality level. Additionally, innovative internet platform (www.tasq.rs) was designed and developed in order to help traditional producers to promote and sell their products on a wider market. The assigned Q trademark of appropriate colour is clearly indicated for each certified product within the producers' profiles on TASQ internet platform, and represents a guarantee of product quality intended to raise customer confidence. Using the experiences, results and established resources within the aforementioned cross-border project, current research activities are focused on further expansion of the base of traditional food producers and their products within the framework of the TASQ internet platform, better connection between small producers and consumers, and therefore a better placement of traditional food products on the hospitality and tourism market of Vojvodina and beyond.

Keywords: Traditional food, Certification mark, TASQ, Internet platform

Acknowledgements: This work was supported by the European Union within the Interreg-IPA CBC Hungary-Serbia Programme (HUSRB/1602/41/0146), as well as by Provincial Secretariat for Science and Technological Development of the Autonomous Province of Vojvodina (142-451-2620/2021-01).

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DIFFERENCES IN DYNAMIC SENSORY PERCEPTION BETWEEN COMMERCIAL CHOCOLATE SPREADS

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Sensory perception is a dynamic process which requires application of appropriate methodology in order to best describe what is perceived by the human senses. Temporal Dominance of Sensation (TDS) is a sensory method that can be used for describing the perception of sensory attributes over time after ingesting a food. This approach is aimed at repeatedly recording the dominant sensations during the tasting period and enables characterization of an entire profile of complex food in a short time. The presented study is an exploratory assessment used to identify similarities and differences among the tested products and to highlight their characteristics in terms of dominant attributes over time. Six commercially available chocolate spreads of different composition were evaluated by TDS by trained sensory panelists (n=12, eight female and four male, 25–45 years old). The assessors were presented with a list of sensory attributes (sweet, bitter, salty, cocoa, hazelnut, milky, vanilla, creamy, grainy, and sticky) previously selected within the Free Choice Profiling sessions, and were asked to choose any attribute that they perceive as dominant. The obtained TDS curves showed different complexity of products sensory profiles. Sweet attribute was the first significant dominant attribute detected in all evaluated samples except in sample S3, for which grainy, creamy and sticky were more dominant sensations than sweetness. Bitterness was recognized as dominant sensation only in two samples (S3 and S5) in the later phases of ingestion, practically in these samples bitterness was experienced as an aftertaste. These two samples are also specific because only in these two samples cocoa attribute was not recognized as significantly dominant. The presented study showed that TDS was useful for comparing products based on their TDS curves. Moreover, it enables highlighting the attributes that contribute the most toward product discrimination and assessment of the influence of time.

Keywords: Dominant sensations, Sensory analysis, Chocolate, Sensory perception

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Contract No. 451-03-68/2022-14/200222).

ICAPP 2022
2nd International Conference
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ANTIMICROBIAL AND ANTIOXIDATIVE ACTIVITY OF SLOVENIAN HONEYS IN RELATION TO THE CONTENT OF PHENOLIC COMPOUNDS

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Honey is a natural product of *Apis mellifera* bees, from flower nectar or secretions from living parts of plants or secretions of insects that suck plant sap on living parts of plants collected by bees, processed with certain own substances, stored, dried and left to mature in the honeycomb. In terms of composition and properties, Slovenian honey must meet the criteria set out in the Rules on Honey (Official Gazette of the Republic of Slovenia, 2011). Bioactive molecules of honey exhibits antimicrobial activity against broad spectrum of microorganisms. In this study, 120 samples of Slovenian honey with determined botanical origin, electrical conductivity, water content, content of total phenolic compounds (TPCs), and antioxidative activity (DPPH and FRAP method) were included. Antimicrobial activity against three Gram-positive (*Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus*) and three Gram-negative (*Campylobacter jejuni*, *Escherichia coli*, and *Pseudomonas aeruginosa*) bacteria was determined by broth microdilution method and studied in relation to the composition of the samples. Dark, mannin/forest honeys (buckwheat, fir, chestnut, and forest honey) exhibited the highest antimicrobial activity, especially against Gram-positive bacteria (*S. aureus*), with minimal inhibitory concentrations (MICs) from 15.6 to >500 mg/mL. Light, nectar/flower honeys (multifloral, acacia, and rapeseed honey) had the lowest antimicrobial activity, particularly against Gram-negative bacteria (excluding *C. jejuni*), with MICs from 62.5 to > 500 mg/mL. Buckwheat honeys had the highest amount of TPCs and antioxidative activity. The results have shown that dark honeys are more antimicrobial active, have higher antioxidant activity and phenolic compounds content than light honeys. Additionally, there is a relationship between antioxidant activity and phenolic compound content in different types of honey, while it was not find a statistically significant relationship between the studied parameters – antimicrobial and antioxidant activity and phenolic compound content.

Keywords: Honey quality, Bioactivity, Antimicrobial activity, Antioxidant activity, Phenolic content

Acknowledgements: The results were achieved within the Program of Measures in the Field of Beekeeping in the Republic of Slovenia in the years 2020-2022, financed by MKGP and co-financed by the European Union.

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COMPARISON OF DIFFERENTE TESTS FOR TEXTURE CHARACTERISTICS ASESSMENT OF DRY FERMENTED SAUSAGES

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Dry-fermented sausage *Petrovská klobása* is traditionally manufactured during winter, within village households, undergoing slow drying and ripening processes. In traditional process fermentation is guided by indigenous microflora, but for the production in different processing condition addition of starter culture could be of great interest to ensure standard quality of final product. Texture characteristics are of great importance for sausage quality, and are affected by numerous factors. Sensory analysis for texture evaluation is only suitable for the final product so, instrumental measurements are especially interesting for texture evaluation during the processes of ripening and drying.

The aim of this study was to determine the influence of starter culture addition on texture characteristics of dry-fermented sausage, and to determine correlation between two mostly used instrumental methods for assessing texture characteristics of sausages: Warner–Bratzler and texture profile analysis during ripening and drying of dry fermented sausages produced in traditional manner (C), and with starter culture addition (SC). The sausages were produced from minced lean pork meat, pork fat and seasonings, and were stuffed in collagen casings. Production in traditional conditions lasted for 90 days and samples for texture analyses were taken after 2, 9, 15, 30, 60 and 90 days of processing.

The starter culture addition had no significant impact on the firmness, hardness and chewiness but at the end of ripening period CS sausages were higher in hardness and chewiness, and lower in firmness comparing with C sausages. No significant differences in texture characteristics could result from thermo-hygrometric conditions in traditional production, i.e. low atmospheric temperature did not facilitate the growth of added starter culture.

Correlation between firmness and hardness was very low, a bit higher was correlation between firmness and chewiness. Both correlations were higher for SC sausages comparing to C sausages.

Keywords: Dry fermented sausage, Warner–Bratzler test, Texture profile analysis

Acknowledgements: This research was financially supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Program (451-03-68/2022-14/200134), as well as by the Provincial Secretariat for Science and Technological Development of the Autonomous Province of Vojvodina (142-451-2039/2022).

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ANTIOXIDATIVE ACTIVITY CORRELATION BETWEEN OIL CAKES (RAW MATERIAL) AND BIOPOLYMER FILMS (PRODUCTS)

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Spoilage of food products is the consequence of different chemical reactions and microbial changes which may endanger consumer's health. Thus, there is an initiative by the food industry not just to ensure the products within the specified shelf life, but to extend the shelf life of foods as well. The use of chemical preservatives creates reluctance among consumers due to potential harmful effects on human health, so consumers prefer minimally processed foods or with natural compounds with pronounced antimicrobial and antioxidant properties. Active packaging with antimicrobial and/or antioxidant activity may control the process of food deterioration, while improving the condition of the packed product.

In order to produce active packaging material, biopolymers from different natural sources are used, because they are good carries of active components. Unlike the direct incorporation of antioxidant components, by using active biopolymer material active components are gradually released into food during storage period, that can contribute to preservation of food quality. Biopolymer materials obtained from agro-industrial by-products are cheap alternative to petroleum-based plastic. Since the by-products are highly nutritious with macro and micronutrients, they can be expected to possess antioxidant activity.

The aim of the study was to investigate the antioxidative activity of composite biopolymer films obtained from different oil cakes (flaxseed, pumpkin, sunflower and plum) and to compare them with antioxidative activity of oil cakes, in order to find correlation between biopolymer films and their raw material. The DPPH radical scavenging activity of biofilms and oil cakes has shown that some of analyzed samples can "scavenge" free radicals that act as free hydrogen atoms or as electron donors, but some of them has not shown any antioxidative activity. Plum seed oil cake (84.70%) has shown the highest antioxidative activity, as well as plum seed oil cake based films (70.47%).

Keywords: Composite biopolymer films, Oil cakes, Antioxidative activity, Active packaging material

Acknowledgement: This study was supported by the Ministry of Science and Technological Development of the Republic of Serbia, Project No. 451-03-9/2022-14/200134.

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PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF CAROB (*Ceratonia siliqua* L.) LEAF EXTRACTS OBTAINED BY GREEN EXTRACTION TECHNIQUES

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In recent years, a growing demand on functional products enriched with naturally derived ingredients has raised interest in finding new sources of bioactive molecules as well as establishing optimal isolation technologies that would allow their fast and efficient isolation. Various green extraction technologies, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) and pressurized liquid extraction (PLE) have emerged due to their main advantages of reducing the extraction time, energy and solvent consumption while achieving higher extraction efficiency and lower degradation of targeted compounds compared to conventional extraction techniques. Carob tree (*Ceratonia siliqua* L.) is a Mediterranean plant belonging to the Fabiaceae family which has recently drawn attention due to the nutritional value of its pods, while other plant parts, such as leaves, have been researched to a lesser extent. Since the health beneficial properties of polyphenols are well known, the aim of this research was to evaluate carob leaves grown in south Croatia as a source of polyphenols by analyzing the phenolic content and antioxidant activity of carob leaf extracts obtained by three different green extraction techniques (UAE, MAE and PLE). The extractions were carried out on a sample of grinded dry carob leaves for 10 min at a temperature of 80°C, sample: solvent ratio 1:20 and 50% aqueous ethanol as a solvent. The frequency in UAE was 37 kHz, microwave power in MAE 500 W and PLE was set to 1 static cycle. The total phenolic content (TPC), hydroxycinnamic acids content (HAC) and flavonol content (FC) were determined by spectrophotometric methods, while the antioxidant activity was determined by DPPH Radical Scavenging and Ferric Reducing Antioxidant Power (FRAP) assays. It was shown that carob leaves can be considered a source of phenolic compounds since the TPC was in the range of 51.86–64.81 mg gallic acid equivalents (GAE) g^{-1}. The extracts have also shown high antioxidant activity with values of 25,10–28,90 mM Trolox equivalent (TE) g^{-1} and 22,92–51,23 mM TE g^{-1} as determined by DPPH and FRAP, respectively. The extraction techniques have significantly influenced all of the examined parameters and MAE was shown to be the most suitable for achieving maximum TPC, HAC, FC and antioxidant activity as determined by DPPH. The results highlight the importance of research of different extraction techniques that would allow the highest recovery effectiveness and utilization of the plants' beneficial properties.

Keywords: Ceratonia siliqua L., Green extraction, Polyphenols, Antioxidant activity

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INFLUENCE OF SOLVENT AND MATERIAL PROPORTION DURING MICROWAVE-ASSISTED EXTRACTION OF *Dictyota dichotoma* AND *Thymus vulgaris* PIGMENTS

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Dictyota dichotoma is a brown alga found in rocky littoral and sublittoral zones from the Atlantic to the western Indian Ocean. Thyme (*Thymus vulgaris* L.) is a Mediterranean herb that belongs to the Lamiaceae family and is known in folk medicine for its various health-promoting properties. Both *D. dichotoma* and *T. vulgaris* contain a variety of biologically active compounds, which include pigments. Pigments, especially chlorophylls and carotenoids, have been the subject of numerous studies due to their potential and utilization for commercial and industrial purposes. They are well known for their industrial applications as food colorants and antioxidants, and have beneficial effects on human health. In order to increase extraction efficiency and to eliminate degradation of the target compounds, novel extraction techniques such as Microwave-assisted extraction (MAE)have been employed. The type of the solvent and the ratio of material in the extraction mixture are important parameters for obtaining higher concentrations of pigments. In this study, MAE was used to isolate pigments from *D. dichotoma* and leaves of *T. vulgaris*. The content of ethanol in the aqueous mixture (30, 50, 70 and 96%) and sample:solvent ratio (1:10, 1:20 and 1:30) were varied, while other extraction parameters (temperature 60 °C; microwave power 800 W; irradiation time 10 min) were kept constant. The content of chlorophylls, carotenoids and total pigments in the extracts was determined spectrophotometrically. Both sources of variation studied had a significant effect on all evaluated pigments. The highest levels of chlorophylls (3.33 mg/g), carotenoids (1.12 mg/g) and total pigments (4.45 mg/g) in the algal extract were obtained using 96% ethanol and a ratio of 1:30. The same conditions were equally effective for thyme extract, where the highest content of total pigments and chlorophylls was 0.46 and 0.40 mg/g, respectively. However, the highest content of carotenoids (0.06 mg/g) in thyme extract was obtained using 30% ethanol and a ratio of 1:30. Algae contained more pigments when compared to thyme, making them an important subject of future research on pigments isolation. The results of this study also confirm the importance of developing optimal isolation conditions that lead to the most effective recovery of pigments.

Keywords: Dictyota dichotoma, Thymus vulgaris, pigments, Microwave-assisted extraction

Acknowledgements: This work was supported by the project "Bioactive molecules of medical plant as natural antioxidants, microbicides and preservatives" (KK.01.1.1.04.0093), co-financed by the Croatian Government and the European Union through the European Regional Development Fund—Operational Programme Competitiveness and Cohesion (KK.01.1.1.04.).

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BIOMASS PRODUCTION IN CYANOBACTERIAL STRAINS OF THE GENUS NOSTOC ISOLATED FROM VOJVODINA PROVINCE (SERBIA)

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Cyanobacteria (blue-green algae) are a group of photosynthetic procaryotes known for the exceptional production of different bioactive compounds. Like the other algae, they are becoming more investigated for various purposes, such as nutrition and biofuel production. However, the commercial use is currently restricted to only a few species, including some from the genus *Nostoc*, traditionally used in nutrition and medicine in some parts of the world. In this regard, the starting point is the selection of strains with faster growth, as well as the optimization of cultivation conditions for the higher biomass yield. Given their photosynthetic metabolism, light is among the most important factors affecting growth, while nitrogen is the trigger of certain molecular mechanisms. In addition, some genera of cyanobacteria including *Nostoc* are N_2-fixing microorganisms, so they do not need a fixed nitrogen source (nitrate, ammonia, etc.) in the growth medium.

This study aimed to investigate the possibility of increased biomass production in four *Nostoc* strains isolated from Vojvodina Province by using continuous illumination and $NaNO_3$ as a nitrogen source. The strains were cultivated for 42 days and during this period the biomass production was determined spectrophotometrically, by measuring the chlorophyll concentration. The cultures under a 12:12 light/dark regime and without $NaNO_3$ in the growth medium were used as the controls. The results showed that continuous illumination increased the biomass production in two strains, approximately 2- and 3-fold, reaching 1.5 mg/mL in *Nostoc* S8, but it also inhibited the growth in the other two strains. On the other hand, the addition of $NaNO_3$ had no significant effect on growth; moreover, in the case of *Nostoc* strain LC1B, the growth was inhibited with $NaNO_3$. Eventually, the most productive strain among the tested was *Nostoc* LC1B, in conditions without continuous illumination and $NaNO_3$, with 2.4 mg/mL. Such results indicate the possibility to increase the production of biomass or specific compounds in *Nostoc* strains by manipulation of cultivation conditions such as light regime and growth medium composition.

Keywords: Biomass yield, Cyanobacteria, Illumination, Nitrogen source

Acknowledgments: The authors acknowledge the financial support of the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/200222 and Grant No. 451-03-68/2022-14/200125).

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DETERMINATION OF TERPENES FROM ESSENTIAL OILS IN PHYTOGENIC FEED ADDITIVES

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Essential oils and their main constituents, the terpenes, are widely studied due to their antioxidant ability and bioactivity, such as antimicrobial, anticancer, anti-inflammatory, and range of other beneficial effects on living organisms. Phytogenic feed additives, also known as phytobiotics or botanicals, are commonly defined as various plant secondary compounds and metabolites having beneficial effects on animal health and production. In this study the development and validation of a method for determining essential oil components was performed by gas chromatography-mass spectrometry (GC-MS). The components tested were p-cymene (from oregano and thyme oil), eucalyptol (from eucalyptus oil), γ-terpinene (from thyme and coriander oil), linalool (from lavender and thyme oil), linalyl-acetate (from lavender oil), camphor (from coriander oil), menthol and menthyl-acetate (from peppermint oil), thymol (from oregano and thyme oil) and carvacrol (from oregano oil) in phytogenic feed additives: RidOfMite®, ImmunoHype® and Patente Herba Plus®. Analytes from three feed additives were extracted with absolute ethanol at room temperature for 15 min. After extraction, samples were diluted with hexane and analyzed by GC-MS under following conditions: analytical column HP-5MS-30 m, 250 µm × 0,25 µm, Inlet Liner (Universal, Wool), injection volume 1 µl, split ratio 20:1, column flow: 1,2 ml/min. Initial temperature was 60°C, and run time was 24,917 min. Calibration range was from 0,1-1,5 mg/l for all compounds. The matrix matched calibration and internal standard were uses during analysis. The method was validated using GC-MS for limit of quantification (200 mg/kg), linearity (R^2>0.99), repeatability (1,68-3,61%), reproducibility (1,75-5,82%), and recovery (95-102%).

Keywords: Terpenes, Phytogenic feed additives, GC-MS

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2nd International Conference
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THE EFFECT OF PARTIAL PROTEOLYSIS ON TECHNO - FUNCTIONAL AND ANTIOXIDANT PROPERTIES OF MODIFIED SOY PROTEIN ISOLATES

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The partial enzymatic hydrolysis is effective and safe method which can be used to improve nutritive, techno-functional and functional properties of soy protein products. This method is affected by numerous factors. The objective of this work was to examine the effect of partial proteolysis on emulsifying and foaming properties of soy protein isolate. Also, the effect of proteolysis on ABTS-radical scavenging activity and iron (III) chelating ability was followed. Spray-dried soy protein isolate was prepared by isoelectric precipitation and modified using three different commercial proteases, Alcalase, Flavourzyme, and Papain. Initial isolate was hydrolyzed for 30, 60 and 90 minutes. Proteolysis was followed by the change of degree of hydrolysis (DH) and by native-PAGE, SDS-PAGE under reducing and non-reducing conditions. The effect of proteolysis on emulsifying properties was followed using emulsion stability index (ESI) and emulsion activity index (EAI). Also, foaming capacity and stability of foams formed using modified isolates were determined. Emulsifying and foaming properties was measured at pH 3, 5 and 7. Modified isolates were characterized with different DH values which were in the range of 11.75±0.08 (30 min, Papain) - 43.30±0.15 (Alcalase, 90 min). Limited proteolysis differently affected emulsifying properties of soy protein isolate. All modified isolates had improved emulsion stability at all investigated pH value whereas their ability to form emulsion was slightly lower or equal to initial isolate but in the range of commercial isolate used as standard. The highest stability had samples modified 30 min using Flavourzyme (366.94±48.85 min). Contrary, limited hydrolysis improved foaming capacity, but these foams were extremely unstable. The used enzymes differently affected the antioxidant properties of soy isolate. Only Alcalase improved radical scavenging activity of the initial isolate whereas the reducing power of all the investigated hydrolysates was lower than that of the initial isolate. Alcalase and Papain improved the ability of isolate to chelate iron (II) ions, whereas Flavourzyme-modified isolates had reduced iron (II) chelating ability. The initial IC_{50} of soy isolate was improved by 31.55-43.87% (Alcalase-modified) and by 21.08-60.40% (Papain-modified).

Keywords: Soy protein isolate, Limited proteolysis, Emulsifying, Foaming, Antioxidant properties

Acknowledgements: This investigation was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia through Grant No. 451-03-68/2022-14/200116.

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EVALUATION OF THE EFFICIENCY OF SANITIZERS ROUTINELY USED IN POULTRY SLAUGHTERHOUSE IN BRAZIL

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Sanitizers are routinely used in poultry slaughterhouses in the process of cleaning equipment to avoid contamination during the production process. The lack of hygiene and improper use leads to the presence of pathogenic microorganisms, mesophilic aerobes and bacteria of the coliform group. That is why it is important to know the efficiency of the sanitizers used in the slaughterhouse, as many of these bacteria end up acquiring resistance to some of these products. The present work aims to evaluate the efficiency of Bracsan, Enztrat®; Chlorine; Whisper; B-Quart-Sept®, Polyhexamethylene biguanide against the following microorganisms: *Staphylococcus* sp. (positive coagulase), *Escherichia coli* and *Salmonella* spp. through antibiograms. The methodology used consists of a disk diffusion test. Data were analyzed by Tukey's test at 5% significance. The results obtained showed that the use of sanitizers, specifically for the slaughterhouse under study, is effective for the disinfection of microorganisms such as *Salmonella* spp., *Escherichia coli* and *Staphylococcus* sp. Standing out Bracsan as the most efficient for the three microorganisms and Enztrat® efficient only for *Staphylococcus* sp. These results suggest that the company should better analyze the cost-benefit of each of the sanitizers applied, since there may be expenses, unnecessary cost for the industry. However, it is suggested that new repetitions should be carried out to prove the tests since on-site monitoring of the action of these sanitizers is necessary. But in a preliminary way, it is concluded that Bracsan has the highest efficiency against the microorganisms tested, while Enztrat® has the lowest. The others have different efficiency for each of the microorganisms tested.

Keywords: Bracsan; Enztrat; Cloro; B-Quart-Sept; Polihexametileno biguanida

Acknowledgements: The authors would also like to thank CNPq for granting a Productivity Scholarship in Technological Extension Level II to Dr MRP, and CAPES (Coordination for the Improvement of Higher Education Personnel) for supporting the development of this research. To the Multiuser Laboratory - LABMULTI - UTFPR Campus Londrina.

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2nd International Conference
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EATING HABITS OF THE YOUNG POPULATION AND INFLUENCE ON THEIR BODY WEIGHT

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Skipping meals, especially breakfast, in addition to excessive intake of high-calorie and fast food and lack of physical activity, represent a new problem that affects the health and body weight of the young population and also has an impact on their later life. The aim of this study was to evaluate daily eating habits of the young population, the regularity in meals intake and types of food, as well as the impact of those habits on their body weight. Data were collected from 390 high voluntary participants (40.2% males and 59.7% females) by a questionnaire survey. Surveyed participants were students grade in elementary schools (12-14 years) and in high schools (18-19 years) located in the province of Vojvodina, Serbia.
The results showed that 69.7% of participants had normal body weight, 20.3% had excessive body weight, 8.1% were obese and 1.9% were malnourished. It was also noticed that there are more obese among the participants from elementary school. Breakfast is completely excluded by 11% of surveyed participants, and 47.8% most often skip this meal. About 40% of participants do not practice any form of physical activity, which confirms that young people have a sedentary lifestyle.

Keywords: Healthy diet, Dietary habits, Young population, Meal regularity

Acknowledgements: This work was supported by the project of the Ministry of Agriculture, Forestry and Water Management, Directorate for Agrarian Payments: "Creation of new functional products by transfer of knowledge between scientific research organization and small food producers", decision no. 680-00-00099/2/2022-02 from 25.05.2022.

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EVALUATION OF THE USE OF OZONE IN THE SANITIZATION OF KNIVES AND IN THE ELIMINATION OF *SALMONELLA SPP*. IN SWINE TONGUE

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The food industry increasingly needs resources to increase product processing technology in order to meet the demands of consumers who demand increasingly stringent hygiene and food safety standards in addition to extending the shelf life. In a slaughterhouse, product contamination can occur in several stages, ranging from the procedures that precede the slaughter and after slaughter. Thus, the hygiene procedure becomes indispensable to guarantee the sanitary quality of the food. In the quest to minimize the effects of chemicals, research has been carried out with the use of ozone for the purpose of sanitization/disinfection, which stands out for its high oxidation potential and does not generate waste. The objective of the research was to evaluate the effectiveness in the elimination of pathogens through the use of ozonized water in the sanitization of knives used in the swine slaughter line and in the water for cooling the porcine tongue. The microbiological analyzes carried out to monitor the contamination of the knives were the counting of aerobic mesophilic bacteria and *Escherichia coli* and, to assess the quality of the tongue, the search for *Salmonella* spp. In the treatment of knives with ozonized water, there was a reduction in the count of aerobic mesophilic bacteria in 90% and 32% of reduction for *E. coli* of the samples and for the porcine tongue, 27,2% of the samples with the presence of *Salmonella* spp. had the elimination of the pathogen after treatment. However, the low efficiency in eliminating *Salmonella* spp. may be related to the control of ozone concentration, temperature and the presence of organic matter in water, indicating the need for further studies.

Keywords: Disinfection and disinfectants, Slaughtering and slaughter-houses, Salmonella spp, Ozone

Acknowledgements: The authors would also like to thank CNPq for granting a Productivity Scholarship in Technological Extension Level II to Dr MRP, and CAPES (Coordination for the Improvement of Higher Education Personnel) for supporting the development of this research.

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ESSENTIAL OILS IN DIETS OF BROILERS AND THEIR INFLUENCE ON MEAT QUALITY

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European Union has recently significantly altered legal regulation in use of supplements, especially in case of antibiotics as growth promoters in poultry, pigs and calves. As a result, the Regulation for total prohibition of antibiotics in animal nourishment has been into effect since 1 January 2006. So far quite intensive investigations have been conducted in order to find an appropriate substitute for antibiotics such as: various organic acids and their salts; probiotics, prebiotics, enzymes and biogenic additives (etheric oils, vegetal extracts, etc.).
The aim of this work is to investigate the possibilities for use of essential oils of vegetative origin as a substitute in nutrition of chickens gaining weight. The obtained results proved that addition of essential oils in nutrition of chickens gaining weight manifested significantly positive effects of more analyzed parameters, especially the thermal sensory characteristics of processed meat permanently from 1st to 30th day of ripening process.

Keywords: Broiler, Essential oils, Meat quality, Sensory analysis

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THE EFFECT OF DIFFERENT STARTER CULTURES ON QUALITY AND SENSORY CHARACTERISTICS ON KASHKAVAL CHEESE

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The kashkaval cheese is a traditional milk product and has a significant place in the milk industry of the Republic of N. Macedonia. In this research work the influence of three different starter cultures of three variants of kashkaval cheese (A, B, C) has been examined on the quality and sensory characteristics of kashkaval cheese. The starter cultures that were used in variant A (TCC 4 - Chr. Hansen) contained the following bacteria strains: *Str. thermophilus Lb. bulgaricus*, the variant B (SH 092 E – Sacco cleriei): *Str. thermophilus, Lb. bulgaricus* and *Lb. helveticus* and the variant C (TREDMIX): *Str. thermophilus, Lb. bulgaricus, Str. lactis* and *Lb. helveticus*. The contents of main components during ripening and storage of cheese were monitored. The impact of the above mentioned three different starter cultures was determined during the process of kashkaval ripening.

Keywords: Milk, Kashkaval cheese, Ripening, Quality

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NUTRITIONAL MARKETING AND CONSUMER BEHAVIOR

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Detecting the behavior of consumers of food products, but also the behavior of people in general is one of the most complex issues in the marketing of companies and therefore requires extensive research, and this is so because of the individuality and complexity of people. Knowing all the impact factors grouped into determinants and discovering the links between them will lead to obtaining a wealth of information on the impacts and behaviors of food consumers. The research subject of this paper is the theoretical development of a new methodology of the so-called Nutrition marketing based on several principles (5N), aimed at the importance of nutritional determinants, but also other important determinants that influence consumer behavior when choosing food products. Including and highlighting the nutritional properties and quality and safety of food products as benefits to human health and well-being are part of the principles of Nutrition Marketing. As one of the concepts of Nutritional marketing, a survey was conducted in R.N. Macedonia on the impact of nutritional determinants on consumers. The analysis of the survey was done by creating multiple models. In the paper, the results of statistical methods for associativity and clustering model are presented. The principles of nutrition marketing and the developed methodology will enable companies to create a successful marketing strategy for food products. The benefit can be threefold: a benefit for companies through greater profits, a benefit for citizens through the consumption of healthy, quality and safe food products, and finally, a benefit for the state.

Keywords: Nutrition, Nutrition determinant, Consumer behavior, Food quality and safety

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TECHNOLOGICAL EVALUATION OF WINE YEAST ISOLATED FROM GREEK WINE

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Wine environment is characterized by a complex consortium of microorganism, which contribute more or less to wine quality. Finding the right yeast candidates for alcoholic fermentation is an essential element for the wine industry. The objective of the current research is to investigate important technological properties of indigenous yeast isolates from Greek wines and their potential use as fermentation starters. For this reason, 26 isolates of different yeast species were scrutinized for their sensitivity to killer toxin, production of non-desirable metabolites such as acetic acid and H_2S, aroma potential and resistance to the antimicrobial agents. All phenotypic tests were based on laboratory microbiological techniques, aiming to the quick selection of the most prominent candidates for winemaking. Additionally, 20 isolates were chosen for their oenological potential in terms of fermentation capacity and sensory contribution. According to our results, the majority of isolates were neutral to killer toxin test, capable to produce acetic acid, and negative to β-glucosidase production. Almost all of the tested yeast isolates showed significant tolerance to SO_2 whereas the production of H_2S varied. Furthermore, after 214 hours of wine fermentation only 35% of the inoculated yeast had lower ability in catabolizing sugars and couldn't lead to dry wines. Wines were clearly grouped in four clusters based on sensory evaluation results. It is noteworthy that all tests were designed to be applicable to the wine-industry and the isolated native yeast isolates exhibited interesting oenological properties affecting directly the total wine aroma.

Keywords: Wine yeast, Greek terroir, Sensory assessment, Alcoholic fermentation

Acknowledgements: This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call " Greece - Israel Call for Proposals for Joint R&D Projects 2019"(project code: Τ10ΔΙΣ-00060).

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MOVING TOWARDS SUSTAINABILITY: VALORIZATION OF BYPRODUCT IN THE BREWING INDUSTRY

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The brewing industry is considered one of the least desirable sectors due to intensive water and energy usage, significant amounts of effluent, solid waste, and a large carbon footprint. Therefore significant brewing companies have developed thereon sustainable programs and defined goals. They have focussed on socioeconomic and environmental sustainability factors. Ecological economics has built the framework for sustainability by reframing basic economic ideas to make them more applicable to environmental challenges. The social factor can play a significant role in driving local communities toward a more sustainable future. However, the environmental aspect significantly influences sustainability, with energy and water consumption as key factors. There are numerous energy-saving approaches, ranging from automation to renewable energy sources such as solar, hydropower, and geothermal power. Reengineering brewing operations might lead to significant water savings. For example, less water during lautering can benefit water and energy usage. Using waste and byproducts in circular exchange can become an input for other industries. These phenomena are known as Kalundborg Symbiosis or eco-industrial parks. Namely, a fish farm, power plant, gypsum wallboard producer, pharmaceutical corporation, cement factory, oil refinery, and local farmers all use byproducts from one another business. For instance, the surplus of CO_2 from the brewing process is captured and fed by algae, enhancing the on-site greenhouse; or made into biochar, a valuable soil amendment for the production of biofuels; or the production of succinic acid by non-photosynthetic microorganisms; or as a substrate for the production of lactic acid; or as a medium for the growth and storage of lactic acid cultures. Brewery solid wastes, spent grain, and hops can be dealt with in several ways. As a nutritional feed, medium for growing mushrooms, raising earthworms for feed, or as a variety of food products. Brewer spent grain has mainly been utilized in manufacturing bakery products such as pizza dough, flakes, flour bread, cookies, cake, waffles, brownies, cereal bars, and in the meat industry for meatballs and meat dumplings. As a non-food, it is used as absorbents, concrete and ceramic materials, paper, bricks, replacement of wood, and bioethanol.

Keywords: Sustainability, Valorization, Byproduct, Brewing

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STABILITY OF MINIMALLY PROCESSED APPLE TREATED WITH LAUREL (*Laurus nobilis* L.) ESSENTIAL OIL

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Minimally processed apples are becoming increasingly popular due to their fresh-like character, convenience, and health benefits. The main problem with such products is browning and microbiological spoilage, which can occur very quickly after processing. To extend the shelf life of minimally processed apples, several methods are being developed, including edible coatings, anti-browning agents, and modified atmosphere packaging. Laurel (*Laurus nobilis* L.) is a plant native to the Mediterranean region that has been traditionally used in folk medicine for its various biological activities (antioxidant, anti-inflammatory, antimicrobial, and antifungal), which can be attributed to various biologically active compounds present in leaf extracts and essential oils (EO). The objective of this study was to investigate the influence of laurel EO application (0 as control, 25, 75, 125, and 175 mg/L) on the shelf life of minimally processed apples during 10 days of storage. Microbiological and sensory analyzes were conducted, as well as measurements of soluble dry matter, pH, and color (*L**, *a** and *b**). With increase of EO concentration, the number of aerobic mesophilic bacteria decreased (from 2.000 log CFU/g in EO-0 to 1.544 in EO-175), while their increase was observed during prolonged storage it was lower than in control samples (after 10 days 2.531 log CFU/g in EO-0 and 2.000 in EO-175). Levels of soluble dry matter and pH ranged from 12.1 to 14.35 °Brix and from 3.54 to 4.18, respectively, and they slightly increased during storage being better stabilized in apples treated with higher concentration of EO. EO had no remarkable influence on the color, although EO-75 caused the smallest color change compared to the control, after ten days of storage. EO treatment influenced all sensory properties, especially taste, but not in an unpleasant way. When considering all obtained results with emphasis on sensory evaluation, concentration of 75 mg/L of laurel EO can be recommended for prolonging the shelf life of minimally processed apples as well as for obtaining new innovative minimally processed product.

Keywords: Minimally processed apple, Laurel essential oil, Browning, Sensory analysis, Microbiological analysis

Acknowledgements: This work was supported by the project "Bioactive molecules of medical plant as natural antioxidants, microbicides and preservatives" (KK.01.1.1.04.0093), co-financed by the Croatian Government and the European Union through the European Regional Development Fund—Operational Programme Competitiveness and Cohesion (KK.01.1.1.04.).

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ENCAPSULATION OF POLYPHENOLS - TECHNIQUES AND APPLICATIONS IN FOOD PRODUCTS

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Over the past decades, polyphenolic compounds have received a lot of attention in both the scientific community and the food industry. The potential health benefits make these compounds interesting for food fortification. However, due to low stability and unpleasant taste at higher concentrations, the use of polyphenols in food products is limited. Encapsulation, a process based on forming a physical barrier around an active substance, is a promising way of overcoming these problems. The number of research studies and reviews focusing on polyphenol encapsulation is on the constant rise. Polyphenol encapsulates tend to display greater stability during processing and storage compared to non-encapsulated polyphenols and, therefore, have a high potential for application in foods. However, papers focusing on the practical application of encapsulated polyphenols are scarce. For that reason, the aim of this work was to present possible applications of such encapsulates in foods, as well as to summarize the most popular techniques used for this purpose. Encapsulated polyphenols can be applied as functional food ingredients and/or food colorants in various products, such as milk products, bakery products, and confectionery. The most commonly employed techniques for polyphenol encapsulation include spray drying and freeze-drying, as well as ionic gelation, complex coacervation, and liposome entrapment. In terms of limitations, the increased cost of industrial production and the low bioavailability of polyphenols and their encapsulates should be further investigated.

Keywords: Polyphenols, Encapsulation, Food application, Food fortification, Food colorants

Acknowledgments: This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant number: 451-03-68/2022-14/200116).

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APPLICATION OF RAMAN SPECTROSCOPY FOR THE CHARACTERISATION OF MILK AND MILK-PHENOLICS POWDERS

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The current trend of food innovations is a development of different milk products enriched with phenolic compounds extracted from food by-products. This represents a promising strategy for food waste recovery and to enhance the beneficial health effects of milk-based functional foods. As one of the promising food ingredients can be skim goat milk enriched with grape pomace seed extract due to improved antioxidant properties of goat milk proteins. So, the aim of this study was the Raman analysis of spray-dried powders of skim goat milk, heated goat milk, and heated goat milk enriched with different concentrations of grape pomace seed extract. The whole statistical procedure (pre-processing and PCA) of Raman spectra was performed independently at Amide I (1700-1600 cm^{-1}), Amide II (1600-1500cm^{-1}), and Amide III (1500-1200cm^{-1}) regions. Raman spectra analysis of amide I and II regions showed a separation between heated and unheated milk due to the loadings for 1650-1653 (α-helix), 1661-1663, 1675, and 1686 cm^{-1} (turn). Furthermore, in the amide I region, the differences between milk enriched with different concentrations of grape pomace seed extract can be observed due to loadings for wavenumbers in the 1612-1644 cm^{-1} region. These differences can be related to the changes in the parallel and antiparallel β-sheet structures, differences in the vibrations of the amino acid residues of tyrosine, tryptophan, and phenylalanine and the vibrations of ν(C=C) bonds from the aromatic ring of free flavan-3-ols (catechin, epicatechin, epigallocatechin). For the amide III region, the applied heat treatment and interactions of proteins with phenolic compounds distinguished samples in loadings for wavenumber in 1236-1257 and 1267-1304 cm^{-1} ranges. These differences can be related to the changes in the α-helix and random coil structures. It can be concluded that PCA separation of analyzed powders in amide I and III regions of Raman spectra caused by the applied thermal treatment, presence of free phenolic compounds, and protein-phenolics interactions, can be used for the characterization of milk and milk-phenolics powders.

Keywords: Goat milk proteins, Grape pomace seed, Phenolic compounds, Raman spectroscopy, Interactions

Acknowledgements: This work was supported by the Science Fund of the Republic of Serbia #GRANT No. 7744714

Materials Design and Applications

ICAPP 2022
2nd International Conference
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CARBON BASED MATERIALS AS ADSORBENTS FOR WATER TREATMENT

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Environmental pollution has been a recognized problem for human health and the ecosystem. Remediation is usually costly and time-consuming, so researchers' attention has been drowning to develop and use materials of the new generation. Based on the available literature it can be observed an increasing trend in carbon-based material usage. Although it is a mainly new approach considered environmentally friendly, there are findings, observations, negative aspects, and conclusions that must be taken into consideration. In this paper, we discuss fundamental knowledge of different types of carbonaceous adsorbents and their possible application in water treatment. A wide range of carbonaceous low-cost and possibly more sustainable sorbents have been considered including different types of chars. Since agricultural production is an important economic sector around the world, the amount of produced waste biomass is significant. Therefore, cost-efficient production of those materials from residual waste biomass may simultaneously address additional environmental problems such as biomass waste management. In order to investigate the adsorption potential for removal of the most commonly employed organic UV filter (3-(4'-methylbenzylidene)-camphor), different types of biochars (originating from *Cannabis satina* and *Paleas*) were investigated. Obtained adsorption isotherms were well described by the Freundlich model. The nonlinearity of the isotherms was below 0.9. In general, all the investigated adsorbents demonstrated higher adsorption affinity for the investigated organic UV filter. This type of research is necessary to obtain safe adsorbents for water remediation.

Keywords: Biochars, Sorption, Water treatment, Organic compound

Acknowledgments: The authors gratefully acknowledge the support of the Provincial Secretariat for Science and Technological Development, Republic of Serbia, Autonomous Province of Vojvodina (Project No. 142-451-2693/2021-01/2).

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MAGNETIC NANOPARTICLES: NEW GENERATION ADSORBENTS FOR ARSENIC REMOVAL

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Arsenic contamination of drinking water sources is a global problem and therefore many countries make a lot of effort to solve this urgent public health issue. Among the different approaches for arsenic removal, nanomaterials, especially magnetic nanoparticles (MNPs), as new generation adsorbents, received a lot of attention. In this study a one-step co precipitation method was applied to produce magnetite nanoparticles and a specially designed magnetic separation unit was used to assess their applicability as part of a real drinking water treatment plant. Batch adsorption tests were carried out to determine the adsorption capacity of the synthesized adsorbent for arsenic removal. Furthermore, continuous adsorption experiments on the magnetic pilot plant were conducted with real arsenic contaminated groundwaters, whereby different doses of magnetite nanoparticles (0.5-2 g/l) and flow rates were applied (7 - 10 l/h). Characterization of the synthesized magnetite nanoparticles shows BET specific surface area of 40.36 m^2/g, while the XRD and FTIR analysis confirmed the formation and presence of magnetite nanoparticles. The results obtained from the dynamic experiments show that 50-70% of arsenic can be removed, depending on groundwater quality, whilst successfully recirculating the magnetite nanoparticles within the magnetic separation unit. Additional investigations are necessary to further improve the design and performance of the magnetic separation unit as a highly promising cost effective solution for small water utilities.

Keywords: Magnetite nanoparticles, Arsenic, Adsorption, Drinking water, Separation unit

Acknowledgements: The authors gratefully acknowledge the support of the Provincial Secretariat for Higher Education and Scientific Research, Autonomous Province of Vojvodina, Republic of Serbia (Grant No. 142-451-2356/2022-01/01).

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MATERIAL SCIENCE AND CULTURAL HERITAGE – A FASCINATING WORLD OF ARCHAEOMETRY, CONSERVATION SCIENCE, AND TECHNICAL ART HISTORY

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Questions about who we are and where we came from have always been intriguing to humanity. Archaeological investigations, archive documents and old artifacts are among the most important material sources of information for the scientists from the humanities: historians, archaeologists, art historians and others. Delicate and valuable, often deteriorated, material testimonies of our past are studied with joint forces of researchers coming from different scientific fields. Science, humanities and art meet to combine knowledge and experiences and to interpret what is not visible to the human eye, to prevent damage or at least slow it down. They use a variety of analytical methods in the effort to extract more information with less impact to the artifact.

Scientists in cultural heritage face the challenge of the field that cannot be explained only by numbers, but has to take into account the creative process of artmaking, local customs and traditions, as well as the history of preservation of the artifact. This makes the research in cultural heritage full of uncertainties, but it offers the possibility of fascinating discoveries.

Keywords: Cultural heritage, Archaeometry, Conservation science

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NEW MATERIALS FOR A NEW BUILDING CULTURE

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Global warming and challenges coming from climate change will be the initiator of construction industry transition, from traditional to sustainable manufacturing. Main reason for this relies on the fact that cement production is responsible for up to 7% of global carbon emissions, and steel manufacturing is responsible for additional 8%.

These facts indicate responsibility and commitment of construction material manufacturers to develop another, resilient method for dealing with the challenges coming from changing environment. The focus is on reducing the use of natural resources and, on the other side, increasing of circularity ratio - building more with less. Locally, company Lafarge, member of the Holcim Group, is implementing globally defined strategy, targeting reduction in CO_2 intensity by 40% until 2030 and reach net zero emissions by 2050.

Scope of this work includes possible ways to approach and to deal with this topic. We need smart solutions. For cement industry, one way is surely significant reduction of operational emissions, but this is long term action which requires significant investments. Also some of those actions are not proven yet. Another way to approach this problem is changing of content of already embodied carbon in cement with direct reduction of clinker factor, through variation in production portfolio. Idea is to create new materials for a new building culture, such as "green" cement, concrete and infrastructure binders, specially designed with the goal to support the sustainable building principles and to minimize negative impact on global climate changes, including biodiversity. However, changing the portfolio also requires application of new technologies and innovations in process, achieving the same or even improved product quality and performance, compared with ones made by traditional manufacturing process.

Keywords: Sustainable development, Cement, Concrete, CO_2 emissions

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SANS INVESTIGATION OF SURFACTANT-POLYMER INTERACTIONS IN POLYMER HYDROGELS

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INTRODUCTION: Recently, the nanophase-separated structure of epoxy hydrogels containing polyoxyethylene (POE) and polyoxypropylene (POP) has been revealed and investigated by SANS. The hydrogels respond sensitively to external stimuli, e.g., change of temperature or presence of a surfactant in swelling solution. The focus of this project is the investigation of the effect of the interaction of ionic surfactants with POE and POP on swelling behavior and structure of the hydrogels.

EXPERIMENT: Stoichiometric epoxy network containing POE and POP was prepared by reaction of α, ω-diamino terminated POP, and POE bis(glycidyl ether). A series of hydrogels were obtained by swelling the network in D_2O and excess volume of solutions of two ionic surfactants: myristyltrimethylammonium bromide (C_{14}TAB) and sodium dodecyl sulfate (SDS) in D_2O. SANS measurements were carried out at the YuMO small-angle instrument at the IBR-2 pulsed reactor (JINR, Dubna, Russia) in the time-of-flight regime.

RESULTS and CONCLUSIONS: A nanophase-separated structure consisting of water-rich and water-poor domains with Bragg's distance ca 110 Å is revealed by SANS from the hydrogels obtained by swelling in D_2O, C_{14}TAB solutions, and subcritical SDS solutions. A significant increase in swelling degree was observed for hydrogels obtained from supercritical SDS solutions. This is accompanied by a change of hydrogel structure from a two-phase morphology to a morphology composed of micelles dispersed in a highly swollen polymer network. Details of hydrogels structure were obtained by fitting experimental SANS profiles to models using hard-sphere potential and rescaled mean spherical approximation. The average radius of water-poor domains of ca 40 Å was determined for hydrogels in the case of two-phase morphology. In hydrogels containing micelles, an average micelle radius of 25 Å (C_{14}TAB) and 17 Å (SDS) was found. Differences in hydrogel behavior and structure are attributed to different degrees of ionization of SDS and C_{14}TAB micelles in the hydrogels.

Keywords: Hydrogels, Ionic surfactants, Neutron scattering

Acknowledgments: This research project has been supported by the JINR-Czech Republic research grant, 2019-2021.

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CHARGE AND MORPHOLOGICAL PROPERTIES OF NANOPARTICLES

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Charging of particles in aqueous electrolyte solutions is important to better understand interactions between biomaterials and surrounding tissue. In this study the surface charge of CeO_2 and TiO_2 nanoparticles was measured by a titration technique. High-resolution transmission electron microscopy imaging was used to determine the morphology of nanoparticles. A theoretical model based on the classical density functional theory coupled with the charge regulation in terms of mass action law was developed to better understand the experimental results [1-4].

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Keywords: Nanoparticles, Charge, Morphology

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GREEN BIOSYNTHESIS OF ZnO NANOPARTICLES USING AGRO-WASTE AND THEIR ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY

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Metal oxide nanomaterials have gained a lot of attention during last decades due to their potential applications in wastewater treatment, energy storage, sensors, food packaging, etc. To date, these materials have been synthesized by different chemical and physical techniques. However many of them employ environmentally unfriendly solvents and toxic chemical compounds. To tackle this problem, use of renewable biomass such as plants and fungi as reducing or stabilizing agents in green synthesis has been considered as more sustainable option compared to toxic chemical compounds. These biological substances also behave as capping agent, which control the size and the shape of the nanoparticles. In this work, ZnO nanoparticles (NPs) have been prepared *via* simple, low cost and ecofriendly method using citrus fruit peel and extracts, *Agaricus bisporus* powder and extract as biological reducing agents. Zinc nitrate and zinc acetate were used as source of zinc ions. Structural and optical properties were investigated by X-ray diffraction analysis (XRD), Zeta potential, Fourier Transform Infrared (FTIR) spectroscopy, UV-visible (UV-vis) spectroscopy and Photoluminescence spectroscopy (PL). Morphological features were characterized by Field Emission Scanning Electron microscopy (FESEM) and High Resolution Transmission Electron Microscopy (HRTEM). Antibacterial and antioxidant activity was tested and evaluated.

Keywords: ZnO, Green synthesis, Citrus extract, Agaricus bisporus, Antibacterial

Acknowledgements: The authors would like to express their gratitude to the Ministry for Education, Science and Technology Development under the contract 451-03-68/2022-14/200053.

ICAPP 2022
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PRINCIPALS OF DIGITAL RESTORATION AND ITS APPLICATION IN CULTURAL HERITAGE

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Even though material science and engineering constitute the core aspects of practical restoration, there are other ways to help preserve cultural heritage. One such approach is digitalization together with digital restoration and reconstruction. Although the main postulates of their application in conservation have been elaborated many times, insufficient attention has been given to their connection with practice. Hence, this paper aims to briefly present the main aspects of digital restoration/reconstruction, upon which, their application in practice will be presented. The basic idea is to show in which ways digital restoration and reconstruction can affect the material object following restoration principles. Four main application niches can be distinguished: documentation, predicatory tool, monitoring, and presentation. The last one can be further divided depending on the targeted audience. Therefore, the goal of the paper is to try and summarize the relationship between digital restoration and practice, as well as present the possible use of digital tools in modern-day conservation.
The so-called "experimental" part, i.e. the case studies will be borrowed from papers that deal with practical solutions, as well as from personal practice related to conservation issues in the basilica of Santa Maria Assunta on the island of Torcello near Venice. The church has three main conservation issues that can be partially (or completely) solved using digital tools. These are double-layered wall decoration in the main apse, dismantling of the crypt's *pergola,* and inaccessible murals in the diaconicon chapel. Since dealing with all three goes beyond the scope of this paper, I will focus on them solely as an illustration of the digital approach discussed in the first part of the paper.

Keywords: Digital Restoration, Digital Reconstruction, Cultural Heritage, Conservation, Restoration Theory

Acknowledgments: I thank my mentor, Prof. Diego Calaon for the support and supervision of my work.

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STRUCTURAL AND FUNCTIONAL PROPERTIES OF MIXED NICKEL ZINC FERRITE NANOPOWDERS

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Mixed nickel zinc ferrite ($Ni_{05}Zn_{0.5}Fe_2O_4$) and double ferrite core/shell composite nanopowders were synthesized by co-precipitation and hydrothermal synthesis. XRD results showed that as-obtained powders were well crystalline. Mixed ferrite was single phase while core/shell composites showed separated phases as expected. All the particles showed features of inverse spinel phase as evidenced by Raman spectroscopy. Magnetic properties presented superparamagnetic behavior of all systems with different values of saturation magnetization. Nickel leaching in water and saline solution was also examined.

Keywords: Nicekl ferrite, Zinc ferrite, Core/shell, Nanopowder

Acknowledgements: Ministry of Education, science and technological developments Grant No. 451-03-68/2022-14/200134.

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NON-DESTRUCTIVE EXAMINATION OF THE PAINTING "MAN WITH A MASK" BY RWANDAN ARTIST GUY KARANGWA OMEGA

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The aim of this research was a non-destructive examination of the painting, "*Man with a mask*", painted between 1974-1975, by Rwandan artist Guy Karangwa Omega (1952-1975) with the objective of determining its technology for future restoration treatments. The ultimate purpose of the combined application of non-destructive techniques (colourimetry, optical microscopy, FTIR and XRF analysis) was obtaining data on the genesis of the work, technique, and manufacturing technology, but also the processes and causes of material degradation (painting was kept in an unfavourable environment since 1975 to today) . Data were collected from various sources to illustrate in detail the impact of the social and political circumstances on art, given the Genocide against the Tutsi in 1994, or even earlier Rwanda`s independence from Belgium in 1962. The obtained data on the elementary chemical composition provided information about the materials used for its production. This in turn led to conclusions about the process of its production half century ago in one of the least developed countries in the world. The analyses enabled further research on the context of the colours and materials origin, that would support further repair of damage, and the preservation of the work, including the prevention of future damage. The results represent a good basis not only for upcoming painting preservation procedures, but also for the formation of a database related to the artists painting technology, given all the challenges of the time and space in which he lived and worked. In addition, this analysis is the first and only scientific examination conducted for an artwork from Rwanda, given limited capacity of its cultural and educational institutions in the field of material cultural heritage protection.

Keywords: FTIR, Painting, Rwanda, Diagnostics, Conservation

Acknowledgements: The authors are grateful for the financial support by the Ministry of Education, Science and Technological Development, Republic of Serbia: project No. 451-03-68/2022-14/200134.

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CHARACTERISATION OF THE MORTARS AND BRICKS FOR CONSERVATION OF THE MEDIEVAL CATHOLIC CHURCH ARAČA

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Arača is the site of a ruined medieval Romanesque church located about 12 km north of Novi Bečej, the Republic of Serbia. The church was built during the rule of the Kingdom of Hungary around 1230 CE and had a history of hardships throughout its existence. It was robbed and destroyed in 1280 CE and reconstructed in 1370 CE. The Gothic tower that is still standing today was added at that time. The Ottoman forces burned down the church in 1551 CE, and it has remained a ruin ever since. Forgotten for many centuries, it finally remerged as an object of archaeological and historical interest in the 20th century. The Provincial Institute for Protection of Cultural Monuments conducted extensive excavation and protection works in the 1970s. Arača was declared a Cultural Monument of Exceptional Importance of the Republic of Serbia in 1990, becoming the only medieval monument of this importance in the region. The Laboratory for the Materials in Cultural Heritage of the Faculty of Technology, University of Novi Sad, in cooperation with the Provincial Institute for Protection of Cultural Monuments from Petrovaradin, did a series of in situ examinations of the mortars and bricks from the Arača locality. Based on the *in situ* examination, some sampling locations were chosen. Different laboratory methods were used for the bricks and mortar characterisation: stereo-optical and digital microscopy, spectrophotometry and colorimetry, mineralogical and chemical analyses, physical-mechanical tests, thermal characterisation, mechanical and chemical separation of aggregates and binders. The obtained results determined the degree of structural damage to the examined materials. The mineralogical composition of the raw materials and technology of the sampled bricks and mortars were determined. The obtained results are intended to be the future material guide for defining the appropriate conservation-restoration treatments for the medieval Catholic church Arača.

Keywords: Conservation, Mortar, Brick, Medieval Church Arača, Diagnostics

Acknowledgements: The authors are grateful for the financial support by the Ministry of Education, Science and Technological Development, Republic of Serbia: project No. 451-03-68/2022-14/200134 and EUREKA PROGRAMME, Advanced CleAning and Protection of TANgible culture heritage, CAPTAN E!13085.

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PHYSICOCHEMICAL CHARACTERIZATION OF Zn DOPED PHOSPHORUS TUNGSTEN BRONZES ZnWPB

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Polyoxometalates (POMs), although having more than a hundred years of history, continue to attract the attention of researchers as catalysts, solid superionic proton conductors at room temperatures, applicable in different electrochemical devices, and also as photochromic, biochemical and biomedical active materials. [1] The best-known group of polyoxometalates (POMs) are the heteropoly compounds (HPCs) with the Keggin anion structure. Heteropolyacids, with a general formula of $H_{3+x}AM_{12}O_{40} \cdot nH_2O$ (x = 0-1; A = P, Si, B, As, Ge; M = Mo, W; n = 30-6) are of special interest as new materials because of their high conductivities. Among them special attention deserves the 12-tungstophosphoric (29-WPA) acid. Heteropolies of acids and salts heteropolises of acids can also be used as starting materials for the production of tungsten bronzes. Phosphate tungsten bronzes (WPB) have been intensively investigated for many applications due to their interesting chemical, optical, electrical, and mechanical properties. These bronzes have a specific structure that results from the collapse of the Kegin anion at a temperatures up to 602°C. This structure is layered and consists of interconnected PO_4 tetrahedra and WO_6 octahedra. In such a structure, pentagonal and hexagonal openings (cavities, channels) are formed in which there is a complete or partial exchange of H^+ ions in WPA. In this work, synthesized 12-tungstenphosphoric acid ($H_3PW_{12}O_{40} \cdot nH_2O$, WPA) was further ionically exchanged with Zn^{2+} ions, which gave 12-tungsten phosphoric acids of the transition metal ($ZnPW_{12}O_{40} \cdot nH_2O$, ZnWPA). ZnWPA was then subjected to thermal analysis, which determined the phase transition temperature (when the Keggin anion collapses). The temperature of collapsing the Keggin anion is about 600°C, and at this temperature, ZnWPA was heated for 10 minutes to obtain phosphate tungsten bronzes doped with zinc (ZnWPB). Physico-chemical methods IR, XRPD and SEM were used to characterize the material. The redox activity of these materials has already been investigated, and the obtained results have encouraged further studies of the possibility of their analytical application.

Keywords: 12-tungstophosphoric acid, Tungsten bronzes, Physico-chemical methods

Acknowledgements: This study was supported by the Ministry of Education, Science and Tehnological Development of the Rebublic of Serbia (contract numbeers 451-03-9/2021-14/200123).

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THE SURFACE CHARACTERIZATION OF THE ANODIZED ULTRAFINE-GRAINED Ti-13Nb-13Zr ALLOY

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Titanium alloys are metal materials widely used in medicine owing to their suitable characteristics such as low density, good corrosion resistance and biocompatibillity. High biocompatibility of the titanium alloy results from the creation of a spontaneous oxide layer with good adhesion and homogeneous morphology. In order to improve characteristics of the metallic materials for application in medicine, electrochemical methods that enable surface nanostructured modification are extensively used, and one of these methods is electrochemical anodization which makes it possible to obtain a nanostructured oxide layer composed of nanotubes on the surface of the metal material.

The tested material was ultrafine-grained Ti-13Nb-13Zr (UFG TNZ) alloy obtained by the severe plastic deformation (SPD) processing using the high pressure torsion (HPT) process. Nanostructured oxide layer on the titanium alloy was formed by electrochemical anodization during the time period from 30 to 120 minutes. Characterization of the surface morphology obtained during different times of electrochemical anodization was done using scanning electron microscopy (SEM), while the topography and surface roughness of the titanium alloy before and after electrochemical anodization was determined using atomic force microscopy (AFM). Scratch test was used to determine the cross profile of the surface topography and critical load during scratching. Electrochemical anodization led to the formation of a nanostructured oxide layer on the surface of the titanium alloy. The obtained results indicated strong influence of the electrochemical anodization time on the oxide layer morphology - with its increase the diameter of the nanotubes increases too, while the wall thickness of nanotubes decreases. Also, electrochemical anodization led to an increase in the surface roughness.

Keywords: Titanium alloy for biomedical application, High pressure torsion process, Electrochemical anodization, Surface morphology, Surface roughness

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DETERMINATION OF THE OPTIMAL THERMAL TREATMENT PARAMETERES FOR THE PREPARATION OF LIGHTWEAR CERAMIC AGGREGATE BASED ON CEMENT KILN DUST AND CLAY MINERALS

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Strict environmental legislation and higher disposal costs have provoked cement industry to seek alternative ways to dispose/utilise aggressive cement kiln dust (CKD), a by-product generated during the calcination process of Portland cement. The composition of CKD depends strongly on raw materials and alternative fuels chemistry, process type and gas velocities in the kiln. Modern plants dominantly apply dry process, which considerably increases the amount of CKD, compared to traditional wet process.
In this research we have focused on CKD reuse as a secondary raw material for the production of lightweight ceramic aggregate, using combination of CKD with clay material and appropriate melting agent. Before preparation of the composite materials with different fractions of CKD, clay and melting agent, detailed thermal and chemical-mineralogical characterisation of collected CKD and clay materials of low quality was performed. After mixing and homogenizing the components, thermal treatment is necessary to consolidate the components into final solid form. Temperature of thermal treatment must be high enough to enable the formation of glassy phase, but on the other hand it should not exceed the decomposition temperature of the salts present in CKD (chlorides, nitrates, and sulphates), to prevent evaporation of harmful gasses in the atmosphere.
To determine the adequate thermal regime, thermogravimetric measurements, coupled with mass spectroscopy (TG-MS), and DSC analyses were performed. Maximal temperature of thermal treatment was set at 800°C. The final composite characteristics were evaluated, including thermal expansion, weight loss, porosity, microstructure, compressive strength, changes of morphology and colour, as well as leaching of soluble salts.

Keywords: Cement kiln dust, Environmental impact, Thermal treatment, TG-MS, Ceramic materials

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COMPARISON OF LIQUID AND LYOPHILIZED SERPYLLI HERBA WASTE EXTRACTS PREPARED AT DIFFERENT pH VALUES

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Polyphenol recovery and physico-chemical properties of the extracts depend on the type of solvents and their pH values. Additionally, due to the presence of different biologically active compounds in plant waste, plant extracts obtained from industrial by-processing can find potential applications in various products. In the present study, Serpylli herba waste extracts were prepared using maceration (60 min), a solid-to-solvent ratio of 1:30, the particle size of plant waste 0.3 mm, and two types of the extraction medium: 50 % ethanol (pH 6) and 50% ethanol with glacial acetic acid (pH 2.5). The lyophilization process was chosen as the next step (-75°C, 0.011 mbar, for 24 h). Comparison of liquid and lyophilized extracts prepared at different pH values was done *via* analyzing total polyphenol content (TPC, Folin-Ciocalteu method), total flavonoid content (TFC, colorimetric assay), antioxidant capacity (ABTS and DPPH assays), zeta potential and conductivity (photon correlation spectroscopy). TPC of liquid extracts prepared at pH 2.5 and pH 6 amounted to 1.38 and 1.23 mg GAE/mL, respectively, while lyophilized parallels had 271.7 and 188.8 mg GAE/g, respectively. The same trend is noticed in the case of TFC: 0.368 and 0.334 mg CE/mL for liquid extracts obtained at pH 2.5 and pH 6, respectively, and 102.5 and 99.7 mg CE/mL for lyophilized parallels. ABTS radical scavenging activity of the liquid extracts at pH 2.5 and pH 6 was 0.767 and 0.750 mmol TE/L, respectively and for the lyophilized parallels was 0.136 and 0.111 mmol TE/g. IC_{50} (concentration for neutralization of 50% of DPPH free radicals) was 1.12 and 1.75 mg/mL for the liquid extracts prepared at pH 2.5 and pH 6, respectively and 0.331 and 0.191 mg/mL for the lyophilized parallels. The zeta potential of the liquid extracts at pH 2.5 and pH 6 was 1.74 and -2.56 mV, respectively, whereas the zeta potential of lyophilized parallels was -3.55 and -18.7 mV. Conductivity of the liquid extracts was 0.864 (pH 2.5) and 0.423 mS/cm (pH 6), whereas for the lyophilized extracts it was 0.199 (pH 2.5) and 0.452 mS/cm (pH 6). The presented results provide the information on physico-chemical properties of Serpylli herba waste liquid and lyophilized extracts that can add value and improve the quality of the existing food, functional food, pharmaceutical and cosmetic products, as well as for drinking water and wastewater treatment.

Keywords: Lyophilization, Polyphenols, Serpylli herba, Waste, Zeta potential

Acknowledgments: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/200287).

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CHITOSAN- AND PECTIN-BASED COATINGS WITH INCORPORATED ACTIVE COMPONENTS FOR APPLICATION IN ACTIVE FOOD PACKAGING

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The subject of this research was the development of biodegradable and eco-friendly material based on natural biopolymers from renewable sources, with the addition of active components for application in active food packaging. The main principle was the incorporation of the active components (lemongrass (*Cymbopogon citratus* L.) essential oil, ZnO nanoparticles, or $Zn(CH_3COO)_2 \cdot 2H_2O$) with antimicrobial activity in the polymer matrix (chitosan, pectin, and gelatin), and their slow release during the time. All of the used components are listed as GRAS (Generally Recognized as Safe) by the U.S. Food and Drug Administration. Different formulations of emulsions and dispersions were processed for mutual comparison. The stability of chitosan and pectin emulsions was determined by using laser diffraction methods. Chitosan emulsions exhibited higher stability during 30 days of storage. The chitosan emulsions and dispersions exhibited a higher antibacterial effect *in vitro* against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. Biopolymer coatings were formed by the spraying of emulsions on existing packaging. The effects of biopolymer coatings on the development of microorganisms on fresh raspberries (*Rubus idaeus* L.) were performed *in vivo* during eight days of raspberry storage at refrigerator temperature. The tested coatings extended the shelf life of stored raspberries from four to eight days. The synergistic effect between lemongrass essential oil and ZnO nanoparticles or $Zn(CH_3COO)_2 \cdot 2H_2O$ was observed both *in vivo* and *in vitro*.

Keywords: Biopolymers, Active components, Emulsions, Active packaging

Acknowledgements: This work was financially supported by the Serbian Ministry of Educations, Science and Technological Development, Contract No. 451-03-68/2022-14/200053.

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BACTERIAL ADHESION ON POLYELECTROLYTE MULTILAYERS

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Polyelectrolyte multilayers (PEMs) are very well-known surface coatings that could be prepared by alternating deposition of positively and negatively charged polyelectrolytes (polycations and polyanions) on a solid surface. In recent years we have developed new PEM strategies which could be valuable for designing soft nanomaterials whose properties can be finely adjusted according to the requirements of specific applications, especially in the field of biomedicine. The emphasis in our studies was on applying PEMs to prevent bacterial adhesion to various surfaces. We investigated the influence of polyelectrolyte multilayer properties on bacterial adhesion capacity [1], as well as the bacterial adhesion capacity of protein-terminating polyelectrolyte multilayers [2]. We also applied this PEM strategy to study the bacterial adhesion capacity of uropathogenic *Escherichia coli* to polyelectrolyte multilayer coated urinary catheter surface [3]. In this case, it was shown that on non-treated PVC surfaces, biofilm was formed, which was not the case for polyelectrolyte multilayer coated surfaces.

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Keywords: Polyelectrolyte multilayers, Bacterial adhesion, Biomedical applications

Acknowledgements: This research was supported by the Croatian Science Foundation under the bilateral Slovenian-Croatian APPLPEMS project (IPS-2020-01-6126).

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INVESTGATION OF THE FORMATION OF MICROPLASTIC PARTICLES UPON GAMMA IRRADIATION AND PHOTOAGING OF PET AND PP

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Microplastics (MP) are plastic particles less than 5 mm in size that are mainly produced by the degradation of large plastics through ultraviolet irradiation, mechanical abrasion, and biological decomposition. They are of great concern because of their harmful effects on ecosystems and human health. Sunlight is a very important factor in the formation of MP, leading to structural defects and the formation of plastic nanoparticles and free radicals. Since the efficiency of natural aging processes is very low, aging can be accelerated by artificial processes, which usually include photoaging. Changes in polymer structure due to UV or gamma radiation have been reported in the literature. In this study, the photodegradation of plastics in aqueous solution and the formation of microplastic particles were investigated by irradiating prisitin and recycled plastics of polyethylene terephthalate (PET) and polypropylene (PP) with a UV lamp at 254 nm (with an intensity of about 0.3 W cm^{-2} for 1 minute) for 15 and 30 minutes, respectively. The samples were also γ-irradiated, with absorbed doses of 10, 50, and 60 kGy. The effects of UV and γ-irradiation on PET and PP samples in water were studied by UV-vis spectroscopy and dynamic light scattering (DLS). The formation of microplastics upon irradiation of PET and PP was studied by scanning electron microscope and Raman spectroscopy. It was found that the absorbance increased with increasing UV irradiation time or absorbed dose for all samples studied. The results also showed the formation of micrometre-sized plastic particles in the photoaged samples, indicating that γ-irradiation has less effect on the formation of microparticles. The results showed the formation of particles in size ranges of less than 100 μm, from which a good Raman spectrum was obtained. This study could be of great help in interpreting the effects of UV and γ-irradiation on PET and PP.

Keywords: Microplastics, Nanoplastics, Polyethylene terephthalate, Polypropylene

Acknowledgements: This work has been supported by the Foundation of the Croatian Academy of Sciences and Arts, project co-financed by the Croatian Government and the European Union through the European Regional Development Fund - the Competitiveness and Cohesion Operational Programme (KK.01.1.1.01.0001) and by the 2018-2.1.12-TET-HR-2018-00003 Croatian-Hungarian bilateral S&T project.

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EFECT OF THE WASTE MATERIALS ADDITION ON PSYCAL-MECHANICAL PROPERTIES OF CEMENT MORTARS

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The paper presents a study on the use of waste materials from Thermal Power Plants in Serbia as replacement components in cement mixtures. In this way, the quantities and costs of their deposition could be reduced, and thus the negative impact on the land, soil, air and environment could be minimized.
The main aim of our research was the comparison of compressive and flexural strengths of the cement mortars with the addition of the fly ash from the Thermal Power Plant Kostolac B and slag from the blast-furnace of the Železara in Smederevo (HBIS Group Iron & Steel). The experimental procedure included tests on the standard mortar samples prisms (4x4x16 cm), and also on the small (laboratory) prisms of size 1x1x6 cm. In order to choose proper quantities of the waste materials, their in-depth characterization was performed: determination of total moisture, granulometric composition, pH value, chemical composition, specific mass, pozzolanic activity, XRD and SEM. Based on the gained results, preparation of the cement mortar mixtures was done by replacing the standard mortar mixtures with 10 mas% of the fly ash or the blast furnace slag. The mechanical properties of the prepared systems: standard cement mortar, cement mortar with 10 mas % cement replacement with fly ash or blast furnace slag, were examined after 28 days and 8 months of hydration. Based on the compressive and flexural strength values, it was noted that the systems with cement replacement by fly ash (10 mas. %) possess better mechanical characteristics compared to the standard cement mortar. In the case of cement replacement with 10 mas. % of slag, the mechanical properties were approximately the same as the values gained for the standard cement mortar.
The obtained results proved that the waste materials from Thermal Power Plants, Serbia can successfully replace 10 mas. % of the cement in the standard cement mortars mixture, which is more than beneficial both from economic and environmental point of view.

Keywords: Fly ash, Slag, Cement, Compressive and flexural strength

Acknowledgements: The authors are grateful for the support funded by the Ministry of Education, Science and Technological Development, Republic of Serbia: project No. 451-0368/2022-14/200134.

ICAPP 2022
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OPTIMIZATION OF HYDROTHERMAL SYNTHESIS CONDITIONS IN ORDER TO OBTAIN PURE BISMUTH SODIUM TITANATE

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Bismuth sodium titanate (BNT) is one of the most interesting lead-free piezoelectric materials. Bismuth sodium titanate is a relaxor-like ferroelectric with a perovskite structure of rhombohedral symmetry at room temperature. BNT shows two phase transitions: paraelectric to ferroelectric at about 320°C (T_C) and has a depolarization temperature at about 200°C (T_d). These transitions are also related to the structural deformations of BNT, i.e. deformation from tetragonal to cubic structure (at T_C) and from rhombohedral to tetragonal phase (at T_d), respectively. In addition, BNT has a strong ferroelectric response (remanent polarization, $P_r = 38$ mC/cm^2) at room temperature, has a high transition temperature, T_C and good dielectric properties ($\varepsilon_r = 692$ and $\tan\delta = 0.045$ at 1 kHz). Due to all the above, BNT is considered to be one of the potentially good candidates for use in piezoelectric applications such as sensors and piezoelectric energy harvesting devices.

$Bi_{0.5}Na_{0.5}TiO_3$ powders were synthesized by hydrothermal method. The main goal of the synthesis was to define the optimal parameters for obtaining the pure BNT powder, and therefore 3 experiment setups were used. In the first setup, the solution inside the sealed reactor was heated externally through the silicone oil at the defined temperature. In the second setup, the sealed reactor with the solution is heated through a heating mantle with pressure and temperature controls. Finally, in the third setup, the sealed reactor with the solution is placed in the dryer at the desired temperature. In addition to the experimental setup, the influence of temperature and reaction time on the morphology and phase composition of bismuth sodium titanate was investigated, too. It has been shown that the third setup is the simplest and most optimal way of synthesis. This experimental setup together with the precise control of process parameters (temperature and time of synthesis) resulted in formation of the desired BNT phase.

Keywords: BNT, Hydrothermal, Piezoelectric

Acknowledgements: The authors would like to acknowledge the financial support from the Program No. 451-03-68/2022-14/200134, Ministry of Education, Science and Technological Development, Republic of Serbia.

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A SENSITIVE VOLTAMMETRIC SENSOR FOR CAFFEIC ACID MADE FROM THERMOLISED MODIFIED UF RESINS WITH INCORPORATED Fe(III) AND Ti(IV) OXIDE PARTICLES

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Domestic high performance electrode material was prepared by thermolysis of *in situ* synthesized urea-formaldehyde (UF) resins modified with Fe(III) nitrate and Ti(IV) oxide in different combinations: iron salt and UF resins (SynFe/UF-TP), titan oxide and UF resins (SynTi/UF-TP) and both metal compounds together and UF resins (SynFe+Ti/UF-TP). For comparison, the thermolysis prepared materials produced by simple physical mixture of metal compounds and UF resins (Fe/UF-TP, Ti/UF-TP and Fe+Ti/UF-TP) were made, too. The surface morphology characterization of all materials were done by SEM. Electrochemical results obtain by cyclic voltammetry and impedance spectroscopy have shown that *in situ* synthesized UF material modified with iron and titan compounds prepared by thermolysis, incorporated in carbon paste electrode (SynFe+Ti/UF-TP@CPE) possesses a better electrochemical response and conductivity than the other materials prepared by the same procedure, or pure CPE without addition of extra material. After optimization of experimental conditions and parameters of differential pulse technique, selectivity of proposed electrochemical method was examined. The proposed sensor with incorporated developed material has proven itself as a selective and sensitive electrochemical platform for determination of caffeic acid (CA). It was successfully applied for the determination of antioxidant capacity, based on CA equivalents, for honey, liqueur and juice samples.

Keywords: Modified urea-formaldehyde resin, Thermolysis, Voltammetric sensor, Caffeic acid

Acknowledgements: Financial support for this study was granted by the Ministry of Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/200123).

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SYNTHESIS OF CARBON NANOTUBES BASED CONDUCTIVE BIOMATERIALS

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In the present paper we report the preparation of multi-walled carbon nanotube (MWCNT) based conductive materials, with poly(lactide) (PLA) matrix and metallic nanoparticles (MNP). For this purpose MWCNT were surface-modified by hydrochloric acid. Series of PLA/MWCNT nanocomposites, with different contents (20, 30 and 40 wt%) of functionalized MWCNT, were synthesized employing a vacuum filtration method. First water dispersion of MWCNT was filtrated, and then PLA solution (20 wt% in dichloromethane) was poured onto MWCNT layer. After evaporation of dichloromethane composite materials PLA/MWCNT were obtained. In order to increased conductivity of obtained composite, different amounts (0,5; 1; and 2 wt%) of metallic nanoparticles (zinc and aluminium oxide) were added into the polymer matrix and hybrid materials PLA/MWCNT/MNP were prepared. FTIR analysis confirmed the structure of obtained samples. DSC analysis shown that a MWCNT had a significant influence on the thermal properties of polymer matrix (raising the Tg and the Tm values), but addition of metallic nanofillers has no influence on the transition temperatures of obtained hybrid materials. Thermo gravimetric analysis estimated that degradation onset temperature for composites with chemically modified MWCNT was much higher than degradation temperature for neat poly(lactide). Conductivity of obtained hybrid materials increases with increasing of metallic nanofiller contents. Higher conductivity was obtained for the hybrid materials with aluminium oxide nanoparticles.

Keywords: Hybrid material, Poly(lactide), MWCNT, Metallic nanoparticles

Acknowledgements: Authors wish to express their gratitude to the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project 451-03-68/2022-14/200134) for financial support.

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INFLUENCE OF MODIFIED MONTMORILLONITES ON FORMALDEHYDE CONTENT IN UREA-FORMALDEHYDE/MONTMORILLONITE COMPOSITES

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The effect of different montmorillonites (KSF and K10) and their modifications (Na-KSF, Na-K10) on content of formaldehyde-FA (free and liberated) modified urea-formaldehyde (UF) composites was investigated. KSF and K10 were modified by sodium chloride (NaCl). A total of four samples were synthesized, with the designations UF/KSF, UF/Na-KSF, UF/K10, and UF/Na-K10, under the same conditions. The content of free FA was determined by the bisulfite method. The hydrolytic stability of modified UF resin was determined by measuring the concentration of liberated FA of modified UF composites after acid hydrolysis. The specific surface area of the tested montmorillonites was determined by the Sear's method. Higher values of specific surface area were obtained for pure KSF (149.4 m^2/g) compared to modified Na-KSF (48.6 m^2/g). Specific surface area for pure K10 was 111 m^2/g, compared to value of 71 m^2/g for modified Na-K10. The amount of free and liberated FA was 0.4%, 0.12% and 1.2% and 2.3%, respectively for UF/KSF and UF/Na-KSF composite. The values for free FA for UF/K10 and UF/Na-K10 composite are the same and amount to 0.6%. It was concluded that the UF/Na-KSF composite has a smaller content of free FA (0.12%) compared to other UF composites. The UF/KSF composite has a higher resistance to acidic hydrolysis and lower liberated FA percent (1.2%).

Keywords: Montmorillonite, Free and liberated formaldehyde, Urea-formaldehyde composite

Acknowledgements: Financial support for this study was granted by the Ministry of Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/200123; 451-03-68/2022-14/200017).

ICAPP 2022
2nd International Conference
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ANTIFUNGAL ACTIVITY OF CHITOSAN FORMULATIONS AS ACTIVE COATINGS OF POLYLACTIC PACKAGING FOIL

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Food packaging is used to preserve the quality of food, ensure food safety, and extend its shelf-life. The main concern of the food sector is to reduce food waste and also food packaging waste. Regarding the objectives and functioning mechanisms of food packaging, new approaches have been developed, from passive to active packaging functions and sustainable (green) production and biodegradable feature of packaging materials. Concerning this, we studied chitosan-based coatings prepared in liquid formulations (1-2,8 %, v/v; primary/quaternary /antioxidant supplements) coated on biodegradable polylactide (PLA) foil by »roll-to-roll« technique to improve its functionality. A standardized procedure for antibacterial testing (ISO 22196:2007) has recently shown excellent inhibition of gram-positive bacteria and also gram-negative *Escherichia coli* and *Pseudomonas fragi*. In this study, antifungal activity against yeasts (*Saccharomyces cerevisiae*) and moulds (mycotoxigenic strains of *Penicillium verrucosum* and *Aspergillus flavus*) has been examined. Physico-chemical parameters such as particle size, polydispersity index, zeta potential, contact angle as wettability measure and other physico-chemical features (XPS, ATR-FTIR analyses) were tested. The impact of physico-chemical characteristics (e.g. type and concentration of chitosan, layering followed by the deposition of antioxidant supplement (e.g. rutin) will be discussed in view of food packaging requests and bioactivity. The latter was excellent regarding antifungal activity against yeasts (e.g. >99,5 % inhibition of *S. cerevisiae* growth), but dependent on the target organism in the case of moulds (up to 95,6% inhibition of *P. verrucosum* and 68,0 % inhibition of *A. flavus* growth), and in correlation with some physico-chemical characteristics, especially contact angle and antioxidant activity. By developing functionalized and biodegradable PLA foils with chitosan-based coatings, we aim to reduce the spoilage of perishable foods, reducing food waste and the detrimental impact of plastic on our environment.

Keywords: Active food packaging materials, Antifungal activity, Chitosan coatings, PLA

Acknowledgements: This work was financially supported by the PRIMA programme, project BioProMedFood (Project ID 1467, and ARRS research programmes (P4-0116 and P2-0118).

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CHEMICAL RECYCLING OF POST-CONSUMER PET BOTTLES AND THE USE OF RECYCLED PRODUCTS FOR ECO-FRIENDLY RESINS

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Due to increasing consumption, the disposal of used PET packaging for bottled water and soft drinks creates severe environmental and economic problems. Recycling is the most suitable option for the treatment of PET waste. Chemical recycling of PET involves depolymerizing post-consumer PET into product monomers and oligomers. Depolymerization of PET can be achieved by methanolysis, glycolysis, hydrolysis, ammonolysis, aminolysis, and other methods. Glycolysis (with ethylene glycol (EG)) is the most popular method to depolymerize the waste of PET. The glycolysis reaction rate depends on several parameters, including temperature, PET/EG ratio, and the type and amount of catalyst. Glycolysis without a catalyst is a prolonged process. PET glycolysis is considered a trans-esterification reaction. In this work, the reaction was conducted at a temperature of 200 °C with a reaction time of 3 h. Natrium acetate was added to the reaction as the catalyst. The second step implies synthesizing resins based on oligomers (the products of glycolyze) with glycerol, the anhydride of phthalic acid, and linseed oil. The mixture was stirred slowly and heated to 180 °C for 30 minutes while the alcoholysis reaction took place. It has been found that resin properties derived from products from glycolysis of post-consumer PET bottles are compatible with the properties of reference resins.

Keywords: PET waste, Chemical recycling, Glycolysis, Resins

Acknowledgements: The authors wish to thank the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant numbers 451-03-68/2022-14/200134) for financial support.

ICAPP 2022
2nd International Conference
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INVESTIGATION OF THE INFLUENCE OF THE INITIAL CONCENTRATION OF HEAVY METALS IN BINARY SOLUTIONS ON THE EFFICIENCY OF ADSORPTION

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In recent years, new, more low-cost and more efficient materials for reducing pollutants in wastewater have been studied. At the same time, most attention is paid to natural materials, most often food waste. There are numerous literature data that testify to the strong adsorption capacity of coffee-derived materials, which are able to adsorb significant amounts of various adsorbates. In many cases, the obtained values were competitive with commercial materials of well-known physical and chemical characteristics, and therefore the approach of using this type of waste was opened, as economically justified, and satisfactory in purpose. This paper presents the results of testing the influence of the initial concentration of copper and chromium ions in binary solutions on the sorption capacity of coffee waste. The characterization of the material was performed, where physical, chemical and physicochemical properties were determined. The content of organic matter in water, cation exchange capacity (CEC), as well as the point of zero charge were determined. Different functional groups present on the surface of the adsorbents, which can participate in the binding of cations due to dissociation in the aqueous medium, were determined by the FTIR method. The effect of biosorbent concentration on the removal of Cu (II) and Cr (III) ions from aqueous solutions was investigated at initial concentrations of 10 mg/L, 50 mg/L and 100 mg/L.

Keywords: Binary solution, Heavy metals, Adsorption, Physicochemical characterization

ICAPP 2022
2nd International Conference
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INVESTIGATION OF POLYURETHANE HYDROGELS FOR USE IN AGRICULTURE

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Polyurethane is a material that is known for its stable structure and good mechanical properties, but also its hydrophobicity and insolubility in water. However, the properties of polyurethanes can be modified by incorporating hydrophilic soft segments (such as poly (ethylene oxide) (PEO)) into the structure of polyurethanes to increase their hydrophilicity, e. i. to obtain a hydrogel. In this work, polyurethane hydrogels of 2.7 functional isocyanates PMDI, poly (ethylene oxide) of molecular weight 2000, 6000, and 10000 g/mol were synthesized, with the appropriate amount of catalyst, dibutyltin dilaurate, polymerization was carried out in tetrahydrofuran as a solvent. As these polyurethane hydrogels have application in agriculture, their very important property is the absence of monomers in the samples and thermal stability. The structure of the samples was examined by infrared spectroscopy with Fourier transform, thermal properties were examined by differential scanning calorimetry (DSC) and thermogravimetry analysis (TGA), the absence of isocyanates was determined by gel permeation chromatography (GPC), the influence of hydrogels for control release of mineral fertilizers was investigated at tomatoes. The FTIR results show that the NCO group reaction is complete and a quantitative reaction is achieved and the DSC results show that the melting temperature varies depending on the molecular weight of the PEO used. GPC showed the absence of isocyanates in samples with PEO 2000 and PEO 6000, but in samples with PEO 10000 the isocyanates were in samples. The results of the TG analysis show that the PU degradation temperature increases with the change of the polyol type.

Keywords: Polyurethane, Hydrogel, Polyol, Thermal properties

Acknowledgements: Financial support for this study was granted by the Ministry of Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/200134).

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APPLICATION OF CERIA-ZIRCONIA-BASED HIGH-ENTROPY OXIDES AS CATALYSTS IN CONVERSION OF ORGANIC MOLECULES

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Efficient Lewis-acid-catalyzed direct conversion of aldehydes to 1,2-diketones in the liquid phase was enabled by using novel ceria–zirconia-based high-entropy oxides (HEOs) as the actual catalysts. HEOs were synthesized in nanocrystalline powder form using a modified citrate sol-gel route. These compounds were further structurally characterized using powder X- ray diffraction (PXRD), Raman spectroscopy, scanning electron microscopy coupled with energy- dispersive X-ray spectroscopy (SEM-EDS), high resolution transmission electron microscopy (HRTEM), BET physisorption measurements and thermogravimetric analysis. The synergistic effect of various cations incorporated in the same oxide structure (framework) was partially responsible for the efficiency of multicationic materials compared to the corresponding single-cation oxide forms. Furthermore, a clear, linear relationship between the Lewis acidity and the catalytic activity of the HEOs was observed. Due to the developed strategy, exclusively diketone-selective, recyclable, versatile heterogeneous catalytic transformation of aldehydes can be realized under mild reaction conditions.

Keywords: Catalysis, High entropy oxides, Lewis acid, Organic conversion

Acknowledgements: Authors acknowledge Croatian Science Foundation (PZS-2019-02-2467) for full financial support.

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SURFACE PROPERTIES OF AMINE CURED EPOXY COMPOSITES WITH DIFFERENT CONTENT OF MONTMORILLONITE

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Surface properties of epoxy based composite polymer materials with addition of organically modified montmorillonite (OMMT) were investigated. Samples were prepared from diglycidilether bisphenole A (DGEBA) and poly(oxypropylene)diamine (Jeffamine D-230), and with 1 wt. %, 3 wt. %, 5 wt. % and 10 wt. % of OMMT (Cloisite 15A), as well as without OMMT. The surface properties were analyzed by atomic force microscopy. The analysis was performed at room temperature, in tapping mode. The surface images were obtained in the range from 1 to 50 μm. Samples were prepared for analysis by treating with liquid nitrogen and pressing at liquid nitrogen temperature. AFM images of materials containing different wt.% of OMMT were obtained in topographic and phase views. Addition of montmorillonite to the epoxy matrix results in significant changes in the surface morphology of the obtained materials. In the AFM phase images, the sample without montmorillonite, as well as the sample with 1 wt.% montmorillonite, has one phase, which means that the sample with 1 wt.% has a homogeneous distribution of montmorillonite. At a montmorillonite content of 3 wt.%, the differences in phases are not so pronounced, but the regions of the hard phase are relatively evenly distributed in the softer phase, i.e. the montmorillonite is evenly distributed in the epoxy matrix. With the increase of montmorillonite content to 5 wt.%, the hard phase starts to dominate, so it can be concluded that with the addition of montmorillonite above 5 wt.%, the formation of agglomerates of montmorillonite in the epoxy matrix occurs, so the distribution of lighter areas in the AFM image of the phase peak uneven. In the case of a sample with 10 wt.% montmorillonite, due to the large mass of montmorillonite present in the system, their accumulation and the formation of entire aggregates occur. From obtained AFM images, it can be concluded that optimal amount for addition of OMMT is 3 wt.%.

Keywords: Epoxy, Montmorillonite, Atomic force microscopy, Polymer composites

Acknowledgements: The authors wish to thank the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant numbers 451-03-68/2022-14/200134) for financial support.

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EFFECTS OF SELENIUM NANOPARTICLES ON PATHOGENIC AND PROBIOTIC FOOD BACTERIA, AND THEIR INFLUENCE ON HUMAN INTESTINAL CELLS

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Selenium is an especially important element for the functioning of antioxidative enzymes, making it one of the essential micronutrients. Although selenium is required in extremely small amounts, often it is lacking from the organism. However, inorganic and organic sources of selenium are known to possess considerable toxicity. Recently, the nanoparticle form of selenium has been shown to exhibit lesser toxicity, and also better antimicrobial and other biological effects, than selenium-containing compounds. Nanotechnology has already found application in many fields, for improving material properties for various purposes, among them, in the process of improving food characteristics. It is possible that selenium nanoparticles can be applied as antimicrobial agents in food, and there has been growing interest in this area of research. We have synthesized selenium nanoparticles (SeNPs) by chemical reduction method, using sodium selenite, ascorbic acid, and bovine serum albumin. The antimicrobial activity of these particles has been assessed by minimum inhibitory concentration assay and colony-forming units count, on *Listeria monocytogenes* and *Salmonella enterica*, which are common bacterial food contaminants. As intestinal microbiota, especially probiotic bacteria, have been shown to play important role in organism functioning, we also tested how SeNPs influence *Lactobacillus rhamosus* and *Lactobacillus plantarum*. To evaluate cytotoxicity, we used MTT assay on the human intestinal cell line (HT-29). Antioxidative properties were assessed by DPPH assay. SeNPs showed higher antibacterial activity on pathogenic bacteria than on probiotic strains. Cytotoxic effect was present only in the highest tested concentrations. The antioxidative activity was considerable, resulting in above 80% reduction of DPPH free radical, at 1 μg/ml of SeNPs. Based on these results, SeNPs are a promising subject for further research in developing materials for food packaging or as antimicrobial selenium additives.

Keywords: Selenium nanoparticles, Antibacterial, Antioxidative, Probiotic

Acknowledgements: Funds for the realization of this work were provided by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Agreement on realization and financing of scientific research work of the Institute of Technical Sciences of SASA in 2021 (Record number: 451-03-9/2021-14/ 200175), and grant number 451-03-68/2022-14/ 200178.

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ADDITIVE MANUFACTURING AND CHARACTERISATION USING DIGITAL IMAGE CORRELATION OF TENSILE PIPE RING SPECIMENS

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Examination of structures and parts of pressure equipment fabricated by additive production techniques is an insufficiently studied area. With the development of new techniques and materials, additive production is becoming increasingly important when it comes to functional replacement parts for different assemblies and devices, in addition to an already established role in rapid prototyping. In order to develop a method for testing the pressure equipment such as pipelines, prototypes of ring-shaped test specimens with a stress concentrator (new pipe ring tensile specimens) are fabricated by different techniques and from various non-metallic and metallic materials. This work focuses on polylactic acid (PLA) specimens fabricated on a Fused Deposition Modeling (FDM) printer. The rings are produced in the axial direction so that the contours of the material obtained by extrusion through the nozzle are loaded with tension during the testing. 3D printed pipe rings are tested on Shimadzu's electro-mechanical universal testing machine. The testing is accompanied by digital image correlation (DIC) on the specimen surface; GOM Aramis system, consisting of two cameras, acquisition equipment, and software, is utilized. This way, the 3D displacement/strain field on the specimen surface is tracked. The obtained results indicate that repeatability of the testing process is possible, and the development of the methodology will be continued in the same direction. In addition to the testing results, this work will include a discussion on the applicability and possible limitations of the proposed testing procedure in analyzing different stress concentrators on pipelines (including corrosion and stress corrosion defects).

Keywords: Pipe ring specimens, Additive manufacturing, Tensile testing, Digital image correlation

Acknowledgments: The Authors would like to thank the Ministry of Education, Science and Technological Development of the Republic of Serbia for providing financial support that made this work possible (by the contract: 451-03-68/2022-14/200105 and 451-03-68/2022-14/200135). The authors would also like to acknowledge the support from European Union's Horizon 2020 research and innovation program (H2020-WIDESPREAD-2018, SIRAMM) under grant agreement No 857124.

2nd International Conference
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INVESTIGATION OF GLASS MOSAIC TESSERAE: CASE STUDY OF THE CITY HOSPITAL FAÇADE IN NOVI SAD

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The main goal of this study was to identify the composition of glass tesserae used to decorate the façade of the City Hospital in Novi Sad (part of the present-day Clinical Centre of Vojvodina) built in 1909. The façade of the hospital has decoration in the glass mosaic technique with two frontally placed monumental figures of angels and between them the year of completion: 1909. The glass mosaic is under restoration. Since there is no data on the technology of the used glass tesserae, it was necessary to identify the composition of the samples of the glass mosaic tesserae. The analysed samples had five colours: white, green with a golden colour leaf between two layers of glass, yellow, orange-red, and purple. Several methods were used in order to obtain a qualitative and quantitative characterisation of the glass samples in terms of elements and mineralogical phases: SEM-EDS, FTIR spectroscopy, VIS colorimetry, and Raman spectroscopy. The obtained results revealed the type of glass from which the tesserae were made, as well as opacifiers and colouring agents added to the glass. Moreover, the composition of the metal leaves in the tesserae was identified to be pure gold. The results demonstrated that good quantitative and qualitative analyses of glass tesserae samples could be performed by non-destructive examination. Furthermore, obtained information on the glass contributed to an improved dating of the tesserae and further increased the knowledge about their production.

Keywords: Glass, Mosaics, Tesserae, Opacifiers, Colouring agents

Acknowledgements: The authors are grateful for the financial support by the Ministry of Education, Science and Technological Development, Republic of Serbia: project No. 451-03-68/2022-14/200134.

Nutraceuticals and Pharmaceuticals

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3D-PRINTING AS A NOVEL TOOL FOR THE PRODUCTION OF FUNCTIONAL PRODUCTS BASED ON STRAWBERRY

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Consumers today are better informed about functional foods (e.g., functional fruit juices) and are more interested in improved health through the foods they consume. They are also increasingly interested in ensuring that the foods they consume are produced using sustainable technologies. Recently, 3D printing technology (3DP) has been extensively developed as it is being proposed and used in many industries as a novel sustainable solution for personalized food production. The 3DP of food is a technology that can use alternative raw materials with lower energy requirements for producing functional products according to personalized consumers' preferences. Such eco-friendly solution allows flexibility in design, creation and production of products such as functional 3D fruit juices without plastic packaging. However, due to the high-water content, fruit juices cannot be printed without the addition of hydrocolloids, which increase the mechanical strength of 3D-printed food products. Therefore, the aim of this work was to develop 3DP strawberry snacks by separately adding different proportions of wheat and corn starch (10, 15, 20%). In addition, different printing parameters (i.e., printing speed, ingredient flow speed, first-layer-nozzle height and line thickness) were investigated for the printability of 3DP strawberry snacks. The content of total phenolic compounds, monomeric anthocyanins and antioxidant capacity were investigated in all 3DP products to gauge nutritive added-value. The results showed that the incorporation of hydrocolloids in the 3DP strawberry juice greatly affected the dimensional stability, as all 3DP products could be printed easily and smoothly while maintaining their 3D design shape after processing. Different starch contents and starch types influenced bioactive compound content and antioxidant activity. Both starches were found to be suitable hydrocolloids that could be used for the production of functional strawberry 3DP-snacks. Since the results show that 3DP products retain their functional properties compared to fresh strawberries, further research is needed to investigate the sensory acceptability and shelf life of these products.

Keywords: 3D printing, Strawberry, Functional food, Bioactive compounds

Acknowledgements: This research was funded by Croatian Science Foundation through the funding of the 'Hurdle Technology and 3D Printing for Sustainable Fruit Juice Processing and Preservation' [number IP-2019-04-2105].

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CO-CREATION AS AN EFFECTIVE TOOL FOR NOVEL NUTRACEUTICALS DEVELOPMENT

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In the context where functional foods and dietary supplements are becoming a major focus of new product development, co-creating ideas for nutraceutical products together with consumers appears to be an interesting alternative approach for both researchers and companies. However, this approach requires a more open and flexible strategy, and it also requires a deep understanding of consumer explicit and latent needs. This strategy has to be followed with a specific methodology and appropriate tools in order to be effective. This lecture provides an overview of methods used in co-creating nutraceuticals together with consumers, which is followed with a brief analysis of a few specific cases and a list of main challenges that are to be expected.

Keywords: Co-Creation, Nutraceuticals, Functional food, Consumers.

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on Advanced Production and Processing

HIGH VALUE PARTICLE MANUFACTURING
from Cell to Drug Encapsulation and Delivery

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Many of the particles shown in the figure below are 'formulation' based. Fine Chemical, Pharmaceutical, Fast-Moving Consumer Goods (FMCG) and more recently Food Industry is in constant need for novel formulations which contain particles of controllable property. In this work the focus will be on encapsulation and manufacturing of highly uniform particles at the right scale using low energy / low shear method based around membrane technology.
Encapsulated particles are primarily used to control the release of components, either to provide a sustained release with time, or a triggered complete release under appropriate conditions, examples include: the masking of fish oil and other non-palatable (but very healthy) components, combined hydrophilic and lipophilic delivery, the sustained release of subcutaneous delivered cancer treatments, such as leuprolide acetate for prostate cancer, and mixed pesticide and fertiliser compounds in the agricultural industries. Cell encapsulation into various spherical matrices opens number of opportunities from cultivated meat to personalised medicine and novel biocatalysts.
Membrane emulsification with micro engineered membranes has been proven to manufacture continuously various formulations from millilitres to tonnes scale and at the presentation specific overview toward the implementation of this new technology in Industry settings will be investigated.

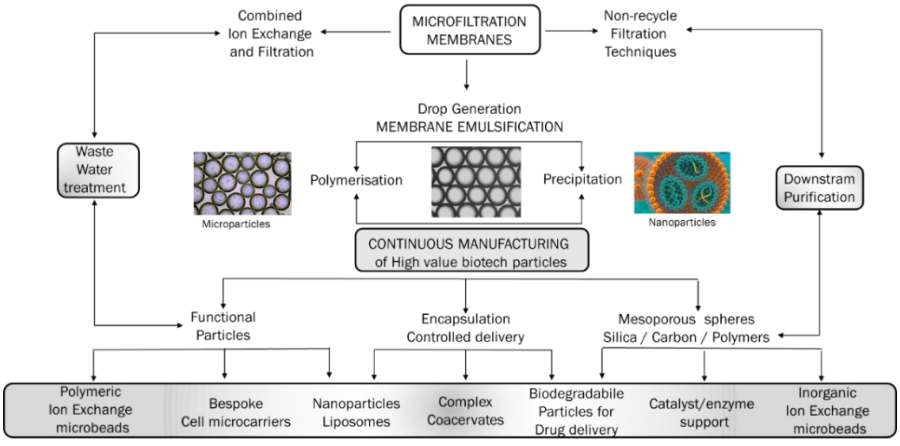

Fig. 1 From nano to microparticles in the High Value Manufacturing arena

Keywords: Bio-Encapsulation, Probiotics/Cells, Controlled release, Drug delivery, Bio-Degradability

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OPTIMIZATION OF SUPERCRITICAL CO_2 EXTRACTION OF LIPOPHILIC BIOACTIVE COMPOUNDS: A CASE STUDY ON MYRTLE BERRIES

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Advanced procedures for extracting various lipophilic bioactive molecules from traditional medicinal plants have recently gained importance in the research and development of functional foods, dietary supplements, or pharmaceuticals. In addition to their well-known role in cardiovascular diseases, the health effects of fatty acids as a major lipid group include the regulation of energy and cholesterol metabolism, potent influence on inflammatory processes, and the effects on the gut microbiota. Phytosterols, another important lipid group, also exhibit the ability to lower total cholesterol and low-density lipoproteins, thereby reducing the risk of cardiovascular disease. They are believed to have anti-inflammatory, antimicrobial, anti-cancer, and immunomodulatory effects, as well as beneficial effects on diabetes and body weight. One of the advanced extraction techniques, particularly suitable for the extraction of these lipophilic compounds, that has recently been used commercially on a larger scale, is supercritical CO_2 extraction. Compared to the conventional solvent extraction methods, this technique offers numerous advantages, which include the preservation of functional properties, the availability of CO_2, and the absence of solvent residues in the extracts. However, since variations in supercritical CO_2 conditions such as temperature, pressure, extraction time, solvent flow rate, etc., affect the extractability of specific components, modelling of process parameters is essential for the development of an effective supercritical CO_2 extraction method. For the optimization of supercritical CO_2 extraction, the response surface methodology is often used as a platform to optimize the extraction and create a process that is efficient from both economic and production perspectives. The complexity of supercritical CO_2 extraction is demonstrated on the case of the extraction of phytosterols from myrtle berries (*Myrtus communis* L.). Regression models developed based on the obtained results for extraction yield, percentage of total polyunsaturated fatty acids, and yield of phytosterols showed good performance and also the suitability of the response surface methodology for modelling these variables. The results demonstrate the potential of supercritical CO_2 for the extraction of bioactive lipophilic compounds, although the extraction parameters are highly dependent on the target compounds and should be optimized based on their properties.

Keywords: Supercritical CO_2, Extraction, Optimization, Fatty acids, Sterols

Acknowledgements: This work was supported by the project "Bioactive molecules of medical plant as natural antioxidants, microbicides and preservatives" [KK.01.1.1.04.0093]

ICAPP 2022
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INNOVATION IN COSMETIC INDUSTRY: FROM FORMULATION TO FINAL PRODUCT

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Cosmetic sector in Serbia faces many challenges especially regarding development of innovative formulations of cosmetic products that are unique on the market. Product innovation is essential in gaining competitive advantage in cosmetic industry. Cosmetic industry is characterized by the constant necessity for releasing of new products and high dependence on new ingredients, especially functional actives. Also, branding is crucial in building an advantage over competitors. In that manner, cosmetic companies are continuously increasing the potential of their existing brands and strive to offer new ones.

Procedures for development of innovative cosmetic products consist of several key segments: flowchart of cosmetic product development, characterization of raw materials, pilot plant/small-scale product production, obtained product testing (including comparative analysis) and optimization of technological procedures for large-scale production.

Keywords: Cosmetic industry, Development, Production.

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CONTAMINATION CONTROL IN PHARMACEUTICAL INDUSTRY

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Cross contamination control and prevention represent the essential requirement in pharma production systems. No matter what kind of production it is (e.g., sterile or non-sterile) appropriate actions must be pre-taken in order to mitigate any, potential cross contamination risk. Pharma production facilities must be constructed in such way that even the layout prevents cross contamination risk (Quality by Design), using the pharma-grade materials which do not emit particles. The production facility must be also equipped with appropriate Air Handlin Unit System (AHU) which will maintain the air in the room in such quality that corresponds to type of product that is being produced in the particular facility or premises. Production premises in pharma industry are called Cleanrooms, and their classification regarding allowable number and size of particles in the room air, are defined by ISO standards. Cleanroom classification determines whether there will be a sterile or non-sterile production. Allowable limit of particles (number and size) and are defined by ISO 14644-1:2015. Every process which takes please in certain Cleanroom must be validated, standardized and controlled.

Keywords: Cleanroom 1, Cross 2, Contamination 3.

Acknowledgements: The authors would like to thank the Ministry of Education, Science and Technological Development, Republic of Serbia, for financial support [Project No. 451-03-68/2022-14/200134].

ICAPP 2022
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POTENTIALLY HARMFUL EXCIPIENTS IN PEDIATRIC ORAL COUGH MEDICINES AUTHORIZED IN SERBIA

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Some pharmaceutical excipients may cause adverse effects or excipient-related contraindications and interactions. The aim of this study was to identify all excipients with known effect (EKE) in pediatric oral cough medicines authorized in Serbia and evaluate EKE labeling in the corresponding Summaries of product characteristics (SmPCs) and Package leaflets (PLs). The study was designed as a post-authorization safety study and safety of excipients was considered in accordance with recommendations of the European Medicines Agency. Out of a total of 64 oral cough medicines authorized in Serbia, 58 (90.63%) of them were approved for pediatric use and all of these pediatric medicines contained one or more EKE. A total of 27 different EKE were identified, from 6 different functional categories (sweeteners, preservatives/antioxidants, electrolytes, solvents, solubilizing/emulsifying agents, and coloring agents) with most of them present in products approved for use across all ages of children and adolescents above the age of 2. A significant number of EKE labeling deficiencies were detected in product PLs and SmPCs, including complete omission of one or more EKE for 74.14% of products, as well as missing or incomplete EKE quantitative (44.83% of products) and/or safety information (44.83% of products). As negative effects of excipients may be more pronounced in the pediatric population compared to adults, it is especially important to consider EKE related safety issues when prescribing and dispensing medicines intended for children. Revision of the product PLs and SmPCs is recommended in order to eliminate deficiencies and improve EKE labeling.

Keywords: Pediatrics, Pharmaceutical Adjuvants, Pharmacovigilance, Risk Assessment.

Acknowledgements: This study was supported by The Ministry of Education, Science and Technological Development, Republic of Serbia [project 451-03-68/2022-14/200114].

2nd International Conference
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REVISITING EXTRACTION OF LYCOPENE FROM TOMATO USING HYDROPHOBIC NATURAL DEEP EUTECTIC SOLVENTS

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Lycopene ($C_{40}H_{56}$), a bioactive lipid-soluble compound, belongs to the class of carotenoids which are one of the most widespread groups of natural pigments. The major edible source of lycopene is the fully ripe tomato fruits. Lycopene, has been authorized within the European Union as a food coloring agent (E160d) in various food products. Its industrial importance stems not only from its coloring attributes but also from its bioactive properties. Depending on the aim of the study, an array of conventional organic solvents (e.g. hexane, acetone etc.) as well as their mixtures, have been used so far for the extraction of lycopene. However, the interest of the scientific community has been focused on the use of alternative green solvents such as the Deep Eutectic Solvents (DESs). The latter ones can be defined as combinations of components that consist of a hydrogen bond donor and a hydrogen bond acceptor that form a network of hydrogen bonds resulting in a liquid with distinct characteristics. In the present study, different hydrophobic natural deep eutectic solvents (HNADESs) based on terpenes (i.e. menthol and thymol) and fatty acids (i.e. decanoic acid and dodecanoic acid) were prepared at different molar ratios, characterized in terms of density, rheological properties and Fourier transform-infrared (FT-IR) spectroscopy and were examined for their effectiveness to extract lycopene from tomato. Response surface methodology was employed to optimize the extraction parameters, namely duration (min) and solvent:solid ratio (v/w) using the most efficient HNADES. Spectrophotometry and RP-HPLC-DAD were employed in order to monitor the process efficiency. The combination of decanoic acid and dodecanoic acid was found to exhibit comparable extraction capacity to acetone. Moreover, recovery of carotenoids from the optimum HNADES extract based on their precipitation caused by the polarity switch of the eutectic mixture upon the addition of an anti-solvent, was also carried out.

Keywords: Hydrophobic natural deep eutectic solvents, Lycopene, Tomato, Response surface methodology, Fatty acids

Acknowledgements: This research has been conducted in the frame of the Operational Program "Competitiveness, Entrepreneurship, and Innovation", under the call RESEARCH–CREATE–INNOVATE ("Development of new tomato cultivars by using omics technologies-Ntomatomics", project code: T2EDK-01332) that was co-financed by Greek national funds and the European Union (European Regional Development Fund).

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EMERGING EXTRACTION APPROACH FOR RECOVERY OF HEMP (*Cannabis sativa* L.) BIOACTIVE COMPOUNDS

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Industrial hemp is a multi-purpose crop used for a wide range of products in many industries. Currently, hemp is being explored for its application in the pharmaceutical industry, as the inflorescences contain a rich spectrum of bioactive compounds. Given the importance of oxidative stress, it is very important to investigate natural antioxidants. The main goal of this study was to determine the chemical composition and antioxidant activity of industrial hemp (*Cannabis sativa* L.) obtained by green extraction techniques. The chemical composition was determined from the essential oil obtained by SFE using GC-MS. MAE extracts were obtained and their chemical composition was determined using UHPLC-DAD MS/MS. Antioxidant activity was determined in extracts obtained by HD, MWHD and MAE. Quinic acid was the main phenolic compound in the SFE raffinate of the Marina variety (40.34 mg/100 g). Helena variety showed the highest antioxidant activity against $ABTS^{+}$ radicals (103.04 μM TE/g) among the extracts obtained by MAE from the primary raw material and from SFE raffinates (88.63 μM TE/g). It can be concluded that hemp is a source of valuable cannabinoids, which are of great interest in terms of their therapeutic potential. The terpenes and phenolic compounds present in hemp are an excellent natural source of antioxidants.

Keywords: Cannabis sativa L., Green extraction techniques, Chemical composition, Antioxidant activity, Cannabinoids

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.

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MODULATION OF THE BIOCHEMICAL ACTIVITIES OF SOYBEAN MILK PROTEIN BY PAPAIN

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Soybean milk protein has been recognized as a high-quality protein. Contradictorily, due to the presence of linear and conformational epitopes in protein structure, there are some limitations have been reported. Objective of the investigation was to understand the effect of the enzyme (papain) and substrate (soybean milk proteins) ratio, such as 0.02:100, 0.057:100, 0.114:100 and 0.229:100 on hydrolysis of soybean milk protein (SMP) and biochemical activity. The hydrolysis of SMP was studied by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The antioxidant capacity was measured by the ferric reducing ability of plasma (FRAP) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assays. The anti-angiotensin enzyme activity was measured by the recombinant angiotensin converting enzyme and substrate Abz-FRK(Dnp)-P. Contributions of Kunitz Trypsin Inhibitor (KTI) and Bowman-Birk Inhibitor (BBI) on antigenicity and *in vitro* digestion of papain-hydrolyzed SMP were studied. Polyclonal antibodies, such as anti-KTI rabbit (Rb) IgG and anti-BBI Rb IgG together with peroxidase-labelled goat anti-Rb IgG secondary antibody were used to identify the antigenicity of KTI and BBI in unhydrolyzed and papain-treated SMP. *In vitro* gastrointestinal digestion simulation protocol (Infogest) was used to understand the digestibility of papain-treated SMP. The hydrolysis of SMP was dependent on the concentration of papain. Antioxidant capacity and anti-angiotensin activity of SMP were increased after the papain hydrolysis of SMP. Mechanism of the anti-angiotensin activity by SMP hydrolysate was non-competitive. The KTI and BBI-specific antigenicity were reduced in SMP with increasing the concentration of papain. However, there was interaction between papain-hydrolyzed SMP and trypsin in native gel, interaction with chymotrypsin was absent. The interaction between trypsin and SMP was reduced due to hydrolysis of papain in a concentration-dependent manner. The digestibility of SMP was increased after its hydrolysis by papain. It may belief that result of present investigation will reduce the limitation of soybean milk consumption.

Keywords: Soybean milk protein, Antioxidant capacity, Anti-angiotensin activity, Antigenic property, Digestibility

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HYPOALLERGENIC PEPTIDES WITH ANTIOXIDANT CAPACITY, ANTI-ANGIOTENSIN ACTIVITY AND ANTIBACTERIAL ACTIVITY FROM LIQUID MILK PROTEIN CONCENTRATE BY ENZYMATIC AND MICROBIAL HYDROLYSIS

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A plethora of information about the beneficial aspects of milk proteins have been published by the International Dairy Federation (IDF). Contradictorily, milk proteins are listed among the "big 8" allergens. Objective of the investigation was to produce hypoallergenic bioactive peptides from liquid milk protein concentrate (LMPC). LMPC was produced from ultra-heat-treated skimmed milk by removing milk serum (whey) as permeate by a tubular ceramic-made membrane, pore size 5 nm. Effects of trans-membrane pressure (TMP) (3-2 bar), recirculation flow rate (Q_r) (100-200 $L \cdot h^{-1}$) and static turbulent promoter on permeate flux of filtration process have been studied. Milk with concentrated proteins from retentate side of membrane was initially hydrolyzed by the different concentrations of trypsin (≥27.78 units per mg of solid at temperature 25 °C), such as 0.008 $g \cdot L^{-1}$ (LMPC-T-0.008), 0.016 $g \cdot L^{-1}$ (LMPC-T-0.016) and 0.032 $g \cdot L^{-1}$ (LMPC-T-0.032) in individual batch-mode operations at temperature 40 °C for 10 min. Inactivation of trypsin in reaction was done at a temperature of 70 °C for 30 min of incubation. Subsequently, the hydrolysis of tryptic-hydrolyzed LMPC (LMPC-T) was performed with lactic acid bacteria (*S. thermophilus* and *L. bulgaricus*) at temperature 42 °C for 6 hr. Microbial hydrolysis of LMPC-T was performed with glucose (LMPC-T-F) and without glucose (LMPC-T-F_G). Subsequently, samples were centrifuged and bioactive peptides in supernatant was considered for analysis. Superior operational strategy in filtration process is herein: TMP 3 bar, Q_r 100 $L \cdot h^{-1}$ and implementation of a static turbulence promoter within the tubular membrane. Antioxidant capacity and anti-angiotensin activity were increased with increase of the concentration of trypsin. Changes of mentioned biochemical activities had similar trend in LMPC-T-F and LMPC-T-F_G. However, antibacterial activity against *B. cereus* and *S. aureus* was noted in LMPC-T, additionally antibacterial activity against *L. monocytogenes* was noted by LMPC-T-F and LMPC-T-F_G. Allergenicity in LMPC was reduced at more than 99% after sequential hydrolysis with 0.016 $g \cdot L^{-1}$ of trypsin and lactic acid bacteria.

Keywords: Peptides, Tryptic hydrolysis, Microbial hydrolysis, Antioxidant capacity, Anti-angiotensin activity, Antibacterial activity

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STUDYING BIOACTIVITY PROPERTIES OF NOVEL CHLOROACETAMIDES BY USING THIN-LAYER CHROMATOGRAPHY

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The priority goal in the early phase of investigating the biological potential of novel compound is assessment of its lipophilicity. For novel derivatives of *N*, *N*-disubstituted chloroacetamides, lipophilicity was determined computationally as well as experimentally by using reverse-phase thin-layer chromatography (RPTLC18F254s) in the presence of two organic modifiers (methanol and dioxane). The influence of the type of hydrocarbon substituent and applied organic modifier on the chromatographic behavior of the tested derivatives was studied. Chromatographic parameters (R_M^0 and m) of chloroacetamide derivatives as assumed measures of their lipophilicity were correlated with the software obtained values of the standard measure of lipophilicity, partition coefficient (log$P$) and selected parameters of ecotoxicity by linear regression and multivariate analysis methods. Appropriate mathematical models were obtained.

Keywords: Thin-layer chromatography, Chloroacetamides, Bioactivity

Acknowledgements: The authors acknowledge financial support of the Ministry of Education, Science and Technological Development of the Republic of Serbia [number 451-03-68/2022-14/200125]

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

MICROENCAPSULATION OF LAVANDIN (*Lavandula x intermedia*) ESSENTIAL OIL BY SPRAY DRYING

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Lavender essential oils possess many different beneficial biological properties, including antioxidant activity and inhibitory effect on many types of foodborne, human and environmental bacteria. Possessing high volatility, poor water-solubility, thermal and chemical instability, essential oils are a suitable candidate for microencapsulation, enabling their applicability as chemical preservatives in various industrial sectors.
The aim of this study was to select an appropriate wall material to be used for encapsulation of lavandin (*Lavandula x intermedia*) essential oil by spray drying. Lavandin essential oil was isolated by steam distillation. Maltodextrin (MD), gum arabic (GA) and whey protein concentrate (WPC) were tested as wall materials. Emulsification procedure involved the addition of essential oil into wall material solution by homogenization at 10000 rpm during 5 min. The core/wall material ratio was 1:4 (w/w). The feed emulsion was injected into a spray dryer at inlet temperature 140 °C and feed flow rate of 0.192 L/h. Total oil content and surface oil content in the microcapsules, oil retention efficiency (RE), encapsulation efficiency (EE), moisture, hygroscopicity and particle size diameter were determined.
The results indicate that MD can be used for lavandin essential oil encapsulation, but only with addition of emulsifiers or other polymer/protein with emulsifying properties. The highest encapsulation efficiency was obtained by using MD with Tween 20 as the emulsifier (83.29 ± 0.1 %), producing a microcapsule with the smallest mean particle size ($D_{4,3}$ = 5.83 ± 0.21 μm), but with tendency of powder aggregation at relative humidity of 75.29 % (25°C). Addition of GA or WPC in a mixture with MD had prevented powder aggregation, but decreased EE (up to 65.03 ± 1.37 % using MD/GA and 77.37 ± 1.2 % using MD/WPC) and encapsulation yield, while moisture and mean particle size were increased.

Keywords: Microencapsulation, Lavandin essential oil, Spray drying

Acknowledgements: This research study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia [number 451-03-68/2022-14/200134].

2nd International Conference
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IMPROVED POLYPHENOLIC ISOLATION FROM AUTOFERMENTED CHAMOMILE FLOWERS BY MICROWAVE IRRADIATION

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Chamomile is one of the most widely used herbs in human history. Its beneficial power and healing action were used by all ancient civilizations, and today they are widely used throughout the world. Its composition has been extensively researched, so today it is reliably known that one of the main active compounds in chamomile is apigenin. Although its amount in the chamomile flower itself is very low and limited, it is possible to increase the content of this important bioflavon through the process of enzymatic transformation, and further by selecting and optimizing the extraction technique it is possible to obtain extracts with a significantly increased content of apigenin. As part of this work, the extraction of autofermented chamomile was performed using microwave irradiation. Autofermentation of chamomile was carried out by the action of its own enzymes, primarily glucosidase, while the extraction was performed under previously optimized conditions. Valorization of functionality of obtained products includes: measuring its antioxidant, enzyme-inhibitor, antimicrobial and antiproliferative properties. In addition, presence of bioactive compounds was determined by applying HPLC-ESI-MS/MS analysis. The obtained results showed an extremely high level of biological activity, even significantly higher compared to standard chamomile extracts. Also the study demonstrates an advantage of microwave extraction for isolation of bioactive components that could find wide application in various branches of industry.

Keywords: Microwave irradiation, Autofermentation, Functional ingredients, Bioactive molecules, Antioxidants, Cytotoxic activity.

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia [number 451-03-68/2022-14/200134] as well as by Leadership Development Center Filip Moris within the project "Run for the Science".

ICAPP 2022
2nd International Conference
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VARIABILITY OF MICROCRYSTALLINE CELLULOSE HAUSNER RATIO AND COMPRESABILITY INDEX DEPENDING ON DETERMINATION METHODOLOGY

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Microcrystalline cellulose is a powder commonly used in pharmaceutical technology as filler in tablet and capsule formulations. Flowability is an important characteristic of powders, because it affects quality of final pharmaceutical dosage form in terms of adequate dosing and therefor therapeutic effect. Hausner ratio and Compressibility index are measures of powder compressibility used to express powder flow characteristics. The aim of this study was to determine effect of graduated cylinder volume and filling mass of Hausner ratio and Compressibility index. In this study 3 graduated cylinders of different volume were used: 10 ml, 100 ml and 250 ml. Each cylinder was firstly filled with microcrystalline cellulose up to1/3 of its capacity, then 1/2 and finally 2/3. Jolting volumeter was used to determine bulk and tapped volume of each powder mass in all of the 3 tested cylinders. Measurements were done after 10, 500, 900 and 1250 strikes. Compressibility index and the Hausner ratio were calculated based on the obtained results, according to 10th European Pharmacopoeia. Statistical tests (ANOVA) were performed with SPSS® program and the differences were considered statistically significant for $p<0.05$.

Both Hausner ratio and Compressibility index were shown to be statistically significantly different when measured in graduated cylinder of 250 ml between 1/3 filling and 1/2 as well as 1/3 and 2/3. In graduated cylinder of 100 ml, statistically significant difference was observed between 1/3 filling and 2/3, and 1/2 and 2/3. In graduated cylinder of 10 ml, there was no statistically significant difference among different amounts of cylinder filling. When filled up to 1/3 of volume capacity, no statistically significant difference of examined flowability parameters was observed between cylinders of 250 ml and 100 ml. For 1/2 and 2/3 of filled capacity, no statistically significant difference was observed between cylinders of 250 ml and 10 ml. Difference between pharmacopeial description of flowability was observed for all three tested cylinders and between tested fillings. Depending on methodology used, the tested powder flowability could be considered either Poor, Very poor or Very, very poor.

Hausner ratio and Compressibility index were shown to depend on determination methodology and therefor results obtained by different methodology can't be considered comparable. Although for the small volume cylinder no statistically significant difference of flowability parameters between filling amounts was determined, difference in pharmacopeial description is still observed.

Keywords: Flowability, Powder, Microcrystalline Cellulose, Flowability Parameters.

Acknowledgements: This study was supported by The Ministry of Education, Science and Technological Development, Republic of Serbia [number 451-03-68/2022-14/200114].

ICAPP 2022
2nd International Conference
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CHARACTERIZATION OF ESCIN-LOADED CHITOSAN/XANTHAN-BASED POLYELECTROLYTE COMPLEXES FOR pH-DRIVEN ORAL DRUG DELIVERY

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Escin is a vasoprotective drug with pH-dependent aqueous solubility, used in the form of gastro-resistant tablets due to its ability to irritate the gastric mucosa. Chitosan/xanthan-based polyelectrolyte complexes can be evaluated as carriers for pH-dependent release of escin since it is known that these complexes can enable pH-dependent release of both hydrophilic and hydrophobic drugs. Polyelectrolyte complexes can be subjected to various drying methods which can affect their characteristics as drug carriers. This study aimed to elucidate the effect of escin content and drying method on the rehydration ability of chitosan/xanthan-based polyelectrolyte complexes and their potential to ensure the pH-dependent drug release. Escin was added to achieve three different escin-to-polymers (chitosan and xanthan) mass ratios (1:1, 1:2, and 1:4) in final complexes which were then ambient-dried or spray-dried. The rehydration ability of dried complexes was checked by measuring the apparent viscosity (at 22.2 s^{-1}) of dispersions obtained after their swelling in solutions at pH 1.2 for 3 h and pH 7.4 for 9 h. Incomplete rehydration at pH 1.2 was observed for ambient-dried complexes at escin-to-polymers mass ratio of 1:1 and 1:2, and for spray-dried complex at the mass ratio of 1:1. On the contrary, at pH 7.4 these samples rehydrated completely and formed dispersions with apparent viscosities of 0.38±0.01 Pa·s, 0.85±0.31 Pa·s, and 1.09±0.11 Pa·s, respectively. Ambient-dried complex prepared at escin-to-polymers mass ratio of 1:4 rehydrated similarly at pH 1.2 and 7.4, with measured apparent viscosities of 1.33±0.10 Pa·s and 1.40±0.07 Pa·s. Spray-dried complexes prepared at escin-to-polymers mass ratios 1:2 and 1:4 rehydrated more completely at pH 1.2, so dispersions at pH 1.2 had higher apparent viscosities (1.20±0.11 Pa·s and 1.37±0.08 Pa·s) than at pH 7.4 (0.84±0.05 Pa·s and 1.13±0.28 Pa·s). These results were in accordance with *in vitro* escin release test. The ambient-dried complex prepared at escin-to-polymers mass ratio 1:1 had the best control and the aimed pH-dependent release of escin with a low amount of drug (4.30%) released at pH 1.2 and significantly higher (37.93%) at pH 7.4. It can be concluded that the rehydration ability and escin release from the investigated complexes are strongly influenced both by escin content and the applied drying method.

Keywords: Chitosan, Xanthan, Escin, Polyelectrolyte complex, pH-dependent drug release

Acknowledgements: This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia through Grant Agreement with University of Belgrade – Faculty of Pharmacy [number 451-03-68/2022-14/200161].

ICAPP 2022
2nd International Conference
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EFFECTS OF BLISTER PACKAGING ON THE VIABILITY OF ORAL PROBIOTIC STRAIN, *Lactobacillus plantarum* LP 299v

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Due to the increasing use of probiotics, scientific experiments related to the production, storage, and use of probiotic products have become more common. The stability of the product is determined by the choice of the probiotic strain, its production and packaging, of which the latter may be the most important in terms of probiotic viability.
This research describes the effects of blister packaging on viability of orally used probiotic strain *Lactobacillus plantarum* LP 299v (DSM 9483).
Strain used was in the form of lyophilized powder, encapsulated and packaged in blisters made of polyvinylidene chloride and aluminum foil. Two samples were analyzed, containing 2 blisters each. Two capsules were taken from each sample for all calculation time points. The probiotic cell count was determined using the agar plate method. After incubation, CFU (colony forming units) were counted and the average final number of *Lactobacillus* per capsule (CFU/capsule) was calculated at the beginning, after 24 months and after 27 months.
The results show continuous decrease of CFU for both samples over time: after 24 months, number of CFU has decreased to 73%, and after 27 months it has decreased to 58% of the initial value.
This research showed that the general packaging materials did not sufficiently protect the product against the external environmental conditions (oxygen, humidity, temperature), thus contributed to reducing the viability of the probiotic strains.
Future efforts should be oriented towards more reliable packaging materials in order to comply with consumer protection regulations.

Keywords: Lactobacillus plantarum 299v, Probiotic, Viability, Packaging

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LIPOSOMES WITH BIOCHANIN A – PREPARATION, CHARACTERIZATION AND *IN VITRO* DRUG RELEASE

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Biochanin A is a natural dietary isoflavone found in plants from the legume family (red clover, chickpeas) with numerous pharmacological and biological activities, such as anticancer, neuroprotective, hepatoprotective, antimicrobial, anti-inflammatory and antioxidant activity. The application of biochanin A in therapy is limited by its poor bioavailability which is a result of poor aqueous solubility, extensive metabolism and rapid excretion from the body. These disadvantages can be overcome by developing novel drug delivery systems with biochanin A. The aim of this work was to prepare and characterize liposome formulation with biochanin A, and to examine biochanin A release from liposomes of different size and lamellarity (multilamellar vesicles and small unilamellar vesicles). Liposomes are prepared by thin-film hydration method followed by extrusion. The characterization of prepared liposomes was performed by determining the vesicle size, polydispersity index and zeta potential on the Zetasizer Nano ZS90. The release of biochanin A from liposomes was examined *in vitro* using the dialysis bag technique. The content of biochanin A in samples was determined using the HPLC method. In order to examine the kinetics of biochanin A release from liposomes, the obtained experimental data were fitted applying appropriate mathematical models. The mean diameter of the prepared multilamellar liposomes with biochanin A was 960.7 ± 4.80 nm, and the polydispersity index was 0.644 ± 0.06. The mean diameter of small unilamellar liposomes with biochanin A was 148.1 ± 1.36 nm, with a very narrow droplet size distribution (polydispersity index was 0.096 ± 0.01), indicating greater stability of extruded liposomes. The zeta potential values were -19.2 ± 1.29 mV and -19.4 ± 1.86 mV for multilamellar and small unilamellar liposomes, respectively. The release of biochanin A from liposomes can be described by Korsmeyer-Peppas mathematical model with diffusion as dominant release mechanism, regardless of their size and lamellarity. Based on the all of the above it can be concluded that liposomes present promising carriers for controlled release of biochanin A.

Keywords: Liposomes, Biochanin A, Dialysis method, Release profile, Mathematical modeling

Acknowledgements: This work was supported by the Republic of Serbia – Ministry of Education, Science and Technological Development, Program for Financing Scientific Research Work [number 451-03-68/2022-14/200133].

2nd International Conference
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OPTIMIZATION OF DIFFERENTIAL PULSE VOLTAMMETRIC METHOD FOR DETERMINATION OF TETRACYCLINE IN REAL SAMPLES WITH rGO-ZnO/GCE

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In this work, a differential pulse voltammetric methodology is optimized using a glassy carbon electrode modified with reduced graphene oxide – ZnO nanocomposite for the determination of tetracycline antibiotic. A Britton-Robinson buffer pH 8, which is used as a supporting electrolyte, provided one intense oxidation peak at +0.98 V *vs*. Ag/AgCl (3.5 mol/L, KCl). The analytical performance of the presented methodology under the optimized voltammetric parameters provides a linearity range of 4-400 µmol/L, detection and quantification limit of 0.632 µmol/L, and 1.915 µmol/L, respectively. Selectivity study showed that the developed methodology does not suffer from the influence of most investigated inorganic ions and organic molecules. In addition, high precision with values of RSD less than 2.5% (n=5 for intra- and inter-day) is achieved. A practical significance of the developed DPV protocol is demonstrated by direct quantitative analysis of the spiked water and human urine sample, with the obtained recovery values near 100%.

Keywords: Tetracycline, Differential pulse voltammetry, Modified glassy carbon electrode, Water, Urine.

Acknowledgements: The authors are grateful to the Ministry of Education, Science and Technological development for the financial support of this work [number 451-03-68/2022-14/200134].

2nd International Conference
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CHEMICAL COMPOSITION AND ANTIPROLIFERATIVE ACTIVITY OF ANISE ESSENTIAL OIL

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Anise (*Pimpinella anisum*) an annual aromatic herb from Apiaceae family, is widely used as a domestic spice. Since ancient time, it is used as flavoring agent for many foods and beverages. Anise is responsible for much of the "liquorice" flavoring in bakery goods, liqueurs and teas.

Aniseeds contains 2-3 % essential oil. The prime constituent of anise oil is *trans*-anethole, consisting of about 90 percent of it and is also responsible for its characteristic aroma. The other constituents may be cis-anethole, anisaldehyde, methyl chavicol, γ-himachalene, zingiberene, and pseudoisoeugenyl 2-methylbutyrate. The chemical profile of anise oil, vary with variety, geographical region, ecological conditions, fruit maturity level and processing conditions. Thus, the aim of this study is determining the chemical profile and antiproliferative activity of anise essential oil from Serbia and the Russian Federation.

The health benefits of anise essential oil can be attributed to its antiepileptic, antirheumatic, antiseptic, antispasmodic, carminative, digestive, expectorant, sedative, stimulant, and insecticide properties. The antimicrobial activity, with great impact on bacteria, fungi, viruses and amoebas are also well recognized.

The cytotoxic activity of the anise essential oil, originated from Serbia and Russian Federation, was evaluated *in vitro* against three different humans cancer cell lines: the human cervix adenocarcinoma (HeLa) cells, the human lung adenocarcinoma (A549) cells, the human colon adenocarcinoma (LS-174 cells) and normal fibroblast (MRC-5) cells. Evaluation of cytotoxicity revealed that both Serbian and Russian juniper oil possess a significant cytotoxic potential against HeLa cells line. The MTT assay determined that cytotoxicity against LS-174 cells were moderate and low to the A549 cells.

Keywords: Anise essential oil, Chemical profile, Cytotoxic activity

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IN VITRO RELEASE OF BIOCHANIN A FROM ELECROSPUN (POLY)LACTIDE NANOFIBRES

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Biochanin A (5,7-dihydroxy-4'-methoxy-isoflavone) is a natural phytoestrogen. It has estrogenic and antiestrogenic as well as other pharmacological activity (antidiabetic, cardioprotective, cytotoxic and antimicrobial). Limited solubility of biochanin A in water and physiological fluids reduces resorption and bioavailability which affects the efficacy of this phytoestrogen. These shortcomings can be overcome by developing modern systems for the delivery of biochanin A. This work involves preparation of formulations of biochanin A with polylactide (2 % and 5 % in relation to the amount of polylactide), using the electrospinning method with certain process parameters (flow rate 2.5-3 cm^3/h, needle-to-collector distance 10 cm, voltage 13-14 kV). The release of biochanin A from electrospun polylactide nanofibers (9 mg) was monitored in Hanks 'buffer pH=7.4 (10 cm^3) at 37 °C using high performance liquid chromatography (HPLC). At certain time intervals, 200 µL of solution was sampled, supplemented with methanol up to 1 cm^3 and analyzed by HPLC. The amount of biochanin A released was increasing with time at a constant rate. Release rates were 0.351 µg/h and 0.429 µg/h for formulations with 2 % and 5 % biochanin A, respectively. The total release time of biochanin A was 23 and 42 days for formulations with 2 % and 5 % biochanin A, respectively. For the *in vitro* release of biochanin A from the prepared formulations, various mathematical models (zero order, first order, Korsmeyer-Peppas, Baker-Lonsdale, Higuchi and Makoid-Banakar) were applied using DD Solver, with the Makoid-Banakar model proving to be the best one (R^2_{adj}. 0.9912 and 0.9976; AIC 60.45 and 33.88; MSC 4.44 and 5.77, for formulations with 2 % and 5 %, respectively).

Keywords: Biochanin A, Polylactide, Electrospinning, Kinetic models, Drug delivery

Acknowledgements: The authors wish to thank Republic of Serbia—Ministry of Education, Science and Technological Development Program, for financing scientific research work [number 451-03-68/2022-14/200133, 451-03-68/2022-14/200134, 451-03-68/2022-14/200113] and Internal project No. 67 (Faculty of Medicine of the University of Niš).

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

OXIDATIVE STABILITY OF RED RASPBERY SEED OIL NANOEMULSIONS: INFLUENCE OF TEMPERATURE, OIL AND ANTIOXIDANTS

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Recently, the innovative carrier systems such as oil-in-water nanoemulsions, are being proposed for delivery of sensitive hydrophobic bioactives aiming to improve their stability and suitability for various applications. The goal of this study was to improve oxidative stability of red raspberry seed oil – RRSO (*Rubus idaeus*, Rosaceae), as a potential pharmaceutic/cosmetic ingredient derived from food industry byproducts, known as a rich source of polyunsaturated fatty acids, carotenoids and tocopherols. Firstly, nanoemulsions were prepared according to the low energy emulsion phase inversion method. Polysorbate 80 was used as an emulsifier, while the concentration of RRSO varied in the oil phase as well as the concentration of antioxidants: lipophilic (butylated hydroxytoluene – BHT or tocopheryl acetate – TA) or hydrophilic (ethylenediaminetetraacetic acid, disodium salt – Na_2EDTA or sessile oak acorn extract (*Quercus petraea*, Fagaceae). Nanoemulsions' physiochemical characterization included droplet size distribution analysis, electrical conductivity and pH value measurements, followed by measurements of primary and secondary oxidation products (lipid hydroperoxides – LH and thiobarbituric reactive substances – TBARS, respectively). Oxidative stability assessment of pure RRSO and RRSO with BHT included analysis of peroxide value – PV, p-anisidine value – PA, and TBARS, during one month of storage at 4, 25 or 40ºC. It was found that RRSOs' oxidative stability was significantly improved when stored at refrigerator after opening or by adding BHT (PV<10, PA<6, % INH_{TBARS} >80%). NEs were physically stable at all temperatures (with nanodroplets <150 nm), but their oxidative stability was highly compromised at 40ºC, or at higher RRSO concentration. However, the formation of undesirable oxidation products was successfully inhibited by employing different antioxidants, with the following order of efficacy: BHT> Na_2EDTA> oak extract, while TA did not exhibit antioxidant effect in the tested nanoemulsions.

Keywords: Nanoemulsion, Red raspberry seed oil, Antioxidant, Oxidative stability, Natural product

Acknowledgements: This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia [number 451-03-68/2022-14/200161].

ICAPP 2022
2nd International Conference
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COMPARATIVE ANALYSIS OF WILLOW LEAF EXTRACTS OBTAINED BY ULTRASOUND-ASSISTED EXTRACTION

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Willow bark extract (*Salix* sp., Salicaceae) has a well-documented medicinal use as analgesic, antipyretic and anti-inflammatory agent. In contrast, willow leaves are mainly considered as waste after bark collection. In recent years, interest in utilization of waste products from medicinal plants is increasing as they represent promising sources of bioactive components. Also, focus is put on environmentally friendly extraction techniques, using green solvents such as water. Ultrasound-assisted extraction (UAE) is a simple, inexpensive, eco-friendly technique which could be used effectively for extraction of phenolic compounds from plant material. Therefore, the aim of this study was to compare the chemical profiles of leaf extracts of six species of the genus *Salix* (*S. alba*, *S. amplexicaulis*, *S. babylonica*, *S. eleagnos*, *S. fragilis* and *S. triandra*) obtained by UAE.

UAE was carried out in ultrasonic bath at 25°C and 40 kHz for 30 min, using water as solvent. Determination of target compounds in the obtained extracts was performed by High Performance Liquid Chromatography methods.

In the analyzed leaf extracts salicin, chlorogenic acid, epicatechin, rutin, quercetin and naringenin were quantified. Salicin was not detected in *S. alba* leaf extract, while in the other samples it was the most abundant component along with rutin. Among the analyzed extracts, leaves of *S. amplexicaulis* and *S. eleagnos* were characterized by the highest amounts of these two compounds. Also, *S. amplexicaulis* leaf extract was the richest in chlorogenic acid and quercetin. Epicatechin was found in highest quantity in leaf of *S. fragilis*, while naringenin in *S. eleagnos*.

The obtained results indicate that leaf extracts of *Salix* species obtained by UAE contain significant amounts of bioactive compounds and could be further explored for pharmaceutical purposes.

Keywords: Salix, Leaf, Ultrasound-assisted extraction

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EFFECT OF HIGH AND LOW HUMIDITY ON THE RELEASE OF LAMOTRIGINE FROM IMMEDIATE RELEASE TABLET FORMULATIONS

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Storage of drugs in different climatic conditions, often without primary packaging, separately in dispensers for weekly or monthly therapy, causes exposure to increased or decreased humidity relative to standard humidity, which threatens the stability of the drug and its safety. The aim of this study was to examine whether there is a difference between the original formulation of lamotrigine tablets with immediate release (original formulation) and selected generic formulations (n = 3) in terms of tablet characteristics (disintegration, hardness, moisture content and weight variation) and lamotrigine release profile when exposed to increased (75% ± 5%) and decreased humidity (30% ± 5%) at room temperature (25°C ± 2°C). Four formulations of immediate release lamotrigine tablets were used. The Fa formulation is original, while the Fb, Fc and Fd formulations are generic formulations. Physical characteristics of LTG tablets were determined according to the European Pharmacopoeia. The dissolution profile comparison was carried out using model-independent (statistical) and model-dependent (kinetic) methods to provide detailed information about dissolution data. Dissolution tests were conducted using a validated UV/VIS spectrophotometric method. Dissolution characteristics of the tablet formulations were evaluated at three points spanning the physiological pH range (pH 1.2, pH 6.8). The Fc formulation showed the largest difference in composition, disintegration and wetting time, as well as in the drug release profile. All formulations had a reduction in hardness in high humidity conditions (75%). Exposure of the formulations to conditions of high and low humidity caused a change in the release profile of LMT and led to the said formulations not releasing more than 85% of the contents in the first 15 minutes, which is allowed by the pharmacopoeia. The effect of high humidity conditions is more pronounced on reduced LMT release compared to low humidity conditions. In pH 6.8 medium, the dissolution profile of the Fc formulation shows a statistically lower release / dissolution of LMT at all time points compared to the Fa, Fb and Fd formulations. The cause of the slower release of LMT from the Fc formulation compared to other tested formulations is probably the presence of calcium carbonate in the formulation. These results clearly indicate that storage of tablet formulations in conditions of high and low humidity not only affect the tablet characteristics of the formulation, but also the dissolution rate of LMT.

Keywords: Lamotrigine tablets, Humidity, Tablet characteristics, Dissolution

Acknowledgements: Project of Ministry Education, Science and Technology of Republic of Serbia [451-03-68/2022-14/200114].

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EFFECT OF BOROXINE DERIVATES $K_2[B_3O_3F_4OH]$ ON THE ACTIVITY OF AMYLASE *IN VITRO*

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Dipotassium trioxohydroxytetrafluorotriborate $K_2[B_3O_3F_4OH]$ is an enzyme inhibitor that can be used as therapy in patients with skin cancer. The reaction of amylase with starch as a substrate was examined, in the absence and in the presence of $K_2[B_3O_3F_4OH]$ by electrochemical method. Electrochemical tests were performed in the classical three-electrode system, using techniques cyclic voltammetry and chronoamperometry. Cyclic voltammetry technique was used to research the influence of different substrate concentration on the activity of the enzyme amylase trapped in a layer of Nafion, immobilized on the glassy carbon (GC) electrode. Using the Lineweaver-Burk diagram, the values of the Michaelis-Menten constants (K_m) and the maximum velocity (V_{max}) were determined without the presence of different concentrations of boroxin. Boroxin has been shown to act as a acompetitive amylase inhibitor. K_m and V_{max} values decrease because boroxin binds to the enzyme/substrate complex, and with this binding the cancer cells cannot continue to grow and divide.

Keywords: Boroxin, amylase, Enzyme kinetics, Electrochemical method

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SUPERCRITICAL CO_2 EXTRACTION AS A SELECTIVE TECHNIQUE FOR OBTAINING INDUSTRIAL HEMP EXTRACTS RICH IN CBD AND TERPENES

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Natural products are increasingly in demand in dermatology and cosmetology for reasons of sustainability and environmental protection. They are also a potential means of improving treatment outcomes for chronic skin diseases and skin care. Industrial hemp (*Cannabis sativa* L.) contains many medicinal properties that are slowly being discovered. Industrial hemp in its natural form, contain very small amounts of CBD, but therefore contain its acid form, which is CBDA. Once this acid form is activated by increasing the temperature, the desired effect and the corresponding decarboxylated analogue (CBD) is formed.

This study is a good example for obtaining highly valuable natural extracts of industrial hemp with green technique - supercritical CO_2 (sCO_2) extraction. Terpenes in the extracts were determined by gas chromatography and mass spectrometry (GC-MS) while high performance liquid chromatography (HPLC) was used to identify and quantify present cannabinoids.

GC-MS analysis indicated β-myrcene chemotype, with abundant α-pinene and β-pinene and variety of other monoterpenes as minor constituents, while the most abundant sesquiterpene was β-caryophyllene. Extracts rich in cannabidiol (CBD) as predominant compound were obtained at higher pressure with decarboxylation as pretreatment before sCO_2 extraction.

Keywords: Industrial hemp, Supercritical CO_2 extraction, Cannabinoids, Terpenes

Acknowledgements: We would like to thank Croatian Government and the European Union (European Regional Development Fund the Competitiveness and Cohesion Operational Programme—KK.01.1.1.02.0010) for funding this research through project TEHNOLOŠKO-INOVACIJSKI CENTAR VIROVITICA (KK.01.1.1.02.0010).

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COMPARISON OF LIQUID AND LYOPHILIZED SERPYLLI HERBA WASTE EXTRACTS PREPARED AT DIFFERENT pH VALUES

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Polyphenol recovery and physico-chemical properties of the extracts depend on the type of solvents and their pH values. Additionally, due to the presence of different biologically active compounds in plant waste, plant extracts obtained from industrial by-processing can find potential applications in various products. In the present study, Serpylli herba waste extracts were prepared using maceration (60 min), a solid-to-solvent ratio of 1:30, the particle size of plant waste 0.3 mm, and two types of the extraction medium: 50 % ethanol (pH 6) and 50% ethanol with glacial acetic acid (pH 2.5). The lyophilization process was chosen as the next step (-75°C, 0.011 mbar, for 24 h). Comparison of liquid and lyophilized extracts prepared at different pH values was done *via* analyzing total polyphenol content (TPC, Folin-Ciocalteu method), total flavonoid content (TFC, colorimetric assay), antioxidant capacity (ABTS and DPPH assays), zeta potential and conductivity (photon correlation spectroscopy). TPC of liquid extracts prepared at pH 2.5 and pH 6 amounted to 1.38 and 1.23 mg GAE/mL, respectively, while lyophilized parallels had 271.7 and 188.8 mg GAE/g, respectively. The same trend is noticed in the case of TFC: 0.368 and 0.334 mg CE/mL for liquid extracts obtained at pH 2.5 and pH 6, respectively, and 102.5 and 99.7 mg CE/mL for lyophilized parallels. ABTS radical scavenging activity of the liquid extracts at pH 2.5 and pH 6 was 0.767 and 0.750 mmol TE/L, respectively and for the lyophilized parallels was 0.136 and 0.111 mmol TE/g. IC_{50} (concentration for neutralization of 50% of DPPH free radicals) was 1.12 and 1.75 mg/mL for the liquid extracts prepared at pH 2.5 and pH 6, respectively and 0.331 and 0.191 mg/mL for the lyophilized parallels. The zeta potential of the liquid extracts at pH 2.5 and pH 6 was 1.74 and -2.56 mV, respectively, whereas the zeta potential of lyophilized parallels was -3.55 and -18.7 mV. Conductivity of the liquid extracts was 0.864 (pH 2.5) and 0.423 mS/cm (pH 6), whereas for the lyophilized extracts it was 0.199 (pH 2.5) and 0.452 mS/cm (pH 6). The presented results provide the information on physico-chemical properties of Serpylli herba waste liquid and lyophilized extracts that can add value and improve the quality of the existing food, functional food, pharmaceutical and cosmetic products, as well as for drinking water and wastewater treatment.

Keywords: Lyophilization, Polyphenols, Serpylli herba, Waste, Zeta potential.

Acknowledgments: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia [number 451-03-68/2022-14/200287].

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EFFECT OF DILUENTS ON PHARMACEUTICAL-TECHNOLOGICAL CHARACTERISTICS OF IMMMEDIATE RELEASE PARACETAMOL TABLETS

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Fillers are a very important group of excipients in tablets obtained by direct compression, as they make up the largest share of the mass of the tablet. Their role is to achieve the practical size of the pharmaceutical dosage form. In this study, the influence of the choice and share of fillers on the mechanical properties of paracetamol tablets and on the dissolution profile of paracetamol was examined.

Four formulations of paracetamol tablets (F1, F2, F3 and F4) were made using a laboratory excenter machine. Each of the formulations contained 7% paracetamol, 0.5% magnesium stearate (lubricant) and 4% sodium starch glycolate (disintegrant). Lactose and microcrystalline cellulose were used as fillers which accounted for 88.5% of the tablet weight. The share of lactose in F1 was 0%, in F2 17.7%, in F3 70.8% and in F4 88.5%, while the remaining share of fillers was microcrystalline cellulose. Methods for determining the bulk angle and bulk and tapping densities were used to test the flowability of the tableting mass. Examination of the dissolution profile was performed in accordance with the 10th European Pharmacopoeia, with paddle type apparatus and phosphate buffer, for 60 minutes. The concentration of paracetamol in the samples was determined via UV/Vis spectrophotometer. Friability, disintegration, resistance to crushing, uniformity of mass and uniformity of content were also tested in accordance with the 10th European Pharmacopoeia.

Examination of dissolution profiles showed that all 4 formulations meet the requirements of the 10th European Pharmacopoeia for immediate release tablets. The difference in release rate was observed only in the first 5 min: F1 released the most (91.95%) and F4 the least (71.76%). These results were consistent with hardness and disintegration tests - formulations with a higher lactose content showed greater hardness and longer disintegration times. Mass variation testing showed that none of the 4 formulations met the requirements of the 10th European Pharmacopoeia, which is related to the poor flowability of the tested tableting masses. The best flowability was observed in the tabulation mass for F3.

The optimal pharmaceutical-technological characteristics were obtained in formulation with a mixture of lactose and microcrystalline cellulose as fillers, where the share of lactose is higher. In order to improve the flowability of the mixture, the tableting mass can be converted into granules, or a silica-type agent can be used.

Keywords: Lactose, Microcrystalline Cellulose, Excipients, Dissolution Profile.

Acknowledgements: This study was supported by The Ministry of Education, Science and Technological Development, Republic of Serbia [number 451-03-68/2022-14/200114].

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ASSOCIATION OF ANISOTROPIC LIPOPHILICITY OF 1-ARYL-3-METHYLSUCCINIMIDES OBTAINED BY RP-HPLC WITH IN SILICO PERMEABILITY PREDICTORS

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Introduction: Certain properties of the whole molecule represent important molecular descriptors in the modeling of complex pharmacokinetic properties. Lipophilicity is a physico-chemical characteristic of a compound that directly affects the biological activity of a chemical entity. Material and methods: Thirteen newly synthesized 1-aryl-3-methylsuccinimides were analysed by reverse phase high performance liquid chromatography (RP-HPLC) for determining their lipophilicity. Chromatographic measurements were conducted on Agilent 1100 apparatus, with DAD detector and binary pump on C18 column (Hypersil ODS, 100mm×2.1; 5 µm). Binary mixture of water and methanol (HPLC grade, JTBaker) with the volume ratio varied from 45:55 to 65:35 was used as the mobile phase. The flow of the mobile phase was 0.8 ml/min with constant temperature at 25 °C. The compounds were detected on λ=205 nm.The online tool pkCSM was applied for determining Caco-2 permeability, logPapp (10^{-6} cm/s), intestinal absorption, IA (%), logarithm of blood-brain permeability, logBBB and logarithm of skin permability, logKp for all analyzed compounds.
Results: Anisotropic lipophilicity expressed as logKw was determined for twelve compunds based on the retention times obtained by RP-HPLC with the variation of the mobile phase. The compund with carboxylic group was completely ionised and thus was elauted with the mobile phase as pre-pick. The association of the logKw values with logPapp (r^2=0.381, p=0.019), logBBB (r^2=0.488, p=0.007) and logKp (r^2=0.536, p=0.004) respectively were linear suggesting that the increment of lipophilicity of the observed compounds was followed by the enhanced permeability through the lipophilic obstacles such as the intestinal membranes, the blood-brain barrier and the skin. The correlation between logKw and the IA was described with a parabolic function in a statistically significant manner (r^2=0.439, p=0.030). Conclusion: Anoistropic lipohilicity of the analyzed 1-aryl-3-methylsuccinimides reflects their predicted permeability through different physiological barriers.

Keywords: Lipophilicity, Permeability, Chromatography

Acknowledgements: The present research is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia [number 451-03-68/2022-14/200114].

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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF CHAGA MUSHROOM EXTRACT GROWING IN SOUTHEASTERN SERBIA

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Chaga (*Inonotus obliquus*) mushroom belongs to the group of medicinal mushrooms – polypores. It is widespread in Russia, China, and Japan. The inner part of the body of the chaga mushroom is used for medical purposes. It contains melanin, flavonoids, beta-glucans, betulin/betulinic acid, pyrocatechol derivatives, steroidal substances (ergosterol), tetracyclic triterpenes (lanosterol and inotodiol), pterins, lignin, polysaccharides, organic acids, as well as a complex of micro- and macroelements. It exhibits antioxidant, adaptogenic, radioprotective, antitoxic, immunomodulatory, hypoglycemic, spasmolytic, diuretic, bactericidal, anti-inflammatory, and anticancer activities. In this study, a hydroalcoholic extract of chaga mushroom from Southeastern Serbia was preliminarily analyzed. The ultrasound-assisted extraction of bioactive compounds was used to prepare 60% (v/v) ethanolic extract. The total antioxidant content of 4.337 g gallic acid equivalent per 100 g of dry weight and total flavonoid content of 0.055 g rutin equivalent per 100 g of dry weight were determined for the extract according to the *Folin-Ciocalteau* method and aluminium chloride colorimetric method, respectively. The antioxidant activity of the extract and BHT as a positive control were determined using the 2,2-diphenyl-1-picrylhydrazyl assay and expressed as a half-maximal inhibitory concentration of 0.296 mg/mL and 36.6 μg/mL. The obtained results indicated the extract had a lower antioxidant activity compared to the BHT standard. Despite this fact, the chaga mushroom is rich in antioxidants so it can be used as a product with added value in the food or pharmaceutical industry. Further studies will be focused on the optimization of these bioactive compounds from the analyzed sample.

Keywords: Chaga, Extract, Antioxidants, Flavonoids, Antioxidant Activity.

Acknowledgments: Siniša Mladenović is a recipient of a Ph.D. scholarship from the Republic of Serbia Ministry of Education, Science and Technological Development. The Republic of Serbia - Ministry of Education, Science and Technological Development, Program for financing scientific research work, number [451-03-68/2022-14/ 200133]. Siniša Mladenović is the recipient of the scholarship from the Ministry of Education, Science and Technological Development.

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POLYPHENOLS PROFILE OF WILD THYME EXTRACTS OBTAINED BY CONVENTIONAL SOLID-LIQUID AND ULTRASOUND-ASSISTED EXTRACTION

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Thymus serpyllum extracts, potentially enriched with a wide spectrum of polyphenols, were subjected to LC-MS-MS analysis in order to determine their qualitative polyphenols profile. Analyses were conducted in the sample with the highest polyphenols yield and antioxidant activity obtained by conventional extraction using 60% of ethanol for 24 h at shaking speed of 150 rpm (Sample CE), sample obtained by ultrasound-assisted extraction at 65 °C for 55 min using 60% ethanol (Sample UAE-CP) and sample obtained at the temperature of 70.28 °C, extraction time of 70 min and using 45% ethanol as a solvent (Sample UAE-OPT). According to results of identification, the Sample UAE-CP contained the highest number of compounds, 31, a total of 30 different compounds were identified in the Sample CE and 27 compounds in the Sample UAE-OPT. Identified flavonoid subgroups were flavanones, flavan-3-ols, flavonols, flavones and isoflavones. Some of them were identified in aglycone form only. On the other side, flavanones were identified not only in aglycone form, but also in a form of their 7-*O*-glucoside, e.g. naringenin-7-*O*-glucoside. Finally, flavonols were identified in various forms, as compounds in aglycone form, then in form of their 3-*O*-glycosides with rutinose, glucose and galactose as the carbohydrate compounds, but in form of glucuronide and hexoside isomer as well. Identified phenolic acids were gallic, vanillic, ellagic, caffeic, 3-*p*-coumaroylquinic, 4-*p*-coumaroylquinic acid, coumaric acid hexoside isomer, *p*-coumaric, *cis*- and *trans*-coutaric acid. Taking into account the discrepancy in the polyphenols profiles of aforementioned extracts, it could be concluded that different extraction techniques under different extraction conditions or using different extraction solvent could be of a great importance for establishing the adequate polyphenols profile of *T. serpyllum* extracts.

Keywords: Thymus serpyllum, Herbal dust, By-product, Antioxidants.

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.

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QUALITY CONTROL OF ANISE AND FENNEL FRUITS IN THE CITY OF NOVI SAD

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Anise (*Pimpinella anisum* L.) and fennel (*Foeniculum vulgare* Mill.) are aromatic plants from the Apiaceae family. The fruits of these plants are widely used in folk medicine, pharmaceutical industry, cosmetics, as natural preservatives and spices.
The aim of this study was to examine the quality of anise and fennel fruits. Herbal material was purchased on the territory of Novi Sad. Samples were first identified by macroscopic (organoleptic) and microscopic analysis. Percentage of foreign matter, loss on drying and total ash were determined in accordance with procedures described in European Pharmacopoeia and measured gravimetrically. Isolation of essential oil was done by the hydrodestilation process on the Clevenger apparatus. The chemical composition of essential oils were analysed by gas chromatography-mass spectrometry with static headspace sampling. Macroscopic and microscopic analysis determined that some samples were counterfeits, and some samples were covered with molds. Also, examination of foreign matter showed that some samples contain forbidden impurities. The results obtained by determining the loss on drying showed that all samples have allowed water content. The results of total ash demonstrated that some samples contain a higher percentage of ash than permitted, indicating an increase in mineral matter. The yields of essential oils in all samples of examined anise and fennel fruits were within the prescribed limits. *Trans*-anetol was identified as dominant compound. Based on the examinations and results obtained, it can be concluded that it is necessary to have continuous quality control of all phases of preparation of plant material (i.e. anise and fennel fruits) for consumption in order to provide their satisfying quality, efficacy and safety on the market.

Keywords: Pimpinella anisum, Foeniculum vulgare, Spices, Quality Control.

Acknowledgements: Project 451-03-68/2022-14/200114.

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PHYSICAL AND BIOPHARMACEUTICAL PROPERTIES OF QUERCETIN EMULGELS FOR DERMAL DRUG DELIVERY

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Natural flavonoid quercetin is widely present in fruits and vegetables. It is a lipophilic compound that exerts various pharmacological effects such as anticancer, antiinflammatory, antioxidation, antianemic, antiplatelet, hypolipidemic and cardioprotective action. Emulgels (emulsion gels) are semisolid dosage forms which consist of a mixture of emulsion and gel, used for dermal delivery of lipophilic drug substances such as quercetin. Nowadays emulsion gel formulations are commonly used because they are easy to use, which enhances patient compliance. Physical characteristics of emulgels depend on proper selection of oil phase, emulsifier and gelling agent. The objective was to formulate a quercetin emulsion gel for topical application with satisfactory physical properties and to examine the influence of gelling agent, oil phase and emulsifier on the emulsion gel characteristics. Eight emulsion gel formulations containing 0.5% quercetin were prepared with concentrations of carbomer 940 as a gelling agent of 0.25% and 0.75%, concentrations of castor oil as an oil phase of 10% and 20%, and concentrations of Tween® 20/Span® 20 mixture (1:2) as an emulsifier of 1,5% and 3%. Emulgels were evaluated for their physical appearance, type of emulsion, pH, spreadability, occlusive properties, quercetin content and its release from formulations. The prepared emulgels had soft creamy consistency, homogenous texture, shiny appearance and favorable tactile properties. Quercetin emulsion gels contained O/W type of emulsion with variable homogeneity and consistency, but all having adequate pH values (6.3-6.5) and quercetin contents (0.50-0.52%). The proportion of gelling agent was the predominant factor in determining the spreadability and the release of quercetin from the formulation. Emulgels exerted mild to moderate occlusive effect and the concentration of oil phase had the greatest impact on occlusivity, although the other components contributed as well. In conclusion, the formulation composition significantly affects the physical characteristics of quercetin emulsion gels. Emulgel with 0.75% gelling agent, 10% oil phase and 1.5% emulsifier can be considered as the optimal quercetin formulation for dermal delivery.

Keywords: Emulsion gel, Drug release, Quercetin, Occlusion, Drug content.

Acknowledgements: This research was funded by the Provincial Secretariat for Higher Education and Science, Autonomous Province of Vojvodina [project No. 142-451-2532/2021-01].

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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF *O. VULGARE* VAR. *VULGARE* AND VAR. *HERACLEOTICUM*

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Oregano (*Origanum vulgare*) is an aromatic plant from the Lamiaceae family. Due to its rich content of polyphenols, flavonoids and other important secondary metabolites, oregano have anti-inflammatory, antioxidant, and antimicrobial properties. This research was aimed to compare the chemical composition of *O. vulgare* var. *vulgare* and var. *heracleoticum* and their antioxidant activity. Investigated extracts were prepared by ethanol maceration. The total phenolic content was determined by the Folin-Ciocalteu method. The total amount of flavonoids was determined spectrophotometrically by analysing flavonoid complex formation with $AlCl_3$. Chemical characterization was performed by high-performance liquid chromatography. The ability of the tested extract to neutralize 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl (OH•) and nitroso (NO•) radicals was tested by the spectrophotometric methods. The total phenolic content was approximately equal in both, *O. vulgare* var. *vulgare* and var. *heracleoticum* (87.53 and 90.5 mg of the gallic acid equivalents/g dry extract, respectively). Also, the total amount of flavonoids was approximately equal in both, *O. vulgare* var. *vulgare* and var. *heracleoticum* (21.8 and 23.23 mg of the quercetin equivalents/g dry extract, respectively). Rosmarinic, ferulic and chlorogenic acids, and rutin were identified as dominant polyphenolic compounds. Moreover, antioxidant activity of both extracts was similar. Half-maximal inhibitory concentration (IC_{50}) for DPPH was 2.28 mg/mL for *O. vulgare* var. *vulgare* and 2.59 mg/mL for *O. vulgare* var. *heracleoticum.* The ability of the tested extract to neutralize NO• was similarly good for both extracts (IC_{50}= 17.71 and 20.53 mg/mL). The tested extracts showed weaker activity in neutralizing OH• (IC_{50}= 285.37 and 192.24 mg/mL). Due to the similarity in chemical composition and antioxidant activity both varieties can be used as medicinal herbal drugs.

Keywords: O. vulgare var. *Vulgare, O. vulgare* var. *Heracleoticum, Chemical Composition, Antioxidant Activity.*

Acknowledgements: The Ministry of Education, Science and Technological Development, Republic of Serbia [Grant Number 451-03-68/2022-14/200114].

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INTERACTIONS IN ESCIN-LOADED CHITOSAN/XANTHAN-BASED POLYELECTROLYTE COMPLEXES: EVALUATION OF DRUG CONTENT AND DRYING METHOD IMPACT

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Escin is an amphiphilic drug with weakly acidic properties, a saponoside with aglycone and glycone parts in the structure. It has high and pH-dependent solubility in water. These characteristics may affect its ability to form complexes with polymers such as chitosan and xanthan gum which also contain both hydrophobic and hydrophilic domains, by establishing electrostatic, hydrogen, and hydrophobic interactions. Also, the drying method can affect the strength and extent of these interactions. This study aimed to investigate the influence of escin content and drying method on the formation and interaction strength and extent in chitosan/xanthan gum-based polyelectrolyte complexes. Escin was added to achieve three different escin-to-polymers mass ratios (1:1, 1:2, and 1:4) in final complexes. The formation of complexes was monitored by measuring the transmittance which decreased continuously from 99.60±2.88% for chitosan solution to very low values: 0.12±0.01% for the escin-to-polymers mass ratio of 1:1, 0.14±0.00% for the ratio of 1:2, and 0.11±0.03% for the ratio of 1:4. This decrease indicated the formation of complexes by establishing interactions between polymers and escin with minor differences between the samples prepared at varying escin contents. Then, the prepared complexes were dried under ambient conditions or spray-dried. Spray-dried complexes were subjected to rotor-stator homogenization before drying. The strength and extent of interactions between chitosan, xanthan gum, and escin in dried samples were evaluated by Fourier-transform infrared spectroscopy. Spectra of dried complexes confirmed the establishment of interactions. The higher intensity of peaks in spray-dried complexes was a consequence of weaker intermolecular interactions, probably due to their weakening during the homogenization and the spray drying process itself. The obtained results indicate that the drying method had a more significant effect on the interaction strength and extent between the drug and polymers in complexes in comparison to escin content, which may affect drug release properties from such carriers.

Keywords: Chitosan, Xanthan, Escin, Polyelectrolyte Complex, Drug-Polymer Interactions.

Acknowledgements: This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia through Grant Agreement with University of Belgrade – Faculty of Pharmacy [number 451-03-68/2022-14/200161].

2nd International Conference
on Advanced Production and Processing

FABRICATION OF INHALABLE NANOPARTICLES USING ANTIINFLAMMATORY DRUGS

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Modern pharmaceutical technology is focused on formulations that are targeted to the exact site at the appropriate time, with maximum efficiency and with reduced side effects. Nanoparticle engineering has been developed and reported for pharmaceutical applications. In this approach, poorly water-soluble compounds are formulated as nanometer-sized (< 1000 nm) drug particles. Nanoparticulate technology offers increased bioavailability, improved absorption, and the potential for drug targeting. The main question of our work is, how can we use and apply the prepared nanosized systems (as predispersions) in drug formulation (to reach local or systemic effect) to get effective therapies for different diseases. Therefore, we should find cost-effective production by new technological processes containing the most important technological and material parameters. In this work, different methods were used to decrease particle size into the micro- or the nanosize range. They can be divided into two main categories: bottom-up and top-down techniques. These two categories are not separated sharply, because the combination of the top-down and the bottom-up technique is necessary to control the particle size. Basically, we focused on the following methods: research and development of dry/wet milling; ultrasonic-assisted nanoprecipitation and preparation of nanocarriers by nano spray drying. We developed inhalable dry powders for nasal and pulmonary application. The applied model drugs were nonsteroidal anti-inflammatory agents such as meloxicam and ibuprofen. Characterization of micrometric and physicochemical properties, structure, compatibility, stability, in vitro, ex vivo and in silico properties were part of the scientific evaluations.The innovative formulations could play a significant role in modern therapies.

Keywords: Nanoparticle Engineering, Nasal and Pulmonary Application, Milling, Sonication, Nano Spray Drying.

Acknowledgements: This work was supported by the Ministry of Human Capacities, Hungary grant TKP2021-EGA-32 and 2019-2.1.11-TÉT-2020-00147 Hungarian-Slovenian Bilateral project [2022-2024].

ICAPP 2022
2nd International Conference
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CHARACTERIZATION OF GRAPE POMACE POLYPHENOLIC EXTRACTS OBTAINED BY AQUEOUS SOLUTIONS OF NON-IONIC SURFACTANTS

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The application of natural polyphenolic extracts in cosmetics requires the use of green technologies and non-toxic extractants. Aqueous surfactant solutions are considered to be one of the most promising green extraction mediums because water is used as the main solvent. Moreover, the surfactants used for extraction remain as an integral component in the final extract, allowing it to be directly integrated into an appropriate cosmetic product (e.g., micellar solutions or emulsions). In addition, wine-making residues represent a cost-effective and viable source of polyphenols. Therefore, this study aimed to investigate the potential of selected non-ionic surfactants for simple green extraction of polyphenols from red grape pomace by quantifying the individual phenolic compounds. Aqueous solutions of Brij S20 and poloxamer 407 (0.5%, 1%, 2% and 3% w/v) and controls (water, ethanol and water:ethanol (1:1) mixture) were used for the extraction of polyphenols from lyophilized red grape pomace (*Cabernet Franc* variety). The HPLC method was used to characterize the phenolic compounds. As surfactant concentration increased, the solubility of the phenolic compounds generally increased, as more micelles are formed at higher concentrations. Compared to water, both 3% surfactant solutions studied extracted higher levels of all identified phenolic compounds, although most non-polar polyphenols (e.g., quercetin, kaempferol, naringenin, resveratrol) were not detected in the water extract, indicating a solid affinity of the surfactant aggregates studied for both polar and non-polar compounds. Both surfactants extracted gallic acid, the most polar phenolic compound, with the same efficiency. On the other hand, less polar polyphenols showed higher extraction affinity to Brij S20 than to poloxamer 407, suggesting that the polarity of micellar interior as well as the steric rigidity of the surfactants determine their solubilization capacity for such compounds. These results suggest that the fine selection of surfactant type could lead to the recovery of extracts with a favorable phenolic profile. Moreover, it could be concluded that the use of aqueous solutions of non-ionic surfactants offers a great opportunity for the development of a suitable cosmetic product.

Keywords: Grape Pomace, Non-Ionic Surfactants, Green Extraction.

2nd International Conference
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ANTIMICROBIAL ACTIVITY OF SUPERCRITICAL EXTRACTS FROM WILD THYME (*THYMUS SERPYLLUM* L.) BY-PRODUCTS

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Antimicrobial resistance to antibiotics is growing, affecting increased burden of the health system and higher incidence of human fatal outcomes. Therefore, the research of novel antimicrobial substances is of crucial importance. Wild thyme (*Thymus serpyllum* L.) extracts were obtained using different conditions of green and environmentally-friendly supercritical fluid extraction. Sample SFE-TSŽ2 was prepared at 350 bar and 50 °C, while SFE-TSŽ7 was extracted at 100 bar and 40 °C. Broth microdilution method was applied to determine how different extraction conditions reflect on sample antimicrobial activity against pathogenic microorganisms. Two Gram positive strains, *Staphylococcus aureus* ATCC 25923 and methicillin-resistant *Staphylococcus aureus* (MRSA), stood out as the most sensitive to both samples (minimal inhibitory concentration, MIC value below 0.02 mg/mL). Bactericidal effect of the sample SFE-TSŽ2 was found for all Gram-positive strains, with minimal bactericidal concentrations (MBC) lower than for SFE-TSŽ7, except for *Staphylococcus aureus* (MRSA) when the same effect of both extracts was found. Significant difference between samples was observed in inhibitory activity against Gram-negative bacterial strains, revealing higher efficiency of SFE-TSŽ2 compared to SFE-TSŽ7. The most sensitive to both samples were *Proteus hauseri* ATCC 13315 and *Yersinia enterocolitica* ATCC 27729 with the same MIC values (0.83 mg/mL for SFE-TSŽ2 and 2.5 mg/mL for SFE-TSŽ7). SFE-TSŽ2 also expressed inhibitory and fungicidal effects against *Candida albicans* ATCC 1231 yeast (MIC 2.5 mg/mL; minimal fungicidal concentration, MFC, 10 mg/mL), while SFE-TSŽ7 showed no activity. Obtained results showed that applied extraction conditions affected antimicrobial activities, most probably due to the differences in chemical compositions.

Keywords: Antimicrobial Activity, Supercritical Fluid Extraction, Thymus Serpyllum.

Acknowledgements: This research was supported by the Science Fund of the Republic of Serbia [grant number 7750168] Novel extracts and bioactive compounds from under-utilized resources for high-value applications-Bio-Utilize and by the Ministry of Education, Science and Technological Development of the Republic of Serbia [contract number 451-03-68/2022-14/200116].

2nd International Conference
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ANTIOXIDANT ACTIVITY OF BLACK SEED (*NIGELLA SATIVA* L.) FATTY OIL OBTAINED BY DIFFERENT ISOLATION METHODS

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Black seed (*Nigella sativa* L.) is one of the cultivated species in various regions of Iran and is widely used in traditional medicine as a natural remedy. Different studies of black seed have shown a wide spectrum of activities such as antioxidant, antimicrobial, antitumor and anti-inflammatory. Since some of these activities are mainly attributed to volatile and fatty oils, the aim of this study was to examine the antioxidant activity of commercial *N. sativa* fatty oil - Natural Black Seed Oil (NSFO) obtained by cold pressing (Health Shop Macedonia) and NSFO obtained by Soxhlet extraction with n-hexane. The fatty oil of *N. sativa* seeds obtained by Soxhlet extraction was isolated in high yield (20.8%). The efficacy of the NSFOs to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was evaluated using a spectrophotometry method. The NSFO obtained by cold pressing showed better antioxidant activity after 20 minutes of incubation, with an EC_{50} value of 3.56 mg/cm^3, compared to the NSFO obtained by Soxhlet extraction, with EC_{50} value of 17.29 mg/cm^3. Despite the fact that NSFOs showed lower antioxidant activity in comparison to the synthetic antioxidant BHT (EC_{50} value was 0.045 mg/cm^3) and phenolic compounds such as gallic acid (EC_{50} value was 0.004 mg/cm^3), these oils can be a good alternative to synthetic additives with fewer harmful effects. Future research should focus on investigating the chemical composition of the obtained fatty oils and their mechanism of antioxidant action.

Keywords: Black Seed, Nigella Sativa L., Fatty Oil, Antioxidant Activity.

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia under the Program of financing scientific research work [number 451-03-68/2022-14/200133]. Aleksandra Milenković is Scholar of the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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COMPARISON OF DIETARY HABITS BETWEEN DIFFERENT FACULTIES IN NOVI SAD

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Unhealthy eating habits in adolescents are manifested by skipping meals, using various diets, eating meals outside the home, and frequent consumption of fast food, sweets, and sweetened beverages. Understanding undesirable dietary patterns in adolescents is one of the measures to prevent many diseases. The aim of this study was to compare the differences in dietary habits of students of medical and non-medical faculties at universities in Novi Sad. The study was conducted in May 2018 and included 514 students aged 19-24 years (133 males and 381 females) from 4 faculties (Faculty of Medicine, Faculty of Pharmacy, Faculty of Sciences and Faculty of Technology) whose study programmes include nutrition topics. The original anonymous questionnaire was used. The questionnaire was designed specifically for this study and contained 26 questions with the offered answers and the possibility to answer some questions. The questions were related to gender, age, year of study, type of study, height and body mass, then questions about general eating and lifestyle habits. Statistical analysis was performed in SPSS20. The results showed that 50% of the students eat 3 meals and only 10% eat all 5 meals a day. A large percentage (12%) of the students in the Faculty of Technology eat constantly during the day compared to the other faculties (4-5%). Half of the students eat breakfast every day, while slightly less than 5% never eat breakfast. Regarding the type of food, only 5% of participants consumed vegetables and only 6% of them consumed fruit more than once a day, with statistically significant differences between faculties ($p<0.01$). At the same time, 92% of participants consumed meat a few times per week and 77% of participants consumed fish at least once per week. A daily water consumption of more than 2 liters was reported by only one third of the students (differences between faculties were statistically significant $p<0.05$), although 85% of the participants indicated that water was their beverage of choice. The results suggest thet there are differences in the dietary habits of students from different faculties. Study programmes that include nutrition topics should focus on increasing the acceptance of proper nutrition habits among their students.

Keywords: Students, Dietary Habits, Novi Sad.

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SOLVENT RESIDUES IN NIFEDIPINE SOLID DISPERSION OBTAINED BY KNEADING METHOD

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Solid dispersions represent one of the most applicable approaches in increasing the solubility of poorly soluble active pharmaceutical ingredients. The kneading method is a simple method suitable in the initial stages of testing and belongs to the group of solvent-based methods for obtaining solid dispersions. Accompanied by other methods from this group, the kneading method can be limiting due to solvent residues.The aim of this study was to quantify the solvent residues in nifedipine solid dispersions with three different polymers (polyvinylpyrrolidone K 30, sodium alginate and polyethylene glycol 4000) in three different ratios, obtained using diluted ethanol by the kneading method. Physical mixtures of nifedipine and the corresponding polymers in proportions of 1:1, 1:3 and 1:5 were obtained using a powder mixer (Farmalabor, Italy). Diluted ethanol (3 ml per 1 g of physical mixture) was used as a kneading medium. The obtained pastes were dried for 24 h in an oven (Memmert, Germany) and 48 h in a desiccator, pulverized, sieved through sieve 355 and stored in a glass containers protected from light before further analyses. The ethanol content in nifedipine solid dispersions was determined on a gas chromatograph coupled to a mass spectrometer and headspace sampler (Agilent Technologies, Germany). Ethanol content above 0.5% was detected in solid dispersions with polyvinylpyrrolidone K 30 (0.75%, 1.40% and 1.47% in ratios 1:1, 1:3 and 1:5 respectively). In solid dispersions with sodium alginate, the content was much lower (about 0.003%), while in solid dispersions with polyethylene glycol 4000, the content was below the limit of detection. The content of ethanol residues in examined nifedipine solid dispersions depends on the used polymer and its proportion. Solvent residues can be a critical parameter when choosing the optimal solid dispersion formulation, especially in cases where solvents of higher toxic potential (Classes 1 or 2) would be used.

Keywords: Ethanol, Ethyl Alchocol, Solvent-Based Solid Dispersion.

Acknowledgements: The study was supported by The Ministry of Education, Science and Technological Development, Republic of Serbia [grant 451-03-68/2022-14/200114].

ICAPP 2022
2nd International Conference
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THE RELEASE OF CURCUMIN FROM THERMO-SENSITIVE COPOLYMER

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Curcumin is a natural polyphenol which shows significant pharmacological effects *in vitro* and *in vivo*. The main barrier to the effective showment of the pharmacological activity of curcumin is its poor water solubility and low bioavailability. In order to eliminate these disadvantages, a matrix system based on a polymeric carrier poly(*N*-isopropylmethacrylamide-*co*-*N*-isopropylacrylamide), p(NIPMAM-NIPAM), with an incorporated inclusion complex of curcumin:2-hydroxypropyl-β-cyclodextrin was developed. The matrix system was obtained by swelling the p(NIPMAM-NIPAM) hydrogel to equilibrium in an ethanolic solution of the curcumin:2-hydroxypropyl-β-cyclodextrin inclusion complex prepared by coprecipitation method in a molar ratio 1:1. The release of curcumin from the matrix system was observed for 48 h under *in vitro* conditions at 37 °C and pH=7.4 by using the HPLC method. The concentration of released curcumin was calculated from the rectilinear part of the calibration curve of the dependence of the peak area on the concentration of curcumin (0.53-106 μg/cm^3). In order to determine the mechanism and kinetics of curcumin release, various mathematical models were applied using the DDSolver package for the Microsoft Excel application. An *in vitro* study about the release of curcumin in solution (10 cm^3) pH=7.4 at 37 °C showed that 2286.4 μg (47.51%) of curcumin was released in 48 h from the swollen gel formed from 50 mg of p(NIPMAM-NIPAM) xerogel. The rate of curcumin release from the matrix system p(NIPMAM-NIPAM) is 262.54 μg/g·h, which provides the possibility of prolonged release of curcumin for 366.5 h. The highest coefficient of determination (R^2) and the lowest AIC (*Akaike Information Criterion*) indicate that the Korsmeyer-Peppas model best describes the release of curcumin from matrix systems, while the diffusion exponent of 0.074 indicates that the mechanism of curcumin release is based on diffusion from polymer matrix. The obtained results show that copolymer p(NIPMAM-NIPAM) is suitable as a carrier for modified release of curcumin in the environment that simulates conditions in the lower part of the gastrointestinal tract.

Keywords: Curcumin, Inclusion Complex, Hydrogel, 2-Hydroxypropyl-B-Cyclodextrin, Korsmeyer-Peppas Model.

Acknowledgements: Republic of Serbia-Ministry of Education, Science and Technological Development, Program for financing scientific research work [number 451-03-68/2022-14/200133].

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

AN INVESTIGATION OF THE INTERACTION OF GELATIN AND CETYLTRIMETHYLAMMONIUM- BROMIDE IN AQUEOUS SOLUTIONS

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Modern formulations of food, cosmetic and pharmaceutical products often include various substances, some of which have a biological effect, while others enable better bioavailability of the active substance or serve as consistency correctors. Polymers of natural or semi-synthetic origin are almost indispensable constituents of most formulations, and they are often present in combination with surfactants. Polymer-surfactant mixtures can form complexes of different properties and lead to improved properties of the final product (increased viscosity, greater stability, better bioavailability of biologically active substances), but sometimes may to compromise the stability of the system, (particle aggregation, turbidity or phase separation), i.e. to product instability. Different polymer-surfactant systems are often used in the processes of microencapsulation, formation of gels, films and coatings. For these reasons, it is necessary to examine in detail their interactions, which enables achievement of desired effects in the final product. In this paper the mechanism of interaction between gelatin and oppositely charged cationic surfactant, cetyltrimethylammonium bromide (CTAB) was defined. The adsorption properties and the charge of the pure compounds and gelatin-CTAB complexes in aqueous solution were determined using tensiometry and by zeta potential measurement. Depending on the CTAB concentration, electrostatic bonding of gelatin and CTAB molecules is possible (dominates at lower CTAB concentrations), as well as their hydrophobic bonding (characteristic for higher concentrations). In the widest concentration interval, both mechanisms take place in parallel (from 0,001% CTAB to 0,005% CTAB). The possibility of application a gelatin-CTAB complex to stabilize oil-in-water emulsion systems was also examined. It was found that gelatin-CTAB complex of the mass ratio 2.5:1 can be used to obtain a relatively stable 20% emulsion of medium chain triglycerides in water (O/W).

Keywords: Gelatin, Cetiltrimethylammonium Bromide, Interactions, Emulsions.

Acknowledgements: This work was financed by Ministry of Education, Science and Technological Development of the Republic of Serbia [Grant No 451-03-68/2022-14/200134].

2nd International Conference
on Advanced Production and Processing

EXTRACTION OF LEMON BALM (*Melissa officinalis* L.) USING NATURAL DEEP EUTECTIC SOLVENTS

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Lemon balm (*Melissa officinalis* L.) is a medicinal plant from the Lamiaceae family of ethnopharmacological importance. Additionally, the medicinal relevance of lemon balm was confirmed in numerous studies that determined its significant biological potential including its antioxidant, antiviral, antifungal, antibacterial, and antiproliferative activities. The most common procedures for obtaining lemon balm-based products involve the use of conventional solvents and methodologies that can be time- and energy-consuming and unfavourable to the environment. Therefore, there is a constant interest in developing new, safe, and efficient approaches to obtaining extracts of lemon balm. Natural deep eutectic systems (NADES) represent natural alternative solvents formed by mixing the hydrogen bond acceptor and hydrogen bond donor at a certain molar ratio. NADES are characterized as highly biodegradable, biocompatible, safe, and low-cost solvents. Considering their safety profile, it is not necessary to remove them from extracts. In this study, NADES (betaine:ethylene glycol (1:3), betaine:glycerol (1:2), and glycerol:glucose (4:1)) were used as solvents in the ultrasound-assisted extraction to obtain extracts of lemon balm. The extraction was conducted at temperatures of 40 and 60 °C and extraction times of 30 and 60 minutes. Furthermore, the obtained extracts were investigated in terms of the content of main polyphenols by applying the HPLC analysis. Dominant compound in the NADES extracts was rosmarinic acid in the range from 163.12 to 2882.82 µg/mL, followed by luteolin-3-O-glucuronide (from 10.50 to 155.26 µg/mL). It was noticed that the contents of rosmarinic acid, luteolin-3-O-glucuronide and luteolin-7-O-glucoside increased with the temperature and extraction time, hence their highest content was obtained in the extract attained under the conditions of 60 °C/60 min with betaine:ethylene glycol. Due to higher viscosity of glycerol:glucose and betain:glycerol mixtures, the mass transfer was decreased causing these solvents to be less efficient for polyphenolic extraction compared to the betaine:ethylene glycol system. The trend of increase in the content of all individual polyphenols with the increase in the extraction temperature and time was also noted in the extracts obtained with glycerol:glucose.

Keywords: Deep Eutectic Solvents, Ultrasound, Polyphenols, Extraction, Lemon Balm.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement [Number 101003396].

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INFLUENCE OF ULTRASOUND, MICROWAVE, AND ENZYMATIC PRETREATMENTS ON SUPERCRITICAL CO_2 EXTRACTION OF *TETRASELMIS* SP. AND *CHLOROCOCCUM* SP.

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The goal of this work was to improve the extraction yield of supercritical carbon dioxide ($scCO_2$) extraction of *Tetraselmis* sp. and *Chlorococcum* sp. microalgae by applying different pretreatments. Moreover, microwave (MW), ultrasound (US), and enzymatic (ENZ) pretreatments (solid/liquid ratio 1:5 (g/mL)) were applied followed by $scCO_2$ extraction at pressure of 300 bar and temperature of 40 °C. The pretreatments were conducted under the following conditions: MW - irradiation power of 800 W and time of 3 min; US - temperature of 60 °C and time of 5 min, and ENZ - incubation temperature of 45 °C and time of 60 min, conducted with Viscozyme enzymatic solution. Additionally, a control $scCO_2$ extraction without pretreatment was performed. The extraction yields of the control $scCO_2$ extraction of *Tetraselmis* sp. and *Chlorococcum* sp. were 0.39% (w/w) and 0.47% (w/w), respectively. MW pretreatment of *Tetraselmis* sp. did not enhance the recovery of the lipophilic fraction, whereas, after US pretreatment, a higher extraction yield (0.61%, w/w) compared to the control was achieved. The most significant yield improvement of the *Tetraselmis* sp. $scCO_2$ extraction was achieved after ENZ pretreatment reaching 2.80% (w/w). Furthermore, the pretreatments were more impactful with regard to *Chlorococcum* sp. MW improved the yield by approximately 50%, while US pretreatment doubled it. Finally, the 5.4-fold higher extraction yield of *Chlorococcum* sp. compared to the control was achieved after ENZ pretreatment.

Keywords: Tetraselmis, Chlorococcum, Supercritical Extraction, Pretreatment.

Acknowledgements: This work was supported by the Bilateral project Portugal-Serbia 5554/2020 (FCT) and 337-00-00227/2019-09/72 (Serbia).

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INCLUSION COMPLEX OF BETULIN WITH HYDROXYPROPYL-β-CYCLODEXTRIN: PREPARATION, CHARACTERIZATION AND TESTING OF PHASE SOLUBILITY

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Betulin is a natural triterpene presented in many plants with numerous biological and pharmacological activities (antimicrobial, antifungal, antiviral, anti-inflammatory and anticancer). The use of betulin in therapy is limited due to its very low solubility in water and aqueous solutions. In order to increase the solubility and the bioavailability of betulin in water, a lot of formulations with various modern carriers have been described in the literature. Cyclodextrin inclusion complexes with pharmacologically active substances represent supramolecular structures with improved characteristics of active agents, which contributes to their greater efficiency. The aim of this work was to prepare and characterize the inclusion complex of betulin with hydroxypropyl-β-cyclodextrin, in order to increase the solubility of betulin in water. The inclusion complex of betulin with hydroxypropyl-β-cyclodextrin was made by the method of thick paste in a molar ratio of 1:2. Structural characterization of the prepared complex, starting substances, as well as the appropriate physical mixture was performed using FTIR and DSC methods. The phase solubility of betulin was tested in the presence of solutions with increasing concentrations of hydroxypropyl-β-cyclodextrin, by Higuchi-Connors method. Differences in the appearance of the FTIR spectrum of the inclusion complex and the betulin standard, as well as the inclusion complex and the physical mixture, show that betulin was incorporated into the cyclodextrin cavities and that the inclusion complex was formed. DSC thermograms of the betulin complex show temperature changes that differ from pure betulin and hydroxypropyl-β-cyclodextrin. Thermograms indicate the formation of new structures with a different regime of temperature changes. The phase solubility diagram belongs to the A_P type, which indicates the formation of a higher order complex in relation to hydroxypropyl-β-cyclodextrin. The obtained results show that the inclusion complexes of betulin and hydroxypropyl-β-cyclodextrin are formed, where the solubility of betulin is increased 1.35 times.

Keywords: Betulin, Inclusion Complex, Hydroxypropyl-β-Cyclodextrin, Physico-Chemical Characterization, Phase Solubility.

Acknowledgements: Republic of Serbia-Ministry of Education, Science and Technological Development, Program for financing scientific research work [number 451-03-68/2022-14/200133, 451-03-68/2022-14/200113] and Internal Project No. 67 (Faculty of Medicine, University of Niš).

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2nd International Conference
on Advanced Production and Processing

PREPARATION AND CHARACTERIZATION OF BETULINIC ACID INCLUSION COMPLEX WITH HYDROXYPROPYL-β-CYCLODEXTRIN

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Betulinic acid is a natural triterpene, which has numerous biological and pharmacological activities such as antimicrobial, antiviral, anthelmintic, anticancer and hepatoprotective. Compared to conventional drugs, betulinic acid exhibits fewer side effects and significantly less toxicity. The lack of betulinic acid is low solubility in water and physiological fluids, which limits its use for therapeutic purposes. Increasing the solubility of betulinic acid can be achieved by making different formulations with specific carriers. Inclusion complexes with cyclodextrins can also successfully solve the problem of betulinic acid solubility. The aim of this paper was to prepare and characterize the inclusion complex of betulinic acid with hydroxypropyl-β-cyclodextrin. The inclusion complex was made by the method of thick paste, in a molar ratio of 1:2. Structural characterization of the developed complex, starting substances, as well as the corresponding physical mixture was performed by FTIR and DSC method. The phase solubility of betulinic acid in the presence of increasing concentrations of hydroxypropyl-β-cyclodextrin in the solution was tested by the Higuchi-Connors method. FTIR spectra of the inclusion complex, betulinic acid standards, hydroxypropyl-β-cyclodextrin, and physical mixtures indicate that an inclusion complex was formed. DSC thermograms of the betulinic acid complex show temperature changes that differ from pure betulinic acid and hydroxypropyl-β-cyclodextrin. A different regime of temperature changes of the complex indicates the formation of a supramolecular structure in which the properties of betulinic acid are changed. The phase solubility diagram belongs to the A_L type, which indicates that the molar ratio of betulinic acid and inclusion complex is 1:1 and that the solubility of betulinic acid is increased in the complex 1.20 times.

Keywords: Betulinic Acid, Inclusion Complex, Phase Solubility, Hydroxypropyl-β-Cyclodextrin.

Acknowledgements: Republic of Serbia-Ministry of Education, Science and Technological Development, Program for financing scientific research work [number 451-03-68/2022-14/200133].

ICAPP 2022
2nd International Conference
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OPTIMIZATION OF SWEET CHERRY EXTRACTION: EVALUATION OF TOTAL PHENOL CONTENT, PROTEIN CONTENT, AND ANTIOXIDANT ACTIVITY OF EXTRACTS

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Cherry fruits are known for their wide range of nutritive and bioactive compounds, including fibers, vitamins, and phytonutrients. Sweet cherry consumption has been associated with several health benefits against human problems such as cardiovascular diseases, cancer, diabetes, and Alzheimer's disease. Particularly, cherries contain abundant phenolic compounds with antioxidant, anti-inflammatory, and anticancer properties as they exert a protective role against oxidative stress and free radical damages. However, there is not a specific method to fully extract all phenolics and other compounds from sweet cherries. Thus, this study aimed to optimize the extraction of cherries through different extraction procedures (maceration and ultrasound-assisted extraction) using different solvents (50% EtOH with and without HCl, and Tris buffer). The resulting cherry fruit extracts were investigated regarding their i) total phenol content - according to the Folin-Ciocalteu colorimetric method, ii) total protein content - evaluated using the Bradford protein assay, and iii) antioxidant activity – characterized by ABTS, DPPH, and FRAP assays. The ultrasound-assisted extraction using 50% EtOH and HCl (pH=2.3) demonstrated the best results for total phenol content (7.51 mg/L), DPPH inhibition (23.7%), and FRAP reducing power (24.5 mmol/g). On the contrary, the highest ABTS scavenging activity was noticed for maceration obtained extract with 50% EtOH and HCl (7.23 mg/mL). Finally, the best protein yield was obtained for cherries when extraction was performed in 0.1M Tris buffer at pH=11.2 (0.23 mg/mL). The optimization methodology in this work is used as a screening test for producing bioactive compounds-rich extracts for the food industry.

Keywords: Sweet Cherry, Extraction, Polyphenols, Antioxidant Activity, Bradford Assay.

Acknowledgments: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia [number 451-03-68/2022-14/200287] and the Science Fund of the Republic of Serbia [number 7739716] CherrySeRB.

Petroleum Refining and Production

ICAPP 2022
2nd International Conference
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EDUCATIONAL AND SCIENTIFIC METHODS IN THE OIL AND GAS INDUSTRY

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Education and scientific methods in the oil and gas industry have been undergoing substantial changes in recent years with the application of innovative technologies such as modern instrumental analysis techniques for petroleum products and construction materials (advanced construction materials, crude oil, additives), digitalization, 3D printing, development of enhanced separation methods, statistical analysis, etc. To meet the future challenges in the oil and gas industry, universities need to considerably reconcile study programs, ensuring the desired progress, flexibility, and practical skills for the young generation. In this paper were analyzed the modifications of the study programs and teaching methods in Russian and Serbian universities. The results showed that the effective implementation of the new teaching methods led to continuous improvement of the quality of education received by undergraduate students. The possible outcomes of international cooperation between Russian and Serbian universities were also taken into consideration.

Keywords: oil and gas industry, advanced construction materials, international cooperation

Acknowledgments: The work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. 451-03-68/2022-14/200134) and the Ministry of Science and Higher Education of the Russian Federation (Project Part of the State Assignment No 0729-2020-0039).

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

CONTROLLED SYNTHESIS OF COPOLYMERS BASED ON HIGHER ALKYL(MET)ACRYLATES AND THEIR APPLICATION AS COMPLEX ADDITIVES TO DIESEL FUEL

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The production of high-quality diesel fuels with an improved set of properties and characteristics is one of the priority areas for the development of the modern petrochemical industry. The main direction of modifying the properties of Euro-5 hydro treated diesel fuel is the use of additives, as which homo- and copolymers are often used. At the same time, important parameters that determine the effectiveness of the polymeric additives are the molecular weight characteristics, composition and structure of polymers. The most promising method for the synthesis of polymer additives with a given structure and properties is controlled radical polymerization or living radical polymerization.

In this work, various methods of controlled radical polymerization (the Reversible Addition-Fragmentation Chain Transfer, Atom Transfer Radical Polymerization, and Metal Free Atom Transfer Radical Polymerization) were used for the targeted synthesis of polymer additives based on stearyl methacrylate and stearyl acrylate in combination with acrylonitrile, glycedyl methacrylate, maleic anhydride and other monomers. Copper and ruthenium complexes, as well as trithiocarbonates of various structures, and iodine-containing agents were used as catalysts and regulators of (co)polymerization.

A number of polymer additives have been synthesized and the influence of their composition, structure, and molecular weight characteristics on the cloud point, freezing point, and filterability limit temperature of diesel fuel has been studied. It has been shown that, depending on the composition and molecular weight, the synthesized copolymers, when introduced into diesel fuel at a concentration of 200 to 1600 ppm, can significantly improve the low-temperature characteristics of diesel fuel and simultaneously increase its oxidative stability. It has been found that the greatest depressant effect is observed when glycedyl methacrylate and maleic anhydride are used as co-monomers to alkyl(meth)acrylates, followed by modification of the resulting copolymers by ring opening. At the same time, additives based on random copolymers are the most promising in terms of reducing the limiting filterability temperature and the freezing point of the fuel.

The proposed additives are not inferior in efficiency to known industrial analogues, and surpass them in the complex nature of the action.

Keywords: diesel fuel, additives, polymerization

Acknowledgements: This work was supported by the federal academic leadership program "Priority 2030" of the Ministry of Science and Higher Education of the Russian Federation".

2nd International Conference
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INFLUENCES OF VARIOUS PROCESS PARAMETERS ON TRIGLYCERIDES HYDROGENATION REACTIONS OBTAINED BY ASPEN SIMULATION

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Considerable efforts have been made to find biofuel that could successfully reduce energy consumption of limited fossil fuel reserves. In that manner, renewable diesel acquired a major attention in both academic and industrial areas. Blends made of petrodiesel and green disel in the amount of 5–30 wt% in most cases exhibited improved characteristics than those of neat petrodiesel. As a mixture of n- and iso- paraffins, green biodiesel exhibits higher heating value, higher cetane number and increased oxidation stability over conventional ester-based biodiesel. Waste vegetable oil hydrogenation reactions were evaluated from the aspect of pressure and temperature influence, hydrogen consumption and kinetic reactions. Hydrotreating of triglycerides proceeded via the hydrocracking of triglycerides into diglycerides, monoglycerides and fatty acids. Then fatty acids were subsequently deoxygenated to hydrocarbons. Hydrotreating of fatty acids occurred primarily via hydrodeoxygenation and the main liquid products were octadecane and heptadecane. Process optimization of triglycerides hydrogenation are simulated using Aspen plus software. When the temperature increased, the conversion of vegetable oil, isomerization selectivity, alkane selectivity, cracking selectivity, together with the selectivity of decarboxylation plus decarbonylation increased. Obtained results showed that hydrodeoxygenation could be promoted by pressure increasing and a higher amount of n-C18 would be formed. Higher reaction temperature is favored because of the higher amounts of formed iso-alkanes in final products which are favorable because giving better cold flow properties of fuel blends.

Keywords: green diesel, triglyceride hydrogenation, refinery co-processing, kinetic, Aspen plus process optimization.

Acknowledgements: Financial support by the Ministry of Education and Science of the Republic of Serbia through projects (Contract No. 451-03-68/2022-14/200135) and Institutional funding of the Faculty of Technology and Metallurgy University of Belgrade is gratefully acknowledged.

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SIMULATION OF MULTIPHASE CATALYTIC REACTOR FOR PROCESSING OF GAS OIL AND LIGHT CYCLE OIL FRACTIONS USING COMSOL MULTIPHYSICS SOFTWARE

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In modern oil refineries, hydrogen refining processes, performed under high pressure and in presence of catalysts, have one of the key roles in processing. Their main role in refinery processing is the removal of sulphuric components, stabilization of the products, and removal of other undesired impurities (hydrotreating reactions), as well as conversion of heavier fractions into lighter ones (hydrocracking reactions). One of key parameters and limiting factors when it comes to yield and selectivity of the process is the reaction that takes place on the surface of the solid catalyst. The hydrodesulfurization takes place in a reactor with a fixed layers of catalyst in the presence of hydrogen, at elevated pressure (typically up to 60 bar) and temperature (up to 633 K). Process of hydrodesulfurization of gas oil and light cycle oil fractions has been modelled and simulated in COMSOL Multiphysics software, based primarily on the catalyst particle. The model includes mass transfer to catalyst particle and inside of the catalyst particle itself, as well as reactions on active centers. The modeling process is based on detail design of the catalyst particle geometry and calculation of mass and energy balance at macroscopic (reactor) scale. The model and obtained simulated data are compared to macroscopic model previously developed using MATLAB software. This model consists of differential species and heat balance equations, as well as corresponding kinetic expressions for hydrodesulfurization of sulphuric compounds. The model is based on detailed thermodynamic phase equilibrium inside reactor, detailed analysis of sulphur distribution by types of compounds and their conversions, as well as the use of Kelvin's equation for catalyst bed wetting calculation.

Keywords: Hydrodesulfuriaztion, Multiphase catalytic reactor, Catalyst particle, COMSOL Multiphysics, Simulation.

Acknowledgements: Financial support by the Ministry of Education and Science of the Republic of Serbia through projects (Contract No. 451-03-68/2022-14/200135) and Institutional funding of the Faculty of Technology and Metallurgy University of Belgrade is gratefully acknowledged.

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ETHYL ESTER BASED BIODIESEL SYNTHESIS UNDER ELEVATED PRESSURE AND TEMPERATURE: PROCESS SIMULATION AND TECHNO-ECONOMIC STUDY

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Non-catalytic biodiesel synthesis under elevated pressure and temperature is highly efficient in terms of triglycerides and free fatty acid conversion, required reaction time and process layout. However, phase equilibrium and mass transfer can play an important role in the final product distribution as well as the presence of reaction intermediates in the final product. This study presents kinetic parameters estimation based on laboratory experiments performed under subcritical conditions in batch reactor, as determined by the standard optimization methods. The kinetic model employed in this study is based on three consecutive and parallel reversible reactions of the second order with six kinetic constants. The novel reactor design based on separation of second liquid phase and process simulation were proposed. The proposed process was compared with ethanolysis at supercritical conditions, heterogeneously catalyzed ethanolysis and alkali catalyzed ethanolysis with pre-treatment of FFA and techno-economic study were performed.

Keywords: Waste vegetable oil ethanolysis, Biodiesel, Optimization Technique, Aspen plus process simulation, Techno-economic study.

Acknowledgements: Financial support by the Ministry of Education and Science of the Republic of Serbia through projects (Contract No. 451-03-68/2022-14/200135) and Institutional funding of the Faculty of Technology and Metallurgy University of Belgrade is gratefully acknowledged.

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2nd International Conference
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EFFICIENT DEMULSIFICATION OF WATER-IN-PARAFFINIC CRUDE OIL EMULSION

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Chemical demulsification is commonly used technique to overcome the problems associated with the formation of water-in-oil emulsions. In this study, emulsion samples were obtained from oil production facilities, differing in their composition and properties. The representative sample was a mixture of two different crude oils. The influences of demulsifier concentration, residence time, and temperature on the efficiency of demulsification of water-in-oil emulsions were studied. The following crude oil properties were experimentally determined or estimated using standard methods: ASTM D86 distillation curve, API density, kinematic viscosity, paraffin content, total asphaltene content, mean relative molecular mass, pour point, and salt content. The properties of blend of two paraffinic crude oils were calculated using Aspen Hysys. For each emulsion prepared, basic sediment and water content of crude oil was measured. Demulsification was performed using bottle test in the wide ranges of demulsifier concentration and temperature. Statistical analysis was conducted in order to select optimal demulsifier concentration and working temperature.

Keywords: Demulsification, Crude oil, Water-in-oil emulsion, Separation of water

Acknowledgements: The authors are thankful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for their financial support, Project No. 451-03-68/2022-14/200134

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2nd International Conference
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CHEMICAL DEMULSIFICATION OF WATER-IN-NAPHTHENIC CRUDE OIL EMULSIONS

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The majority of crude oil reserves are paraffinic crudes. The naphthenic crude oil reserves are found in rare oilfields. However, Serbia has several naphthenic crudes. The emulsion of water-in-naphthenic crude oil is stabilized by naturally occurring emulsifiers in the crude oil such as waxes, asphaltenes, associated solids, resins, and some organic acids and bases. Removing emulsified water from a water-in-crude oil emulsion is critically required for pipeline quality, transportation, and downstream processing. Chemical demulsification is commonly used for treating oil-field emulsions. In this paper, the effect of the concentration of five commercially available demulsifiers on dehydration efficiency was investigated. Emulsions were prepared and collected in the oil field according to the analysis of well fluid flow and average water content. Demulsifiers were tested with a standard bottle test, over a broad range of temperatures and concentrations. The effectiveness of demulsifiers was followed by observing the dependence of dehydration efficiency on time. For all investigated demulsifiers, this dependence was sigmoidal. Based on this dependence and statistical analysis, the optimal demulsifier concentration, residence time and temperature were determined.

Keywords: Demulsification, Crude oil, Emulsion, Statistical methods

Acknowledgements: The authors are thankful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for their financial support, Project No. 451-03-68/2022-14/200134

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

INFLUENCE OF SURFACE TENSION ON EXTENDER OIL PERFORMANCES

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Many authors have investigated effect of ingredients' properties on rubber properties. However, due to the complex mixing mechanisms of rubber compounding ingredients, reliable mathematical models for the estimation of rubber properties are scarce. As far as we know, there are no published results regarding the effect of surface tension on the rubber compounding operation. Mineral extender oils used for rubber compounding in this study were: solvent neutral oil, naphthenic oil, residual aromatic extracts, treated residual aromatic extract, and paraffinic oil. Physicochemical properties of mineral oils were experimentally determined or calculated. The surface tension of examined mineral oils was determined in a wide temperature range by the modified method using a stalagmometer. In this study, the influence of API gravity, and refractive index of mineral oils on its surface tension was examined. To obtain reliable predictive models for calculating surface tension statistical methods such as linear and multivariate regression were applied. Experimental results for the dependence of surface tension on temperature were compared to calculated surface tension values by Miqueu, Baker-Swerdloff, and Abdul-Majeed equations. Miqueu equation gave the best fit in surface tension values for all investigated mineral extender oils, compared to Baker-Swerdloff and Abdul-Majeed equations. The developed regression equations showed that the oils with higher API density exhibited lower values of surface tension. It can be assumed that those extender oils will more easily form droplets and disperse among the rubber ingredients. It can be concluded that the surface tension of extender oil can be estimated by the proposed regression equation based on API density and refractive index (*Adj.* R^2=0.9957).

Keywords: Rubber, Extender oil, Mineral oils, Surface tension

Acknowledgments: The authors are thankful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for their financial support, Project No. 451-03-68/2022-14/200134

2nd International Conference
on Advanced Production and Processing

ESTIMATION OF PETROLEUM PRODUCTS PROPERTIES

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Petroleum products are obtained by primary and secondary refining processes. A modern deep conversion petroleum refinery requires numerous analyzes of the physical and chemical properties of petroleum blending components and products such as gasoline, kerosene, diesel, fuel oil, etc. Determining the physicochemical properties of petroleum blending components and products requires a variety of laboratory analyzes which are often expensive and time-consuming. In this study, statistical methods were applied to estimate properties of petroleum blending components and products. The results showed that neural networks provide the most reliable models while multivariable regression analysis allows more flexible statistical models to be obtained.

Keywords: petroleum, petroleum products, refinery, neural networks

Acknowledgments: The authors are thankful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for their financial support, Project No. 451-03-68/2022-14/200134

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

IMPROVEMENT OF STABILIZATION TECHNOLOGY OF WEAKLY CONSOLIDATED COLLECTOR

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Sand removal in the formation fluid, related to the broken structure of loosely bound (consolidated) collectors is a serious problem and represents an important aggravating factor for many oil and gas fields. Particles from the collapsing collector are deposited in the lowest section of the well and in the collection and preparation system. This leads to a decrease of well productivity, an increase in the erosive wear of the equipment, and a significant increase in costs for disposal of the accumulated slop. In the period from 2018 to 2022, worldwide known stabilization technologies were tested, the most efficient technology was selected for field tests and after the evaluation of field tests results technology was improved in order to eliminate the observed problems. In this work, the results from performed laboratory tests with common technologies are presented. Field tests were conducted with the selected stabilization technology (resin treatment). These results as well as the development of an idea concept for the improvement of the resin treatment were used to propose a new technical solution. This approach was evaluated on the basis of field test results. By implementing all the proposed technical solutions, the effectiveness of resin treatment in the near-well zone has been increased. This can be confirmed by the preservation of collector productivity, stabilization of the near-well zone of the layer, and prevention of sand appearance in the formation fluid.

Keywords: Petroleum production, Sand removal, Resin Treatment

Acknowledgements: This work was supported by Scientific-Technological Center NIS-Naftagas Novi Sad.

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

MATRIX FORMATION FOR APPLICABILITY OF SUFFOCATION SOLUTIONS AND FLUIDS

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The aim of this investigation is to reduce oil losses through the selection of an adequate working fluid that would be used during overhaul works and thus reduce the risk of disrupting the production characteristics of the petroleum production well. Furthermore, one of the goals is to accurately define the criteria/parameters that influence the selection of suffocation fluid. During the last year, around 90 overhaul works were carried out using water-based working fluids. In numerous wells, there was an extended duration of VNR and disruption of production characteristics of the layer due to negative effects caused by the interaction between the working fluid and the reservoir. These negative effects can be avoided or significantly reduced by using adequate working fluids and additives. In this work, the most important working parameters were identified. These working parameters can affect the choice of suffocation fluid, related to compatibility with rock, as well as the formation of water and oil. Based on the laboratory results, the limit values of those parameters were determined, when there is a risk of deterioration of the production characteristics of the wells. The focus of the investigation was on multidisciplinary laboratory tests, such as core analyses (mineralogical, swelling and filtration tests), chemical analyses (analysis of layer water composition, oil composition, thermostability, corrosive activity, etc.), and geochemical analyses (SARA analysis). By reliable selection of the additives that are added to the working fluids and optimization of their application, it is possible to reduce the negative effects on the production characteristics of the layer that can occur during the overhaul works.

Keywords: Petroleum production, Suffocation fluid, Wells, Additives

Acknowledgments: This work was supported by Scientific-Technological Center NIS-Naftagas Novi Sad during the last five years.

Sustainable Development, Chemical and Environmental Engineering

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

HOW DOES USE OF PESTICIDES IN PROTECTION OF OILSEED RAPE PRODUCTION AFFECT BEE HEALTH AND SAFETY OF BEE PRODUCTS?

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For more than ten years, we have been witnessing a decrease in the number of honey bees. The use of pesticides is recognised as one of the globally identified reasons. Extensive studies around the world have shown that exposure to pesticides can contribute to the deterioration of bee health and the contamination of bee products. Although international regulations and policies regarding the use of pesticides are becoming stricter, especially in the EU, and many active substances are even banned, there are frequent cases of uncontrolled pesticide use. Thus, our research aimed to test the presence of residues of an organophosphate pesticide, chlorpyrifos and two pyrethroids—tau-fluvalinate and lambda-cyhalothrin—in samples collected on three apiaries (A, B and C): pollen, honey and bee bread, as well as in the bees which were grazing in oilseed rape fields. A modified QuEChERS (Quick Easy Cheap Effective Rugged Safe) approach was used for sample preparation, and the detection of the pesticides was done using GC-MS (Gas Chromatography-Mass Spectrometry). In apiary A, chlorpyrifos was detected in the highest concentration in rapeseed flowers (1958.0 µg/kg), followed by pollen (334.5 µg/kg), dead bees (17.83 µg/kg) and honey (15.71 µg/kg), whilst lambda-cyhalothrin and tau-fluvalinate were not detected. Chlorpyriphos in pollen, perga and bees was also detected in apiary B, in average concentrations of 548.63 µg/kg, 46.83 µg/kg and 105.13 µg/kg, respectively. The presence of lambda-cyhalothrin in a concentration of 60.11 µg/kg of pollen was detected in this apiary, while the presence of tau-fluvalinate was not determined. In the samples from apiary C, chlorpyrifos was not detected, but in brood honeycombs lambda-cyhalothrin, tau-fluvalinate 1 and tau-fluvalinate 2 were found in the following concentrations: 69.90 µg/kg, 114.16 µg/kg and 105.36 µg/kg, respectively. The obtained dana indicate that it is necessary to establish constant monitoring of bee exposure to pesticides. This would ensure that the application of laws and regulations governing the use of pesticides in both Serbia and the EU ensures successful protection of pollinators and enables their role in agroecosystems. Only with adequate and prescribed application of pesticides is it possible to simultaneously provide protection of oilseed rape, a very important agricultural crop, and contribute to the reduction of mass bee deaths.

Keywords: Chlorpyrifos, Pyrethroids, Residues, Bee products, Bees

Acknowledgements: This study was funded by Ministry of Education, Science and Technological Development of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2021, Contract No: 451-03-9/2021-14/200031 and by Provincial Secretariat for Agriculture, Water Management and Forestry of Vojvodina, Contract No: 104-401-4672/2021/01.

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CHALLENGES IN SUGAR PRODUCTION – FOOD SAFETY MANAGEMENT SYSTEM

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Sugar industry is one of the largest among the food industry. One plant can process around 8000 tons of sugar beet daily and produce 1150 tons of sugar. In Serbia, it can be said, that sugar is a strategic product. Also, the wastes and by-products, such as molasses, sugar beet pulp, sludge from the mentioned industry are valuable, since they are raw materials for other technological processes. The sugar plant in Pećinci few years ago started a new production line, which is unique in Central Europe and Balkan area, for betaine production from molasses. In this study the high product safety requirements for sugar industry products will be introduced.

Due to unambiguous impact of globalization on modern business, one of the requirements set in front of manufacturers is standardization and certification of food safety and quality systems. The objective of this work is individual analysis of the GFSI approved schemes (FSSC 22000 - Food Safety System Certification, BRC, IFS) applied for sugar industry plant.

An overview of the GFSI Standards - Global Food Safety Initiative and key factors which collaboratively drive continuous improvement in food safety management systems around the world with a vision „Safe food for consumers everywhere" will be presented. Further, it will provide an overview of the impact of the globalization process on sugar industry and applied GFSI approved schemes.

Keywords: Sugar, Molasses, Betaine, Food safety, GFSI, FSSC 22000, IFS, BRC

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A STEP TOWARD COMPLETE UTILIZATION OF EGGSHELL WASTE

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One of the five major challenges of sustainable waste management in the agri-food industry by using the "zero-waste" model of the circular economy is the development of innovative waste transformation techniques for the production of chemicals, fine chemicals, bioactive compounds, enzymes and functional materials. These products have at least twice added value of products obtained from currently dominant outdated waste management strategies which are not in accordance with sustainable development. Eggshell waste is one of the wastes produced by agri-food industry whose majority instead being re-used, ends in landfill sites, additionally burdening the environment. Situation is quite concerning if one considers that at least 7,894,962 t of eggshell waste was generated annually worldwide of which 777,000 t in the European Union in 2018. Keeping in mind current state of eggshell waste utilization/landfilling and the need for the development of innovative waste transformation techniques, we have designed "*3 from 1*" process of complete utilization of eggshell waste oriented toward "zerowaste" model. The process included eggshell waste washing, acid solvation and few additional steps for preparation of powdered calcium salts and eggshell membranes. After complete utilization of eggshell waste: clarified adherent egg white protein solution, three different types of eggshell membranes and three different calcium salts: calcium chloride, calcium acetate and calcium hydrogen phosphate of high purity were produced. In addition, spry-drying for the production of anhydrous calcium chloride was implemented. Based on results obtained, it can be concluded that designed "*3 from 1*" process has great potential to be used not only for the complete utilization of eggshell waste, but also for the production of high-value added products from it.

Keywords: Eggshell waste utilization, Adherent egg white protein, Eggshell membranes, Calcium salts

Acknowledgements: This work has been fully supported by Croatian Science Foundation under the project IP-2020-02-6878.

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THE ADSORPTION OF CATIONIC DYES FROM AQUEOUS SOLUTION USING HUNGARIAN AND INDONESIAN RICE HUSK

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The textile industry is one of the largest global pollutants and consumes many chemicals and water transformed into wastewater. For example, cationic dyes used in the textile industry include methylene blue and basic red 9. These dyes can cause negative effects on humans and living organisms. Wastewater treatment is needed to remove pollutants in dye wastewater. One of the best techniques is adsorption because of low-cost and simple process. The present study investigated the using Hungarian and Indonesian rice husk as a bio-adsorbent to remove methylene blue and basic red 9 from aqueous solutions. The characterization of RH was investigated by zeta potential, and fourier-transform infrared spectroscopy analysis. The adsorption studies were carried out by batch experiments using different parameters, such as pH, adsorbent dose, initial concentration, contact time, and temperature. The results showed that the equilibrium was obtained at the contact time of 120 min. The maximum dye removal has occurred at pH 10 for MB and pH 7 for BR9 using a 500 mg adsorbent dose at the initial concentration of dyes 30 mg/L. Adsorption of MB and BR9 follows the pseudo-second-order kinetic. Therefore, raw rice husk could become a potential adsorbent in methylene blue and basic red 9 adsorption.

Keywords: Adsorption, Basic red 9, Bio-adsorbent, Methylene blue, Rice husk

Acknowledgements: We would like to thank the Tempus Public Foundation from the Hungarian Government for its funding support.

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MONITORING OF BIOMASS FERMENTATION EFFICIENCY BY DIELECTRIC MEASUREMENT

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There are known several biological processes for the utilization of biomass, such as ethanol fermentation or anaerobic digestion. Some of the core challenges of enzymatic hydrolysis and fermentation are the on-line and real-time monitoring of process efficiency, detection of optimum process time and end product inhibition, and predicting end-point of biodegradation processes. Determination of the changes in dielectric behavior of bio-systems is suitable for these purposes, but the applicability of dielectric measurements is not investigated in details in biomass biodegradation and utilization processes.
Our research focuses on the determination of the frequency dependent change of reflection coefficient (Γ), dielectric constant (ε') and dielectric loss factor (ε") in the frequency range of 200-2400 MHz during batch enzymatic hydrolysis of cellulosic biomass (using corn cob feeds and grits as substrate for enzymatic degradation by enzyme blend of xylanase and cellulase), lab-scale yeast ethanol fermentation (applying separated and simultaneous saccharification and fermentation process, as well), and lab-scale batch mesophilic anaerobic digestion process (using municipal and food industry originated wastewater sludge as feed).
Our results verified that the decomposing of high molecular weight components, therefore the increment of the concentration of smaller and more polar compounds, furthermore the liberation of ionic components from cell membranes and intracellular space and/or the destruction of extracellular polymeric substance, and the change of binding energy of water molecules to macromolecules (ratio of free to bounded water) have effect on the dielectric behavior of the high water contented suspensions and substrate-enzyme-microorganisms-end/side products contented systems. It can be concluded that the dielectric constant (ε') measured at a frequency range of 300-500 MHz was the more sensitive parameter to monitor the biodegradation process and fermentation efficiency of cellulosic biomass and wastewater sludge, as well.

Keywords: Dielectric parameters, Biomass, Fermentation, Anaerobic digestion, Efficiency

Acknowledgements: The research is supported by János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00161/21/4) and UNKP-22-5 (Beszédes S.) New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research Development and Innovation Fund.

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IS THERE ALTERNATIVE TECHNOLOGY WHERE USE OF NaOCl IN WASTEWATER TREATMENT DOESN'T HAVE HARMFUL CONSEQUENCES?

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Almost all industrial wastewater should be treated before being discharged or reused. Widely used household bleach sodium hypochlorite is excellent low price disinfection agent and could be used in wastewater treatment processes. However, large amounts of residual sodium-hypochlorite at the end greatly increase chemical oxygen demand and additional processes should be applied to remove it from treated wastewater.
Treatment of textile industry wastewater with diluted sodium hypochlorite could be successfully done in microreactor systems. Microreactor systems are relatively new technological processes. One of the advantages of this technology is improved diffusion process, thus smaller amounts of chemical agents are needed. As a result, outlet wastewater could be directly discharged into recipient. In this paper, simulated wastewater from textile industry was treated with diluted sodium hypochlorite in microreactor systems and experiments showed that highly contaminated textile wastewater could be purified to fulfill national laws requirements to be released in environment.

Keywords: Wastewater, Microreactor system, NaOCl.

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-9/2021-14/200287 and 451-03-68/2020-14/200287)

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BIO-OIL MODEL SYSTEM SEPARATION BY VACUUM DISTILLATION: OPTIMIZATION WITH ASPEN PLUS

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Finding alternative sources of energy is of great importance for the current and future sustainable development of humanity. Bio-oil, a complex mixture of various chemical compounds, can be obtained from biomass, a renewable source of energy, through the pyrolysis process, from which fine chemicals can be extracted, as well as components that can be used as a source for obtaining hydrogen. Also, bio-oil can be used as a substitute for liquid fossil fuels for obtaining heat and steam. However, the components need to be separated and purified using technologies such as catalytic cracking, hydrodeoxygenation, extraction, catalytic esterification, column chromatography, and vacuum distillation, the latter proving to be the best because it is simple, efficient, and allows for easy large-scale application. Vacuum distillation process should be optimized due to competing cost effects that appear as pressure of the process is lowered. Namely, lower operational costs for energy are accompanied by grater capital costs for sizable column and condenser.
Aspen Plus is a powerful tool for simulation, design, and optimization, and it was used in this work to optimize vacuum pressure of the process. Due to the complexity and varying composition of bio-oils, as well as to verify the experimental results of vacuum distillation, a model system of bio-oil can be used instead of the oil itself. A bio-oil model composed of seven the main characteristic components of bio-oil: distilled water, ethanol, acetic acid, furfural, phenol, guaiacol, and 3-methyl-1, 2-cyclopentadione, was separated by vacuum distillation. Experimental results of vacuum distillation process of the bio-oil model system separated on a small-scale distillation device obtained from literature were used to check results of simulations obtained in this work.

Keywords: Bio-oil model system, Vacuum distillation, Rectification, Aspen Plus, Optimization.

Acknowledgements: This research study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. 451-03-68/2022-14/200134).

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APPLIED STATISTICS AS A TOOL IN QUALITY CONTROL ON ADVANCED PRODUCTION

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Nowadays, the application of statistical methods takes an increasingly important role in the management of companies, especially in the process of increasing quality, reducing costs, and increasing productivity. The purpose of this paper is to present Statistical Process Control (SPC) through a concrete example in production and processing and to find applications in other areas of technology as advanced production and higher control. In the process of encouraging small and medium-sized companies in their development, it is necessary implementation of computer technology in every segment of the processes. This paper will help to better understand this SPC through its statistical calculation, but also its implementation in companies by offering a suitable solution for its application through code. Applied statistics is a powerful tool in the area of statistical control process and it is very popular in the industry. The main objective of the control chart is to improve manufacturing processes by revealing the variability which is related to certain (specific) reasons. Once the underlying cause of variation is determined, managers take action to fix the process. Applications of statistical methods is in order to visualize, interpret and anticipate outcomes over collected data. It means to enabling managers to make objective decisions based on analysis and experience and based on the analysis and processing of statistical data.

Keywords: Applied Statistics, Statistical Process Control, SPC

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APPLICATION OF RESPONSE SURFACE METHODOLOGY TO THE ULTRAFILTRATION OF DAIRY WASTEWATER

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The dairy industry generates large volume of wastewater due to the high water consumption during technological operations, washing and cleaning. Membrane technologies are promising methods to treat wastewaters. The main disadvantage of membrane filtration in dairy wastewater treatment is the membrane fouling. One way to reduce fouling is to increase the surface shear rate of the membrane, i.e. change the flow properties (stirring and using 3D printed spacers).
In this study we used a response surface methodology to determine the optimal parameters of ultrafiltration on dairy wastewater. The classical method (one-at-a-time) provide for changing one independent variable while maintaining all others at a fixed level which is time-consuming and expensive. The application of the response surface method enables the simultaneous examination of several variables, so the procedure enables faster, more economical and efficient optimization.
Polyether sulfone membrane was employed with 50 kDa molecular weight cut-off. Ultrafiltration experiments were performed model dairy wastewater with different transmembrane pressures, stirring rate and 3D printed spacers. The response surface was analyzed and the optimal parameters were determined.

Keywords: Fouling, Spacer, Response surface methodology, Ultrafiltration

Acknowledgements: The authors are thankful for financial support provided by New National Excellence Program of the Ministry of Human Capacities.

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APPLICATION OF MEMBRANE SEPARATION AND ADSORPTION FOR NUTRIENT RECOVERY FROM DAIRY WASTE WATERS

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Abstract

The water protection is one of the most important current environmental challenges facing humanity. The objective of reduce to zero the generation of waste in productive activities, by applying the concept of circular economy, has led to the emergence of proposals for the production of biomaterials capable of contributing to environmental protection, and that do not imply huge cost investments. As a good example of these proposals, biochar had to be mentioned as a material with the excellent adsorbent properties, with the low production costs, and that is made with any type of organic matter such as agricultural waste. In this work, a nanoparticle-modified ultrafiltration membrane was used as a pre-treatment method prior to ammonium adsorption. As adsorbent for ammonium removal, alkaline modified biochar produced from banana leaves were used. The characterization of biochar was performed. The kinetics study was performed applying the isotherm models based on the batch experimental data. The general results obtained after the combination of membrane filtration and adsorption are promising, and reflect a satisfactory ammonium removal percentage, proving that biochar might be a good adsorbent for nutrient recovery from wastewaters.

Keywords: Ammonium removal, Water treatment, Membrane filtration, Biochar, Adsorption

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APPLICATION OF MIXED MATRIX MEMBRANES FOR FOULING MITIGATION DURING MEMBRANE FILTRATION OF WASTEWATERS

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Membrane separation processes offer several benefits over conventional methods in industrial wastewater and water treatments, however, fouling issue limits their applications. Several materials may cause fouling, depending on the chemical characteristics of the membrane material and the pollutant, or the characteristics of the water matrix, among them proteins and protein-like substances are known to induce severe membrane fouling. There are several methods for fouling mitigation, both physical and chemical methods. Enhancing the performance of polymeric membranes by nanomaterials has become of great interest. The present work aimed to present the effect of modification of polyvinylidene fluoride (PVDF)–membranes by incorporating TiO_2 and/or $BiVO_4$ nanoparticles and/or carbon nanotubes (CNTs). Their photocatalytic performance under UV or visible light was also investigated. All modified PVDF membranes exhibited higher hydrophilicity (lower contact angle of water droplets) than that of the neat membrane used as a reference. The membranes were characterized by AFM, XPS and SEM, in order to reveal their surface properties, then tested with protein or oil containing wastewaters. The hybrid membranes had better antifouling properties as they had lower irreversible filtration resistance than that of the neat membrane. Hybrid PVDF membranes containing TiO_2/CNT/$BiVO_4$ showed the highest flux and lowest irreversible resistance during the filtration of the BSA solution or oily wastewaters. In case of BSA solutions PVDF-TiO_2/$BiVO_4$ membranes provided good flux recovery ratio under visible light (70% for the PVDF mixed with 0.5% TiO_2 and 0.5% $BiVO_4$).

Keywords: PVDF, Nanocomposite membranes, Fouling, Visible light regeneration

Acknowledgements: The research was supported by the project TÉT_2017-2.3.7-TÉT-IN-2017_00016.

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VALORIZATION OF BLACKBERRY AGRO-RESIDUES UNDER THE BIOREFINERY CONCEPT FOR WASTEWATER TREATMENT

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The employment of lignocellulosic biomass in the biorefinery concept is gaining popularity since it allows the production of a varied range of products such as biofuels, pulp and paper, biomaterials, and biochemicals. In biomass deconstruction, pre-treatments are often needed as first steps, causing changes in their physicochemical properties. These changes include increased porosity and surface area, decreased cellulose crystallinity, and hemicelluloses contents. This paper addresses the possible use of solid blackberry residues from an organosolv delignification process as bio absorbent in wastewater treatment. The chemical profile of the original blackberry agro-residues was determined following TAPPI standards: 15.9% of extractives, 19.1% of lignin and 64.9% holocellulose (37.2% α-cellulose and 27.7% of hemicelluloses). The organosolv delignification was conducted in a reactor, with the following conditions: a mixture of 55% H_2O and 45% ethanol (v/v), a 1:10 solid:liquid ratio at 150°C for 2 h. After filtration, the remaining solid fraction was dried and used in adsorption experiments to remove Cr(VI) ions from aqueous solutions. Batch experiments were performed by mixing 1 g/L of adsorbent and chromium solutions (initial concentrations 10-700 mg/L and pH 2) for 5 min to 48 h. The kinetic study demonstrated fast adsorption during the first several hours while the equilibrium is reached after 48 h. However, after 6 h, 73.7% of the total adsorbed amount of Cr(VI) was removed from the solution at an initial concentration of 50 mg/L. Equilibrium studies were performed at the contact time of 24 h and indicated the maximum capacity of 120 mg/g. Langmuir model appeared to be the best for fitting experimental data with the q_{max} of 126.6 mg/g and R^2=0.9822. Overall, blackberry wastes are promising raw material for removal of Cr(VI) ions.

Keywords: Adsorption, Heavy metals, Chromium, Blackberry

Acknowledgments: Financial support from: i) the Ministry of Education, Science and Technological Development of the Republic of Serbia (Program No. 451-03-68/2022-14/200134), ii) Fundação para a Ciência e Tecnologia, Portugal: CEF: UIDB/00239/2020; CEABN: UIDB/50027/2020, research contract of Ana Lourenço (DL 57/2016/CP1382/CT0007), a doctoral fellowship to Ricardo Costa (2020.07451.BD) and Duarte Neiva through the project Acacia4FirePrev (PCIF/GVB/0145/2018). We are also gratefully acknowledging the supported by the LignoCOST Action 17128.

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A TRIETHANOLAMINE:CHOLINE CHLORIDE DEEP EUTECTIC SOLVENT AS A COSOLVENT IN THE ETHANOLYSIS OF *BRASSICA NIGRA* L. SEED OIL

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Black mustard (*Brassica nigra* L.) seeds oil (BMSO), characterized by a high content of erucic acid (C22:1), belongs to inedible oils. Since BMSO has a high percentage of monounsaturated and branched fatty acids, it is a suitable raw material for biodiesel production. Also, green cosolvents, like deep eutectic solvents (DESs), can improve the biodiesel production process. This study reports the influence of the triethanolamine:choline chloride DES (2:1 mol/mol) on the BMSO ethanolysis over calcined CaO as a catalyst, under the following reaction conditions: temperature of 50, 60, and 70 ºC, ethanol-to-oil molar ratio of 12:1, as well as TEOA:ChCl DES and calcined CaO content of 20% and 10%, respectively. At 70 ºC, the ChCl:TEOA DES provided a high content of fatty acid ethyl esters (FAEE) (98.46±0.7%) after 1.5 min, compared to the control reaction (without the presence of DES), where the maximum FAEE content (98.05±0.6%) was achieved within 4 h. BMSO ethanolysis was described by the kinetic model of the pseudo-first order and the model of variable reaction order concerning TAG and the autocatalytic behavior of the ethanolysis reaction. Both kinetic models, with great accuracy, fitted the experimental data. As a result, physicochemical properties of the obtained biodiesel were within the limit values prescribed by the quality standard EN 14214. Also, the reusability of calcined CaO was proven even in four cycles with the FAEE content of over 90%.

Keywords: Brassica nigra L., Deep eutectic solvents, Ethanolysis, Kinetic modeling, Triethanolamine

Acknowledgments: This work has been funded by the Republic of Serbia - Ministry of Education, Science, and Technological Development, Program for financing scientific research work, number 451-03-68/2022-14/200133. This research is also a part of Project 0-14-18 of the SASA Branch in Niš, Serbia.

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TERPENE PROFILING OF WHITE WINE MADE OF REGIONAL GRAPE VARIETY- GRAŠAC

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Volatile organic compounds (VOCs), comprising a group of several hundred compounds, are important flavor components of wines. The perception of flavor in finished wines is result of two processes: (*i*) grape variety and the cultivation practice in vineyard each season, and (*ii*) winemaking practices. Terpenes play an important role in white wines aroma, representing a group of flavor compounds characteristic for specific grapes used for wine production. This study deals with terpenes profiling of white wines produced from the same Grašac grape variety that is characteristic of the Northern Balkans region. Wine samples were taken in wineries (n = 10) located in northern Serbia. The samples differed in their vintage, covering the period of 10 years (2012-2022). For the characterization of the wine terpenes profile, a quick and simple fully-automated method based on headspace solid-phase micro-extraction (HS-SPME) in conjunction with a gas chromatography-mass spectrometry (GC-MS) was used. Different chemical classes of VOCs were identified, such as alkanes, esters, alcohols, aldehydes, ketones, aromatics, alkenes, acids, and other volatiles. Although screening analysis showed the presence of a large number of VOCs in selected wine samples, the following compounds among terpenes were identified (with frequency of occurrence above 20%): sabinene (21%), α-terpinene (79%), o-cymene (100%), D-limonene (100%), γ-terpinene (100%), terpinolene (100%), terpinen-4-ol (86%), α-cubebene (86%), γ-elemene (100%), β-farnesene (36%), α-muurolene (100%), α-farnesene (36%), tridecanal (29%), and α-calacorene (29%). The presence of terpinen-4-ol in high relative abundance (>15%) was observed in the wine samples of vintage that covers the period from 2020 to 2022, which is in line with the previously published studies. γ-Elemene dominated in the wine samples, which vintage covered the period from 2012 to 2020. For wines produced in 2020, the relative abundance of terpinen-4-ol and γ-elemene is comparable. Principal component analysis separated the wine samples into two groups based on relative terpene concentrations. The first group covers the wine samples produced before 2020 (old wines) and the second group comprises wine samples produced after 2020 (young wines).

Keywords: Grašac grape variety, Terpenes profile, HS-SPME-GC/MS, Wine aging

Acknowledgements: The results presented here are obtained within the project entitled "Promotion of the Grašac/Grašac white grape variety as an autochthonous variety" supported by the Ministry of Agriculture, Forestry and Water Management, Republic of Serbia.

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HYDRODYNAMICS AND MASS TRANSFER IN SIEVE-PLATE EXTRACTION COLUMNS

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In this paper the literature relating to sieve-plate liquid extraction columns and relevant hydrodynamic characteristics have been surveyed. Mass transfer characteristics during drop formation, rise and coalescence, and related models were also reviewed. Important design parameters i.e. flooding, dispersed-phase holdup, drop size distribution, mean drop size, coalescence/flocculation zone height beneath a plate and jetting phenomena were investigated under non-mass transfer and mass transfer conditions. Drop size distributions were best described by the functions (relations) proposed in the literature. Experimental values of the slip velocity, dispersed-phase holdup as well as mean drop size in two-phase liquid-liquid (L-L) systems from different literature sources were also analyzed and discussed. Changes of the Sauter mean drop diameter as functions of dispersed-phase holdup and phase velocities were presented and remarked. The correlations for the dispersed-phase hold-up, mean drop size in the preferred jetting regime and in the non-jetting regime, and coalescence zone height were described. Separation efficiency was estimated through Murphree model. The results provided empirical equations to predict the extraction efficiency, which exhibited good correlation with experimental data. The mass transfer and capacity correlations of Treybal, with some modifications, were found to represent adequately the efficiency and maximum throughput capacity of the columns tested here. The overall volumetric mass transfer coefficient increases with an increase of the dispersed phase superficial velocity. The overall volumetric mass transfer coefficient is normalized for a given content of the dispersed phase but is independent on the dispersed phase superficial velocity in the range of tested operating parameters.

Keywords: Sieve-plate, Extraction column, Hydrodynamics, Mass transfer, Separation efficiency

Acknowledgements: This research study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. 451-03-68/2022-14/200134).

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INTRODUCING GREEN EXTRACTION TECHNIQUE FOR OBTAINING BIOACTIVE COMPOUNDS FROM ORANGE PEEL (*CITRUS SINENSIS* L.) HERBAL DUST

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The citrus processing industry produces a huge amount of waste, which represents almost 50% of the weight of the fresh fruit. Orange peel waste (OPW) generated during citrus processing is a rich source of bioactive compounds. Orange peel powder (OPP) represents the milled OPW fraction of particle size lower than 0,315 mm, which cannot be further used in filter tea production, but can be valorized and used as a potential source of fats, mono- and disaccharides, organic acids, polysaccharides, enzymes, flavonoids, terpenes and pigments. Conventional extractions can be applied for the isolation of these compounds but the question is whether they are profitable in terms of extraction time, required amount of solvent and efficiency. The use of supercritical carbon dioxide extraction (SFE-CO_2) is widely recognized as safe and efficent solution which can be applied to replace these conventional methods. According to the green concept, SFE-CO_2 is not only faster and more efficient, but also prevents the degradation of thermolabile compounds, avoiding application of toxic solvents, enabling production of solvent free extracts. Therefore, in this study, the efficiency of Soxhlet extraction (SE), as conventional method, and SFE-CO_2 as advanced method for the isolation of volatile compounds from OPP was examined. During the SFE-CO_2 process, the extraction time was reduced to 4 h, the temperature was kept constant (40 ºC), and the extraction pressure (100-300 bar) was varied. The obtained extracts were analyzed using GC-MS method. The dominant compounds identified were fatty acids (linoleic, hexadecanoic and oleic), 7-Methoxy-8-(2-oxo-3-methylbutyl)coumarin and osthole.

Keywords: Orange peel, Herbal dust, Supercritical extraction, Bioactive compounds

Acknowledgements: This study was funded by Ministry of Education, Science, and Technological Development. Project number: 451-03-68/2022-14/200134.

ICAPP 2022
2nd International Conference
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UTILIZATION OF GINGER (*ZINGIBER OFFICINALE*) HERBAL DUST USING SUPERCRITICAL EXTRACTION TECHNOLOGY

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Extensive filter tea production in the last decade has increased the amount of by-products and wastes generated by this industry. Due to the particle size (less than 0.315 mm), this material can't be packed into the filter tea bags and is called herbal dust. According to previous studies, highly useful compounds such as polyphenols, aromatic compounds, chlorophylls, and carotenoids have been recovered from different herbal dusts. Therefore, herbal dust represent the useful material for further processing into the high value products. Ginger (*Zingiber officinale*) is commonly used for the treatment of digestive disorders, cough, cold and various health issues. In addition, ginger is the great antioxidant and possesses potential against inflammation and muscular pain. Therefore, the research of this study is focused on the valorization of ginger hebal dust as a potential source of valuable bioactive molecules through the establishment of an optimized, environmentally friendly extraction process. Supercritical carbon dioxide (SFE-CO_2), and Soxhlet extraction were employed for delivering ginger extracts with high concentration of non-polar and low polar bioactive constitunets. SFE-CO_2 was performed at the pressures from 10-30 MPa, process temperature was 40ºC. Two different flow rates of CO_2 were investigated. The highest extraction yield was obtained by SFE-CO_2 (7,74%) at 300 bar and at lower flow rate. The yield obtained with the Soxhlet extraction was lower, 6,60%, indicating higher efficiency of SFE-CO_2. To study the time impact the fractionating of supercritical extarcts at selected pressure has been done. Chemical profiles of all extracts were determined by GC-MS.

Keywords: Zingiber officinale, Supercritical extraction, Herbal dust, Fractionation, Bioactive compounds

Acknowledgements: This study was funded by Ministry of Education, Science, and Technological Development. Project number: 451-03-68/2022-14/200134.

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

MICROENCAPSULATION OF *BACILLUS SUBTILIS* NCIM 2063 BY SPRAY DRYING: PROCESS OPTIMIZATION

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Spray drying is a promising technology for microencapsulation of microorganisms that increases the resistance and durability of the final product. The aim of this work was to assess the individual and combined effects of important spray drying variables on the microencapsulation of *Bacillus subtilis* NCIM 2063, and to provide statistically significant model that simulate the system behaviour. Microbial powder formulations were prepared using spray drying with maltodextrin as a carrier. The three independent process variables: maltodextrin concentration (10-50 g/L), inlet air temperature (110-140 °C) and feed flow rate (6-10 mL/min) were varied, according to Box-Benkhen experimental design. The number of viable cells (CFU/g) and encapsulation efficiency (%) were monitored as response variables. All factors had a significant effect on the product viability and encapsulation efficiency. An increase in concentration of maltodextrin has a positive effect on the viability, while increasing the feed flow rate can reduce the negative impact of temperature. Encapsulation efficiency undergoes the same dependency pattern as viability. The experimental data log values were fitted to a quadratic model that predicts the viability off cells in spray dried powder. The model was statistically significant, adequately fitted and reproducible. A maximum number of viable cells ($1.99 \cdot 10^9$ CFU/g) and a maximum encapsulation efficacy (94.38 %) are achieved at 110 ° C, 8 mL/min of feed flow rate and 50 g/L of maltodextrin concentration. In conclusion, determined relation between the process variables and the most important formulation characteristics greatly facilitates the selection of drying conditions with other plant growth, promoting microorganisms and encourages future research on this subject.

Keywords: Microencapsulation, Spray-drying, Bacillus subtilis, Optimization

Acknowledgements: We would like to acknowledge the financial contribution of the Republic of Serbia - Ministry of Education, Science and Technological Development, Program for financing scientific research work, number 451-03-68/2022-14/200133.

ICAPP 2022
2nd International Conference
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KINETIC MODELS APPLICATION ON ADSORPTION OF HEAVY METAL IONS BY POPLAR AND FIR WOOD SAWDUST IN FIXED-BED COLUMN

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In our paper, the adsorption potential of poplar and fir wood sawdust to remove copper and cadmium ions from aqueous solution was investigated using a fixed bed adsorption column. Experiments were performed in laboratory column filled with 5 g of sawdust. Four kinetics models, Thomas, Adams and Bohart, Yoon–Nelson and Volborska model were applied to experimental data to predict the breakthrough curves and to determine the characteristic parameters of the column useful for process design. Model constants were evaluated for application in column design by linear and nonlinear regression techniques. The predictions of the nonlinear Thomas and Jon-Nelson model correspond better to the experimental data than the other two methods, while in the case of linear models they all show approximately the same agreement with the experimental data.

Keywords: Kinetic models, Adsorption, Heavy metal, Fixed-bed column

Acknowledgements: The work has been supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia under grant no 451-03-68/2022-14/200134.

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

ZEIN FILMS FROM NANOPARTICLE SUSPENSIONS WITH ENCAPSULATED CARVACROL

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Zein is water–insoluble, edible, plant protein, isolated mainly from corn. It can form different colloidal structures, such as nanoparticles, films, fibers, etc., which makes it a desirable material for use in food and pharmaceutical formulations. Because of its hydrophobicity, it is suitable for encapsulation of hydrophobic active substances. Carvacrol is a major component of the essential oils derived from oregano, thyme, marjoram, and summer savory, and is generally recognized as a safe food–grade additive. It is an antioxidant and antimicrobial agent. In this work, different concentrations of carvacrol were encapsulated in zein nanoparticles, by antisolvent precipitation technique. Such obtained suspensions of zein nanoparticles with carvacrol were cast in silicon molds in order to prepare zein films with co–encapsulated carvacrol. Such prepared films were further tested for their physical, optical, and mechanical properties, and benchmarked against zein films without carvacrol. It was shown that zein films can be prepared from zein nanoparticle suspensions with different concentrations of carvacrol co–encapsulated. There was no significant difference in thickness of films with and without carvacrol. Water solubility of all films was below 10%, and water absorption wasn't more than 3%. Addition and concentration of carvacrol did not affect mechanical properties of films. However, carvacrol addition had some effect on optical properties of films.

Keywords: Zein, Edible films, Carvacrol, Encapsulation

Acknowledgements: The work was financed by the Ministry of Education, Science, and Technological Development of Republic of Serbia (Grant No. 451-03-68/2022-14/200134).

2nd International Conference
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ARTIFICIAL NEURAL NETWORK MODEL FOR TURBULENCE PROMOTER-ASSISTED MICROFILTRATION PROCESS OF STARCH SUSPENSIONS

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Microfiltration is the most popular membrane technology to remove dispersed particles during the drinking water and wastewater treatment processes. However, the performance of microfiltration is seriously obstructed by the deposition of the rejected particles on the membrane surface, well known as the phenomenon of membrane fouling. To reduce the negative effects of membrane fouling, different types of turbulence promoters are extensively applied during various crossflow microfiltration processes, providing a significant improvement of the permeate flux.

The aim of this study was to develop a model based on the artificial neural network (ANN) concept to predict flux improvement during crossflow microfiltration of starch suspensions under different operational conditions (suspension flow rate, transmembrane pressure and suspension concentration). To obtain a single neural network to simulate flux improvement, the models with three different training algorithms (Levenberg–Marquardt training algorithm, Bayesian regularization and Scaled conjugate gradient method) and two sigmoid activation functions in the hidden layer (Log-sigmoid and Hyperbolic tangent sigmoid transfer function) were tested. To compare the performance of various ANN configurations, three statistical parameters were used (Coefficient of determination, Mean square error and Mean absolute percent error).

When Levenberg–Marquardt training algorithm was used, ANNs with Log-sigmoid transfer function had higher R^2 than ANNs with Tangent-sigmoid. For the case of Bayesian regularization and Scaled conjugate gradient method, ANNs with Tangent-sigmoid transfer function tended to have higher R^2 compared to the Log-sigmoid transfer function. The highest accuracy (R^2=0.97355) was achieved for the combination of Bayesian regularization and Tangent-sigmoid transfer function.

Keywords: Artificial neural network, Microfiltration, Starch suspension, Static mixer

Acknowledgments: The authors wish to express their sincere gratitude to the Ministry of Education, Science and Technological Development of the Republic of Serbia for their financial support (Project No. 451-03-68/2022-14/200134).

ICAPP 2022
2nd International Conference
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STRUCTURING OF ECO-FRIENDLY POLYURETANE HYBRID FILMS WITH TITANIA NANOPARTICLES

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Recently, thermoplastic polycarbonate-based polyurethane elastomers present very attractive environment engineering materials, due to their unique chemical composition, biodegradability, biocompatibility and desired end-use properties. The modification of polycarbonate-based polyurethanes with titania (TiO_2) nanoparticles allows the structuring of hybrid films important for the sustainable coating industry, concerning environmental protection and regulations related to the removal of organic solvents pollution. In order to meet the strict requirements related to their end-use properties, a proper selection of starting all aliphatic components (macrodiol, chain extender and diisocyanate) as well as the use of adequate preparation technique is of a great importance. According to literature, TiO_2 nanoparticles are very promising strengtheners of thermal stability and flame retardancy of polyurethanes. In this work, a series of eco-friendly thermoplastic polycarbonate-based polyurethane hybrid films were prepared by addition of different titania nanoparticles ratio in one-step procedure, using all aliphatic starting reactants. The influence of nanofillers presence addition on the structure and hydrogen bonding of PC-PU hybrid materials was studied by Fourier transform infrared spectroscopy (FT-IR). Thermal properties of prepared nanocomposite films were analyzed by differential scanning calorimetry. It was found that thermal characteristics of prepared eco-friendly polyurethane hybrid films suitable for sustainable coating design, are dependent on the titania content as well as the phase separation degree between hard and soft segments of prepared elastomers.

Keywords: Sustainable coatings, Eco-friendly polyurethane hybrid films, Titania nanoparticles, Thermal characteristics

Acknowledgements: The authors wish to express their sincere gratitude to the Ministry of Education, Science and Technological Development of the Republic of Serbia for its financial support (Project Number: 451-03-68/2022-14/200134).

ICAPP 2022
2nd International Conference
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ICP-OES AND UHPLC-ESI-MS ANALYSIS OF HYPERFORIN EXTRACTS FROM *HYPERICUM PERFORATUM* L.

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Hyperforin is a polycyclic polyprenylated acylphloroglucinol, a bioactive ingredient of *Hypericum perforatum* L. In addition to its antioxidant, an anti-inflammatory, photodynamic and anticancer property, hyperforin is used to treat depressive states and feelings of sadness. The instability of hyperforin to light and oxygen reduces its potential use in finished products in the form of dietary supplements. Also, the way and extraction conditions affect the final hyperforin content and therefore its activity. The aim of this work was to isolate hyperforin rich extract that can find further application in final products and to carasterise its chemical content. Extraction of hyperforin from *Hypericum perforatum* L. was carried out by conventional maceration and sonication method with four different solvents: n-hexane, petroleum ether, methanol and ethanol. The extract samples were analyzed by ultrahigh liquid chromatography coupled with diode array and electrospray ionization mass spectrometry (UHPLC-ESI-MS) and inductively coupled plasma-optical emission spectrometry (ICP-OES) method. The applied UHPLC-ESI-MS method can be characterized as selective and fast method of analysis. The active compound content in extracts increase on solvents polarity: n-hexane> petroleum ether>ethanol>methanol. The highest hyperforin concentration of 3.5680 mg per g of dry plant material was detected in methanol extract obtained by maceration method. ICP-OES analysis showed that extracts of ethanol and methanol isolated by maceration have the highest mineral content (K>P>Mg>S>Ca>Na>Zn>Si>Cu). Based on obtained results, in terms of nutritional benefits as well as active compound high concentration, isolation of hypeforin should be preferably carried out by methanol.

Keywords: UHPLC-ESI-MS, ICP-OES, Hyperforin, Hypericum perforatum L.

Acknowledgements: Authors wish to express their gratitude to the Republic of Serbia - Ministry of Education, Science and Technological Development, Program for financing scientific research work, number 451-03-68/2022-14/ 200133.

2nd International Conference
on Advanced Production and Processing

THE INFLUENCE OF HYDROCHAR ON THE RUBBER PRODUCTS AGING

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The rubber products are commonly used in a great number of applications and favoured when their highly deformable characteristics are required. They are commonly obtained using the recipe that includes Sulphur, essential additives and reinforcement agent (filler). Traditionally used filler is carbon black (CB), due to its graphite crystal structure that improves the final products' characteristics and its low cost. The CB's shortcoming is that its production is highly dependent on the fossil fuels, and, due to current rapidly growing energy demand, rubber industry has a rapid need to find the environmentally friendly and sustainable fillers for CB replacement, such as hydrochar. As the process of hydrothermal carbonization can convert any organic matter into a stable carbon form, hydrochar can be obtained from all types of biomasses in a high pressure stainless steel reactor. Hardwood waste biomass was used to prepare hydrochar by hydrothermal carbonization treatment. The hydrochar and CB were blended using the commercial recipe for natural rubber mixture. The amount of hydrochar and CB was varied, while the total filler amount was kept at 50 phr. The examination of hydrohar influence on the rubber aging was performed in the oven with a circulating gas, at a constant temperature and varied retention time. Dynamic-mechanical properties were tested after aging and the results showed that with longer aging time, higher hydrochar content caused decreasing storage and loss modulus values. On the other hand, the obtained results showed that the mixture containing low hydrochar amount did not significantly differ from rubber matrix with pure CB. The results of this study revealed that certain amount of hydrochar can potentially replace CB, without causing the significant rubber products aging, and enabling rubber industry to follow up the strict environmental protection demands.

Keywords: Hydrochar, Carbon Black, Natural Rubber, Rubber Aging

Acknowledgements: The authors would like to acknowledge to the Ministry of Education, Science and Technological Development of the Republic of Serbia for their financial support, Project No. 451-03-68/2022-14/200134.

ICAPP 2022
2nd International Conference
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CIPROFLOXACIN REMOVAL USING SEPIOLITE-BASED ADSORBENTS

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Ciprofloxacin (CIP) as a broad-spectrum quinolone antibiotic is extensively used in daily life and can be detected in municipal and pharmaceutical wastewaters, as well as natural waters. In this study, natural mineral sepiolite (SEP), SEP modified with hexadecyltrimethylammonium-bromide (HDAB) and SEP composites with graphene oxide (GO) were used as adsorbents for the removal of CIP from aqueous solutions. The composites were prepared at a mass ratio SEP to GO of 2 : 1, using SEP and SEP modified by [3-(2-aminoethylamino)propyl]trimethoxy-silane (APT). Thermogravimetric analysis, X-ray diffraction and nitrogen adsorption-desorption were used to characterize the obtained adsorbents. The adsorption of CIP was investigated at the pH value close to pH of natural waters. The concentration of CIP in aqueous solution was determined by UV-Vis spectroscopy. GO showed the highest adsorption capacity (~ 150 mg/g). The capacity of SEP was about 60 mg/g, while the SEP modification with HDAB has led to a decrease in the adsorption capacity (~ 35 mg/g). The composite SEP/GO showed the higher adsorption capacity (~ 110 mg/g) than the composite SEP-APT/GO (~ 40 mg/g). The capacity of the composite SEP/GO was higher than of mixture of SEP and GO, which indicated a certain degree of GO exfoliation and intecalation of sepiolie particles, as it was shown by X-ray diffraction. Equilibrium data for all adsorbents, except for HDAB modified sepiolite, fitted well to the Langmuir isotherm model.

Keywords: Adsorption, Ciprofloxacin, Sepiolite, Graphene oxide

Acknowledgements: The authors acknowledge funding from the Ministry of Education, Science and Technological Development of the Republic of Serbia through the contracts No. 451-03-68/2022-14/200287, and 451-03-68/2022-14/200135.

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

REVIEW OF SIMULATION TOOL PACKAGES FOR LIQUID – SOLID TWO PHASE FLOW

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Mixtures containing liquid and solid phase are still a challenge for numerical simulation and numerous software packages are used for these purposes. The solution is a combination of the Discrete Element Method (DEM) and Computational Fluid Dynamics (CFD). Therefore, in this paper, the approach of numerical simulation instead of conventional experimental determination of particle velocity in two-phase flow will be analyzed. Available scientific literature and software packages will be examined and commented. Governing equations of continuity and momentum will be introduced and their accuracy of estimation will be analyzed comparing with experimental results. This way, the main goal will be the possibility of using numerical simulation for problems that are difficult or impossible to determine experimentally. The Euler – Lagrange approach is reviewed, as it treats particles as discrete elements, which calculates interactions between the solid and fluid phase and collisions among particles, for each particle independently. Discrete phase model in ANSYS Fluent gave a demonstration of relatively good prediction of critical velocities in two – phase flows. For modeling macroscopic particles, that requires special treatments as taking into account effects like fluid volume blockage, the proper evaluation of drag forces, collision effects and friction dynamics, Macroscopic Particle Model is evaluated and discussed. Coupling methods, between DEM and CFD is innovative, promising approach to model two phase flows. Two different coupling methods are reviewed, coupling between ANSYS Fluent and ROCKY DEM – simulation of fluidized bed, and coupling between ANSYS Fluent and EDEM – simulation of abrasion effects on process equipment. Simulations generated realistic solid phase trajectories, relatively good values of velocities and pressure drops that showed good accordance to literature correlation.

Keywords: Numerical simulation, Liquid – solid flow, CFD, DEM

Acknowledgements: The authors would like to acknowledge to the Bilateral project between Republic of Serbia (Ministry of Education, Science and Technological Development) - Project No. 451-03-812/2021-14/8 and Republic of Turkey (TUBITAK) – Project No. 220N413 for the financial support.

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

TREATMENT OF MWF AEROSOL BY WASTE FIBERS

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Metalworking fluids, MWFs, are applied during different machining processes (like boring, milling, grinding, drilling, etc.) to lubricate and cool the contact surface between working and metal pieces. MWFs could be straight oils, applied as oil, or soluble oils (semi-synthetic and synthetic) applied as water emulsions. During the application of MWF, a huge amount of MWF aerosol is generated in the working environment. MWF aerosol represents a significant hazard to the health and safety of the workers. Exposure to MWF aerosol may cause laryngeal cancer, asthma, bronchial hyper-responsiveness, lipoid pneumonia, lung cancer, dermatitis, etc. Therefore, it is necessary to use an effective separation method for the treatment of MWF aerosol. In this paper, the separation efficiency of coalescent filtration with a filter bed formed from waste fibers is investigated for the treatment of MWF aerosol. The filter bed is formed from waste fibers from carpet production. MWF water emulsion made of 10% soluble oil is used to generate MWF aerosol. Different velocities of ventilation air are applied: 1 m/s, 3 m/s, 4 m/s, 5 m/s. The results show high separation efficiency, in the range of 92%-100%, which confirms that coalescers with waste fibers from the carpet industry could successfully be used for the treatment of MWF aerosol.

Keywords: MWF aerosol, Occupational health and safety, Coalescer, Waste fibers

Acknowledgements: The Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. 172022, Grant No. 451-03-9/2021-14/200156 supported the work. We sincerely thank CEEPUS - Central European Exchange Program for University Studies for supporting mobilities between the University of Novi Sad and TU Wien within the network "CIII-SI-0708-08-Chemistry and Chemical Engineering".

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2nd International Conference
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SILK FIBROIN SOLUTIONS – CHARACTERIZATION AND INTERACTIONS WITH OTHER MACROMOLECULES

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Silk fibroin can be isolated from silk fibers, which are produced by cultivated *Bombyx mori* silkworm. Silk fibers mainly consist of two proteins, sericin and fibroin, and their content depends on the type of cocoons. The isoelectric point of fibroin varies in the range pH 3.6–5.2, depending on the solution preparation conditions. Native fibroin is soluble in a number of solvents and insoluble in water, but it can be regenerated in aqueous solution, and then further processed into sponges, films, hydrogels, etc. Silk fibroin has a wide application due to its high mechanical properties, high biocompatibility with living tissues, low biodegradability and minimal inflammatory reactions. The aim of this paper was to prepare and characterize an aqueous solution of fibroin and to investigate interactions with other macromolecules. Degumming is the process of removing sericin from silk fibers and is the first step in preparing a fibroin solution. Fibroin was dissolved in 9.3M LiBr and then dialyzed against water. Intrinsic viscosity of the prepared solution was determined with glass capillary viscometer in the temperature range from 10 to 70°C. The isoelectric point of fibroin was determined by measuring the zeta–potential and turbidity of silk fibroin solution at different pH values. Interactions of fibroin and other macromolecules at different pH values and at different mass ratios of macromolecules were also investigated. The isoelectric point of fibroin is at about pH 4.2. According to the flow times of the solution, the fibroin solution was found to be stable for up to three weeks if stored in the refrigerator.

Keywords: Silk fibroin, Isoelectric point, Viscosity, Macromolecule interactions

Acknowledgements: This work was financed by Ministry of Education, Science and Technological Development of Republic of Serbia, 451-03-68/2022-14/200134.

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

RAPID REMOVAL OF TOLPERISONE FROM WATERS BY APPLICATION OF HETEROGENEOUS PHOTOCATALYSIS

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Tolperisone hydrochloride (TLP) is a piperidine derivative and belongs to the group of muscle relaxant pharmaceuticals. TLP affects spasticity through interaction with upper motor neurons (central acting muscle relaxants). It is commercially available under different trademarks (e.g. Biocalm®, Muscodol®, Mydeton®, Mydocalm, etc.) and used in various treatments of rheumatological, orthopedic and traumatic neurological disorders. TLP is extremely hazardous for water and should not be allowed to reach the ground water, water course or sewage system, even in small quantities. Moreover, it is dangerous to the drinking water if even extremely small quantities leak into the ground. Advanced oxidation processes are proved to be capable as method that may remove up to 100% of various drugs from waters. Heterogeneous photocatalysis is based on the application of different semiconductors as photocatalysts (such as TiO_2, ZnO, etc.). This technology has key advantage of including operation at ambient conditions with catalyst that are inexpensive, commercially available in various crystalline forms, with particle characteristics and photochemically stable. Accordingly, for removal of TLP from water, the photocatalytic experiments in the presence of two types of photocatalyst, namely TiO_2 and ZnO with simulated solar irradiation and UV light were carried out. The findings obtained by using chemometric analysis showed that the complete TLP removal can be reached using TiO_2 as photocatalyst (1.0 mg/mL), after 60.83 min of UV irradiation. To confirm removal of TLP from samples and to detect the eventual photodegradation intermediates, LC–ESI–MS analysis was done.

Keywords: Heterogeneous photocatalysis, Tolperisone, Chemometric analysis

Acknowledgements: This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200125) and by the Science Fund of the Republic of Serbia (Grant No 7747845, In situ pollutants removal from waters by sustainable green nanotechnologies-CleanNanoCatalyze).

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APPLICATION OF INDIRECT PHOTOLYSIS AND PHOTOCATALYSIS IN THE REMOVAL OF THE ACTIVE COMPONENT OF THE DRUG CIPROCINAL® FROM WATER

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Ciprofloxacin is a broad-spectrum antibiotic and belongs to the class of fluoroquinolone antibiotics of the second generation. It is one of the most effective and best-selling antibiotics in the world, with over 300 different registered drugs. Due to its widespread use, ciprofloxacin was detected in wastewaters, surface, ground, as well as in drinking water. Removal of antibiotics and non-metabolized antibiotic residues is still a challenge in the field of environmental protection. Poor biodegradability of antibiotics contributes to their long-term retention in the environment and thus leads to toxic effects on the global ecosystem and public human health. Bearing in mind the complexity of most samples taken from the environment (samples of wastewater, soil, sludge, etc.), the selection of an appropriate method for pollutant removal plays an important role. In this work the effectiveness of direct and indirect photolysis, as well as photocatalysis of ciprofloxacin present in the commercial formulation (drug Ciprocinal®) under UV irradiation was investigated. In the case of indirect photolysis process, effect of different concentrations of H_2O_2 and $KBrO_3$ on the ciprofloxacin removal was studied. Along with that, the research focus of this study was determination of the influence of the different concentrations of selected electron acceptors (O_2, H_2O_2 and $KBrO_3$) on the photocatalytic degradation of the drug active compound in the presence of ZnO as photocatalyst. The obtained results showed that the highest removal efficiency of ciprofloxacin was achieved by application of photocatalysis in the presence of ZnO and $KBrO_3$. The degradation efficiency of ciprofloxacin was monitored by ultrafast liquid chromatography with a diode array detector.

Keywords: Photocatalysis, Indirect photolysis, Ciprocinal®, Ciprofloxacin, UV irradiation.

Acknowledgements: This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200125) and by the Science Fund of the Republic of Serbia (Grant No 7747845, In situ pollutants removal from waters by sustainable green nanotechnologies-CleanNanoCatalyze).

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OPTIMIZATION OF BIODIESEL PRODUCTION FROM WHITE MUSTARD SEED OIL BY CORN STALK ASH

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The synthesis of fatty acid methyl esters (FAMEs) was performed from white mustard seed oil over ash obtained by burning corn stover. The corn stover, a waste of corn harvest, can be used to get thermal energy, and the ash generated by combustion can be used as a catalyst in the production of biodiesel. The process conditions of the methanolysis (catalyst concentration, methanol/oil molar ratio, and reaction time) were optimized according to a 33 experimental design with five central points. A second-order polynomial equation was used to model the experimental data on FAME content. Analysis of variance (ANOVA) showed that the most significant influence on the FAME content is the catalyst concentration, reaction time, and finally, the methanol/oil molar ratio. The optimal conditions for the methanolysis of white mustard oil to achieve the maximum FAME content were a catalyst concentration of 17.5% (based on oil weight), methanol/oil molar ratio of 9.7:1, and reaction time of 50 min. The predicted value of FAME content based on the second-order polynomial equation (97.7%) was close to the experimental obtained value of MEMK content (97.1%), indicating that the applied model was acceptable for predicting MEMK content in the tested range of the reaction conditions.

Keywords: Biodiesel; Methanolysis; Modeling; White mustard seed oil.

Acknowledgements: The present work has been funded by the Ministry of Education, Science and Technological development of the Republic of Serbia, Program for financing scientific research work, No.451-03-68/2022-14/200133. It is also a part of the Project 0-14-18 of the SASA Branch in Niš, Serbia.

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THE KINETICS OF THE BIODIESEL PRODUCTION FROM RAPESEED OIL OVER CALCINED WASTE FILTER CAKE FROM SUGAR PRODUCTION

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This work deals with the kinetics of biodiesel production by the transesterification of rapeseed oil with methanol in the presence of a catalyst prepared by calcining waste filter cake from an industrial sugar production from sugar beets. The reaction conditions of the rapeseed oil transesterification were as follows: the methanol-to rapeseed oil molar ratio of 9:1, the catalyst loading of 4–10% of the oil weight, and the reaction temperature of 40–60 °C. The oil was recovered from the rapeseed by a screw press followed by vacuum filtration to remove solid residues. The waste filter cake, consisting mainly of $CaCO_3$ and the precipitated organics, was calcined at 900 °C for 2 h before the use. The kinetics of the transesterification reaction was described by a model combining the changing mechanism and the first-order rate law for triacylglycerols (TAGs) and fatty acid methyl esters, respectively. A good agreement between the model and the experimental data was proved by the mean relative percentage deviation for TAG conversion degree of only ±7.43% (based on 42 data). Finally, the properties of the produced biodiesel were determined and compared with the biodiesel quality standard.

Keywords: Biodiesel; Catalyst, Kinetics, Transesterification, Waste filter cake

Acknowledgements: The present work has been funded by the Ministry of Education, Science and Technological development of the Republic of Serbia, Program for financing scientific research work, No.451-03-68/2022-14/200133. It is also a part of the Project 0-14-18 of the SASA Branch in Niš, Serbia

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BLENDS OF PALM AND CASTOR OILS FOR BIODIESEL PRODUCTION

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The production of biodiesel depends on the availability of oily feedstocks. Therefore, shifting its production from a single feedstock to oil blends is promising to ensure the sustainability of biodiesel production. Moreover, using oil blends for biodiesel production could improve the fuel quality at a minimum effort and cost. For instance, blending the palm oil containing a high content of saturated fatty acids with non-edible castor oil with a high content of ricinoleic acid could produce biodiesel with favorable characteristics like good oxidative stability and lower cloud point. The biodiesel production from the blends of used palm and refined castor oil via methanolysis catalyzed by calcium oxide was studied. The calcium oxide was calcined at 550 °C for 2 h before use. The methanolysis of oil blends was conducted in a stirred batch reactor at 60 °C under atmospheric pressure. This study aimed to estimate the effect of the palm:castor oil blends ratio (50:50, 70:30, and 90:10 wt%) and catalyst amount (1, 5, and 9%) on the reaction rate and methyl ester content. Blending the used palm oil with castor oil enhanced the reaction rate. Increasing the castor oil content from 10 wt% to 50 wt% increased the reaction rate and methyl ester content. This positive effect of castor oil could result from its high miscibility with methanol. Notwithstanding that at the beginning, the methyl ester content increased with an increase in catalyst amount, their content in the final period of the reaction did not change significantly. The exponential change of methyl esters content indicated that mass transfer resistance did not occur at the beginning of the reaction. It suggested the efficient mixing of the reaction mixture was favored by the increased miscibility of castor oil with methanol. A high methyl ester content (>96.5 %) was achieved within 50 min.

Keywords: Biodiesel, Calcium oxide, Methanolysis, Oil blends

Acknowledgments: The present work has been funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Program for financing scientific research work, No.451-03-68/2022-14/200133 and No.451-03-68/2022-14/200383. It is also a part of the Project 0-14-18 of the SASA Branch in Niš, Serbia

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BRILLIANT GREEN DYE BIOSORPTION USING IMMOBILIZED SPENT BREWER'S YEAST

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In this research, a carrier matrix for immobilization of *Saccharomyces cerevisiae* was designed. The immobilized yeast was used for the removal of brilliant green dye (BG) from aqueous solutions. The yeast from the beer industry was washed, dry, and immobilized in alginate by extrusion technique. The obtained beads have about 3.5mm in diameter measured on a light microscope. The influence of different factors such as contact time, pH, adsorbent dosage, and initial dye concentration, on the adsorption of BG onto immobilized yeast, was investigated. The beads were also coated with 0.4 and 0.8 % chitosan layer and analyzed for BG sorption. FTIR analysis was performed to determine the characteristics of the material and material - BG interaction. The research has shown that 40 g/L alginate-yeast beads (AY) had higher q (amount of BG adsorbed by yeast, mg/g) compared to sorbent concentration of 60 g/L. The pH of the solution showed to play an important role in the sorption process. The percentage of BG sorption by yeast immobilized in alginate was similar for pH 4 and pH 8, but significantly lower for pH 6. The coated beads showed significantly higher sorption compared to uncoated alginate-yeast beads. The FTIR spectrum of all samples with BG showed shifts in bands to higher and/or to lower with the appearance or disappearance of some bands, compared to samples without dye, which indicate the adsorption of the BG dye.
It can be concluded that immobilized yeast in an alginate carrier is good sorbent material for brilliant green dye. Also, alginate-yeast beads coated with a 0.8 % chitosan layer show the highest sorption compare to all used beads.

Keywords: Brilliant green, Biosorption, Yeast immobilization, Alginate, Chitosan.

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/200287)

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POTENTIAL USE OF CYANOBACTERIA *NOSTOC* SP. IN BIOREMEDIATION OF HEAVY METALS-CONTAMINATED EFFLUENTS

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Cyanobacteria are well known for their ability to remove a variety of pollutants from waters, including toxic heavy metals (HMs). This study investigated the potential remediation of wastewaters by cyanobacteria *Nostoc* sp. Five heavy metal ions were selected for the experiment including Cd^{2+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, and Zn^{2+}. Removal efficiencies of HMs by cyanobacteria *Nostoc* sp. using bioaccumulation and biosorption processes were evaluated and compared. Results confirmed the high efficiencies of the investigated species of cyanobacteria for the removal of the target contaminants which were concentration and contaminant-dependent. Live cells of *Nostoc* sp. have the highest affinity to bioaccumulate Pb (98.15%) and Cu (95.14%) from the solution. However, the biosorption capacity of dried biomass of *Nostoc* sp., besides Pb (92.27%) and Cu (96.00%), was high for Cd (91.00%) removal as well. Living cyanobacterial cells of *Nostoc* sp. were able to accumulate 82% of Zn, while dead cyanobacteria biomass adsorbed 87% of Zn. In the case of Ni, cyanobacteria *Nostoc* sp. did not show significant bioremediation potential. Through the bioaccumulation process, removal of Ni was only 38%, while the biosorption process was more efficient with 63.80% of Ni removal. These results indicate the potential of *Nostoc* sp. cyanobacteria as an efficient agent for pollution control. Furthermore, the data obtained represent a base for further investigation towards the development of a suitable biosorbent system that could be used for industrial effluent treatment.

Keywords: Biosorption, Bioaccumulation, Toxic metals uptake, Wastewaters, Cyanobacteria.

Acknowledgments: This work is a result of the research funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. 451-03-68/2022-14/200134).

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IMMOBILIZATION OF HORSERADISH PEROXIDASE ONTO BIO-LINKED MAGNETIC PARTICLES WITH *Allium cepa* PEEL WATER EXTRACTS

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In this paper the suitability of bio-magnetite as a solid support for covalent immobilization of horseradish peroxidase (HRP) across glutaraldehyde was examined. The activity of immobilized HRP at different pH values (4-9) and temperatures (20-80°C) and reusability were examined. Bio-linked magnetite nanoparticles (bio-MNPs) was synthetized by co-precipitation method from Fe(II) and Fe(III) sulfate salts in the presence of water extract of the *Allium cepa* peel. The water extract showed 81% of antiradical potential (according to DPPH assay), which is connected with the high polyphenol content. According to the FTIR analysis, the bio-magnetite contains oxygenic functional groups suitable for binding to glutaraldehyde, after which the enzyme could be covalently immobilized. The immobilized enzyme showed high activity at ambient temperature and pH7 (30 U/g) and shows high activity (around 80%) at a wide range of pH (5-8) and temperature (20-50°C). The immobilized HRP onto bio-MNPs was remarkable stable towards temperature and pH perturbations, compared to the free enzyme form. On the other hand, immobilized HRP showed low reusability. After first washing cycle enzyme retains 50% its activity, while after the third washing cycle retains only 22%.

Keywords: Bio-linked magnetite nanoparticles, Enzyme immobilization, Enzyme activity, Allium cepa peel water extracts

Acknowledgements: This work was supported by Ministry of Education, Science and Technological Development of Republic of Serbia (Grant No. 451-03-68/2022-14/200134, 451-03-68/2022-14/200125) and COST Action CA18130 ENFORCE-TXRF.

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APPLICATION OF MAGNETITE NANOPARTICLES AS ADSORBENT FOR ARSENIC(V) REMOVAL FROM WATER

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Magnetite nanoparticles (MNPs) are group of nanoparticles that can be manipulated by the effect of magnetic fields and have great potential application in environmental processes. Selective magnetic separation of MNPs from solutions is technologically important because it simplifies the process of water treatment and reducesits cost. In this work the synthesis of MNPs and their application for As (V) removal from water was investigated. The MNPs were synthetized from Fe^{2+} and Fe^{3+} sulphate salts by co-precipitation method in alkali conditions (pH 10.0) at 80°C. XRD analysis confirmed the presence of magnetite nanoparticles with mean crystallite size of 10 nm. MNPs showed high efficiency in As (V) removal from water solution of initial concentration of 1069 µg/L. Using MNPs in concentration of 5 g/L almost all the amount of arsenic ions from water was removed after 2 h (residual concentration of As was 1.71 µg/L). After 30 min of adsorption process the residual concentration of arsenic in the water was < 10 µg/L, thus it can be concluded that, in terms of arsenic content requirements for drinking water quality standard, has been satisfied. Additionally, the results of the kinetic studies were analyzed using three kinetic models, while the adsorption equilibrium was tested by means of four different adsorption models.

Keywords: Magnetite nanoparticles, Arsenic removal, Adsorption, Water treatment

Acknowledgements: This work was supported by Ministry of Education, Science and Technological Development of Republic of Serbia (Grant No. 451-03-68/2022-14/200134, 451-03-68/2022-14/200125). Part of this work was supported by the COST Action CA18130 ENFORCE-TXRF.

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2nd International Conference
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ANALYSIS OF THE FOULING OF CROSS-FLOW ULTRAFILTRATION MEMBRANE

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Membrane filtration is a widely used technique in many industries, especially for treating the waste stream as well as in the production of drinking water. Numerous organic and inorganic matters cause fouling of the membrane in pressure-driven membrane processes and decreasing process capacity. Mechanisms of fouling can be various such as pore blocking or formation of the layer of cake on the membrane surface. It is indicated by the appearance of concentration polarization. One of the organic foulants of the membrane is humic acid, while silica particles, can appear as inorganic foulants. The aim of this paper was to investigate the influence of organic-inorganic mixture on the mechanism of ultrafiltration membrane fouling. A ceramic membrane of 100 kDa cut-off was used for the cross-flow ultrafiltration. The investigation was carried out at different transmembrane pressure and feed flow rates. The concentration of humic acid and colloidal silica were varied. Fouling was analysed testing the models for four mechanisms of fouling: cake filtration, complete, intermediate and standard blocking of the pore. Experimental data were fitted with chosen models for cross-flow ultrafiltration. It has been observed that the fouling of the membrane is a combined cake formation mechanism with the dominant occurrence of the intermediate pore blocking. Moreover, it was noticed that the mechanism depends on working conditions, mostly on the feed flow rate. Further, it was found that unlike an organic-inorganic mixture, which causes the surface fouling, the organic mixture, mostly causes the intermediate blocking of the pore.

Keywords: Modeling, Membrane fouling, Humic acid, Colloidal silica

Acknowledgements: This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Project No.451-03-68/2022-14/200134.

2nd International Conference
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THE INFLUENCE OF FERTILIZERS ON THE SOYBEAN SEEDS QUALITY

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Soybean crop becomes more and more interesting to farmers and consumers due to its seeds, which represent valuable raw material for a wide range of food products. Because the quality of soybean seeds is related with the crop technology, this research was directed towards the study of the fertilizers' influence in this context. A field experiment was conducted at Research and Development Station for Agriculture (RDSA) Turda, Romania. Caro TD, an early soybean variety, registered in 2015 at RDSA Turda station was used as biological material. The experiment included a wide range of organic and chemical fertilizers, treatments being applied according to the recommendation of each producer. Dry matter, ash content, protein content, lipids content, total carbohydrates, total flavonoids, total phenols and total carotenoids content were determined, as well as major isoflavones (glycitin, daidzein, glycitein and genistein). Descriptive statistics was used to emphasize the influence of treatments on the seed quality features. It was established that generally, ecological products significantly increased the values for studied parameters; an increase in isoflavone content was observed in the treatment based on basic fertilization ($N_{20}P_{20}K_0$) combined with seed treatment and foliar bio-fertilizer with active humic fertilizer + bio-fertilizer with NPK, amino acids and microelements. The protein content was positively correlated with: glycitin, daidzein, glycitein and genistein; positive correlations were established between lipid content and total phenols and total carotenoids content.

Keywords: Soybean seeds, Quality, fertilizer, Isoflavone, Carbohydrates, Flavonoids.

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BIOMASS HARVESTING FROM *Bacillus* BACTERIA FERMENTATION BROTH WITH NATURAL FLOCCULANTS

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Biocontrol research is mostly done to replace or reduce the use of synthetic chemicals due to concerns about the effects of these chemicals on human health and the environment. For the cultivation and downstream processing of microbial broth, low-cost and ecologically friendly technologies are needed in order to produce biomass on a wide scale for low-value applications, such as agricultural production. Thus, harvesting bacterial biomass represents a significant challenge. Flocculation is considered a cost-effective and promising harvesting method. The choice of flocculants also depends on the desired microbial biomass product. Inorganic metal salts are restricted in their use as they can contaminate the biomass along with the effluent quality during flocculation. The use of some chemical flocculants can also have adverse effects. To overcome these problems, natural polymers or natural flocculants have been used for harvesting.

The aim of this work is to evaluate egg shells and three types of chitosan as flocculants for *B. amyloliquefaciens* biomass harvesting. Experiments were performed to determine the optimum flocculant dose, at pH value 5.0, using three different types of chitosan type A: ≥75% deacetylated; molecular mass 100,000-300,000 Da; type B: ≥75% deacetylated, molecular mass 310,000-375,000 Da and type C: ≥90% deacetylated, molecular mass 600,000-800,000 Da, as well as egg shells.

It was found that chitosan A had slightly higher flocculation efficiency than chitosan B and C. The highest degree of flocculation efficiency (97.04%) was achieved when chitosan A concentration was 120 mg/L. The highest degree of flocculation efficiency (90.83%) was achieved when egg shells concentration was 10 g/L. It can be concluded that different types of chitosan and egg shells could be successfully applied as the flocculating agents for separation of *Bacillus amiloliquefaciens* from the cultivation broth.

Keywords: Chitosan, Egg shells, Flocculation, Bacillus amiloliquefaciens

Acknowledgements: Research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-68/2022-14/200134, and by the Science Fund of the Republic of Serbia, PROMIS, #6064541, BioSolAfla.

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PHOTOCATALYTIC EFFICIENCY OF ZnCr-MIXED METAL OXIDES IN CORRELATION TO REACTION PARAMETERS

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Various organic pollutants are discharged into the water from chemical, textile and pharmaceutical industries every day. The photocatalytic wastewater treatment is an efficient, cheap and eco-friendly process for the removal of organic toxic dyes, such as methylene blue, which is one of the most common toxic dyes in wastewater. Photocatalysts ZnCr mixed oxides were synthesized and thermally treated at 500 and 900 °C. Their efficiency in photocatalytic degradation of methylene blue and the influence of the reaction parameters were studied. High photocatalytic activity of both samples was observed, where the complete decolorization (100%) and partial decolorization (45%) of the aqueous solution of methylene blue were achieved after 6 hours for ZnCr 900 and ZnCr 500, respectively. In order to determine the influence of the pH on the photocatalytic effieciency, the photodegradation reactions were conducted using better performing photocatalyst at different pH values, measuring the photodegradation efficiency at the same time intervals. The optimal pH resulting in the most efficient photodegradation for ZnCr 900 photocatalyst was observed at pH 12, which could be explained by the correlation with different textural and structural properties of photocatalysts. The results showed that the obtained ZnCr mixed oxides are highly efficient in the methylene blue photodegradation and have a great potential for the application in the photocatalytic wastewater treatment processes.

Keywords: Photocatalytic water purification, Methylene blue, Mixed metal oxides

Acknowledgements: This work was supported by the Science Fund of the Republic of Serbia, project no. 7737365, ZERO-WASTE CONCEPT FOR FLOOD RESILIENT CITIES – Ø-Waste-Water

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THE GENETIC PROGRESS OF THE HYBRIDS CREATED AT ARDS TURDA REGARDING THE BIOCHEMICAL COMPOSITION

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Maize (Zea mays) is one of the main crops worldwide in terms of yield, harvested area and production quantity. For the cultivation of this plant a special importance must be given to the quality of the grains. Of the total dry matter, the predominant biochemical elements of maize are starch, protein, fat, sugar, fiber and ash. The researches of the Maize Breeding Laboratory from the Agricultural Research and Development Station Turda were oriented both, towards the creation of productive hybrids, adapted to the cultivation area, and towards obtaining genotypes with a superior quality. In the present study, 11 extra-early, 13 semi-early and 13 early hybrids were analyzed. All hybrids are created at ARDS Turda. This study presents the results on the percentage of protein, starch, fat and fiber. The analyzes were carried out using Tango FT-NIR spectrophotometer. From the group of extra early hybrids, the following hybrids were noted: HD 115 for protein content, Turda 100 for starch, Turda 145 for fiber and Turda 199 for fat. From the group of semi-early ones, the following hybrids were noticed: Turda 228 for protein content, Turda 160 for starch, Elan for fiber and Turda 213 for fat. Regarding the early hybrids, the highest content of biochemical elements was found in: Turda 260 for protein, Turda 332 for starch, and Saturn for fiber and fat.

Keywords: Maize, Hybrids, Protein, Starch, Fat

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ANTIOXIDANT CAPACITY OF HERZEGOVINIAN WILDFLOWERS EVALUATED BY UV-VIS AND CYCLIC VOLTAMMETRY ANALYSIS

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Considering vast cultural and traditional heritage of the use of aromatic herbs and wildflowers for the treatment of light medical conditions in Balkans, comparsion of antioxidant capacity of wildflowers extracts from Herzegovina was studied using both cyclic voltammetry and spectrophotometry. The cyclic voltammograms taken in the potential range between 0 V and 800 mV and scan rate of 100 mV s^{-1} were used for quantification of electrochemical properties of polyphenols present in four aqueous plant extracts. Antioxidant capacity expressed as mmol of gallic acid equivalents per gram of dried weight of sample (mmol GAE/g) was deduced from the area below the major anodic peaks (Q400 pH 6.0, Q500 pH 4.7, Q600 pH 3.6). Results of electrochemical measurements suggest that the major contributors of antioxidant properties of examined plants are polyphenolic compounds that contain ortho-dihydroxy-phenol or gallate groups. Using Ferric reducing-antioxidant power (FRAP) and 2,2'-azino-bis spectrophotometric methods (3-ethylbenzthiazoline-6-sulfonic acid) radical cation-scavenging activity (ABTS) additionally determined antioxidant capacity. The Folin-Ciocalteu procedure was applied to determine total polyphenolic content (TP). Measurements of total flavonoid (TF) and total condensed tannin (TT) content were also performed to obtain a broader polyphenolic profile of tested plant materials.

Keywords: Cyclic voltammometry, Spectrophotometry, Plant extracts, FRAP, ABTS.

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

PREPARATION AND DETERMINATION OF STABILITY AND PHYSICOCHEMICAL PROPERTIES OF TERPENE-BASED HYDROPHOBIC EUTECTIC SOLVENTS

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Terpene-based hydrophobic deep eutectic solvents are considered to be relatively non-toxic, less volatile, and environmentally friendly, which makes them promising alternatives to conventional solvents in liquid-liquid extraction of non-polar analytes from an aqueous environment. In this paper, series of different hydrophobic "deep" eutectic solvents based on L-menthol and Thymol (as H-bond acceptors) and a number of organic acids (as H-bond donors), with chain lengths from 8 to 18 C atoms, were prepared. Chemical stability of ten solvents was determined 24 hours after preparation and only four solvents formed a homogeneous transparent liquids and no crystallization at room temperature was observed. Considering that in the previous measurements of the extraction efficiency, the best results were obtained by the solvents with the composition: Men:DecA (1:1) and Men:OctA (1:1), the physicochemical properties (viscosity, density), electrical conductivity, thermal analysis (TG/DSC), water content and FTIR characterization for these solvents were carried out. FTIR spectra of prepared solvents compared to individual components, confirmed formation of H bonds between donors and acceptors, in both cases. The results of Karl-Fischer titrations showed low water content (236 ppm for the Men:DecA (1:1) while for the Men:OctA (1:1), measured 258 ppm water), which confirms their hydrophobicity. Also, measured difference between solvent densities compared to water, enable macroscopic separation of phases during extraction. Furthermore, viscosities lower than 100 mPa·s, makes them acceptable solvents in industrial scale processes. Thermal stability of prepared solvents compared to starting materials, also contribute to their acceptability for investigated purposes.

Keywords: Hydrophobic deep eutectic solvents, Menthol, Thymol, Decanoic acid, Octanoic acid

ICAPP 2022
2nd International Conference
on Advanced Production and Processing

THE INFLUENCE OF FILTRATION OF COMMON BEAN CRUDE EXTRACT ON ITS COAGULATION ABILITY

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A common bean crude extract which can be used as natural coagulant for water and wastewaters purification contains a significant amount of suspended particles. The hypothesis of this work was that these particles may promote water or wastewaters clarification by building into flocs during coagulation and flocculation and increasing their weight. Besides that, skipping of filtration would simplify and reduce a cost of natural coagulant preparation.
Common bean dry seeds were ground to a fine powder by using a laboratory mill and sieved through 0.4 mm sieve. The fraction with particle size smaler than 0.4 mm was used in experiments. Fifty grams of seed powder was suspended in 1 L of either NaCl water solution (0.5 mol/L) or distilled water. The suspensions were stirred using a magnetic stirrer for 10 min to accomplish extraction. Halves of both extracts were remained as this (crude extracts of active components) and other halves were filtered through a rugged filter paper (Macherey-Nagel, MN 651/120) to obtain filtrates. Obtained crude extracts and filtrates were applied as coagulants in model waters whose pHs were 10 and initial turbidities 50 and 100 NTU.
Filtrate obtained with distilled water achieved higher coagulation activity compared with the crude extract obtained with the same extragens in both model waters. Additionally, the crude extract even increased the residual turbidity of treated model waters at certain applied doses. This result indicates that unfiltered crude extract introduces fine suspended particles into model waters which are difficult to sediment and thus remain in treated water and decrease seemingly coagulation efficiency of natural coagulant.
The coagulation behaviours of the filtrate and the crude extract obtained with 0.5 mol/L NaCl were more complex compared to those obtained with distilled water, ie. at certain doses unfiltered crude extract exibited higher coagulation activity than filtrate. Nevertheless, the filtrate showed the highest coagulation activity in both examined model waters: 56.9 % in the model water of initial turbidity 50 NTU and 39.2 % in the model water of initial turbidity 100 NTU. Obtained results speak to filtration as a necessary step of natural coagulant production.

Keywords: Common bean, Natural coagulant, Coagulation and flocculation, Water and wastewater treatment.

Acknowledgements: The financial support from the Ministry of Education, Science and Technological Development, Republic of Serbia (Project No. 451-03-68/2022-14/200134) is greatly acknowledged.

2nd International Conference on Advanced Production and Processing
20th-22nd October 2022, Novi Sad, Serbia

ICAPP

International Conference
on Advanced Production and Processing

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Thank you for Mr. Branislav Bogdanović dipl.ing. and for Biljana Bogdanović dipl.ing., members of the ALUMNI CLUB of Faculty of Technology Novi Sad for their donation of sweets for ICAPP2022.