IBSC

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8th International PSU – UNS Bioscience Conference

Towards the SDG Challenges

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BOOK OF ABSTRACTS

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PROGRAM IBSC 2021
25-26. NOVEMBAR 2021.

25th NOVEMBER, 2021	
DAY 1 9:00 - 18:00 (CET)	
REGISTRATION	**8:45 – 9:00 CET**
09:00 – 09:20	**OPENING CEREMONY:** • Prof. *Milica Pavkov Hrvojević*, Dean of the Faculty of Sciences, University of Novi Sad, Serbia • Prof. *Anchana Prathep* Dean of the Faculty of Science, Prince of Songkla University, Thailand • Prof. *Neda Mimica-Dukić*, President of the Scientific Committee, Faculty of Sciences University of Novi Sad, Serbia
09:25 – 10:05	**PLENARY LECTURE 1:** **Prof. Snežana Đorđević** Division of Bioscience, University College London (UCL), UK **Title: *Evolutionary and Functional Links between Neuropilins and VEGF***
10:10 – 10:50	**PLENARY LECTURE 2:** **Prof. Anchana Prathep** Prince of Songkla University (PSU), Faculty of Sciences, Thailand **Title: *Seagrass Biodiversity and Conservation for Sustainable Development Goals: A Case Study from Thailand***
10:55 – 11:35	**PLENARY LECTURE 3:** **Prof. Imre Vass** Hungarian Academy of Sciences; Institute of Plant Biology, Biological Research Center of HAS, Szeged, Hungary **Title: *Application of Plant Phenotyping Methods in Research and Breeding***
11:40 – 12:20	**PLENARY LECTURE 4:** **Prof. Viktor Nedović** Ministry of Education, Science and Technological Development of the Republic of Serbia, University of Belgrade, Faculty of Agriculture, Serbia **Title: *Encapsulated Bioactives for the Food Industry***
BREAK	

13:00 – 16:00	**Track 1: Biology and Environment** **Invited lectures:** **13:00 - 13:20** Sari Kontunen-Soppela, University of Eastern Finland (UEF), Joensuu, Finland: "*Acclimation and Adaptation Capacity of Northern Silver birch (Betula pendula) Populations to Climate Change*" **13:25-13:45** Carmen Arena, University of Naples Federico II, Naples, Italy: "*Plants in Extreme Environments: Focus on Space*" **13:50-14:10** Adriana Basile, University of Naples Federico II, Naples, Italy: "*Biomarkers of Heavy Metal Pollution in Bryophyta*" **14:15-14:35** Biljana Basarin, University of Novi Sad, Faculty of Sciences, Serbia: "*Detailed Analysis of Extreme Heatwaves in Serbia, South-East Europe*" **Oral presentations (T1)** **14:40-14:50** Aleksandra Tubić, University of Novi Sad, Faculty of Sciences, Serbia: "*Adsorption mechanism of magenta printing dye on polyethylene microplastics*" **14:55-15:05** Kringpaka Wangkulangkul, University Prince of Songkla, Faculty of Science, Thailand: "I*nfluence of monsoon-driven oceanographic variability on recruitment of rocky intertidal dominant sessile species*" **15:10-15:20** Thanwarat Sangkuanun, University Prince of Songkla, Faculty of Science, Thailand: "*The roles of dragon fruit oligosaccharide on immunity of freshwater crustacean, Daphnia magna*" **15:25-15:35** Milica Stankovic, University Prince of Songkla, Faculty of Science, Thailand: "*Seagrass ecosystems as nature-based solution for climate change mitigation*" **15:40-15:50** Apisara Nakpan, University Prince of Songkla, Faculty of Science, Thailand: "*Habitat use by the cryptic sea slug Elysia pusilla (Bergh, 1872) (Sacoglossa) in a tropical Halimeda macroloba Decaisne, 1841 meadow*"
	Track 3: Biochemistry, Molecular Biology and Biomedicine **Invited lectures:** **13:00-13:20** Vassiliouss Roussos, Department of Pharmacy, National and Kapodistrian University of Athens, Greece: "*Algal Bioactive Metabolites for Biomedical Applications*" **13:25-13:45** *Silvia Rocha*, University of Aveiro, Chemistry Department, Aveiro, Portugal: "*Rethinking Plants as Excellent Sources of Health and Wellness-Promoting Compounds: Inspired by Nature and Science*"

13:00 – 16:00	**13:50-14:10** Sergej Tomić, University of Belgrade, INEP- Institute for Aapplication of Nuclear Energy Department for Immunology and Immunoparasitology, Belgrade, Serbia: "*Immunological profiles of COVID-19 patients reveal promising indicators and therapeutic targets for severe forms of the disease*" **14:15-14:35** Wipawadee Sianglum, Prince of Songkla University, Faculty of Science, Thailand: "*Proteomic Analysis of Antimicrobial Effects of Lupinifolin in Vancomycin-Resistant Enterococci*" **Oral presentations (T3)** **14:40-14:50** Arnon Chukamnerd, Prince of Songkla University, Faculty of Medicine, Thailand: "*Genomic analysis of carbapenem-resistant Acinetobacter baumannii clinical isolates using whole-genome sequencing data*" **14:55-15:05** Tatjana Majkić, University of Novi Sad, Faculty of Sciences, Serbia: "*The effects of Plantago l. water extracts on mRNA expression of enzymes involved in cyclooxygenase pathway of arachidonic acid metabolism*" **15:10-15:20** Jelena Bašić, University of Niš, Faculty of Medicine, Serbia: "*The influence of glucokinase regulatory protein gene polymorphisms on lipid profile in acute ischemic stroke patients*"
	Track 2: Biotechnology and Bioengineering **Invited lecture:** **14:40 – 15:00** Jelena Pejin, University of Novi Sad, Faculty of Technology, Serbia: "*Triticale in Beer Production*" **Oral presentations (T4)** **15:05-15:15** Petra Djuza, BioSense Institute, University of Novi Sad, Serbia: "*A deep learning-based prediction model for soybean yield*" **15:20-15:30** Viruja Ummat, Teagasc Ashtown Food Research Centre, UCD School of Biosystems and Food Engineering, University College Dublin, Ireland: "*Ultrasound assisted depolymerization of sulfated polysaccharide (fucoidan) from seaweed*" **15:35-15:45** Ermenegilda Vitale, University of Naples Federico II, Italy: "*Light quality and biostimulant application: a sustainable approach to improve antioxidant properties and photosynthesis in soybean (Glycine max l. Merril) sprouts*" **15:50-16:00** Živan Mrkonjić, University of Novi Sad, Faculty of Technology, Serbia: "*RSM and ANN optimization of polyphenols recovery from Thymus serpyllum herbal dust using microwave-assisted extraction*"
16:00 – 18:00	**PARALLEL POSTER SESSIONS** **T1, T2, T3 & T4**

26th NOVEMBER, 2021	
DAY 2 9:00 - 17:00 (CET)	
REGISTRATION	**8:45 – 9:00 CET**
09:00 - 09:40	**PLENARY LECTURE 1:** **Prof. Antonio J. Meléndez-Martínez** Universidad de Sevilla, Nutrition and Food Science, Toxicology and Legal Medicine **Title: *Carotenoids and Derivatives: Versatile Compounds for Nature and the Agro-Food Industry***
09:45 - 10:25	**PLENARY LECTURE 2:** **Prof. Declan Troy** The Agriculture and Food Development Authority (TEAGASC), Ireland **Title: *Emerging Technologies and Consumer Perception for Sustainable Meat Processing***
10:30 – 11:10	**PLENARY LECTURE 3:** **Prof. Gianluca Polese** Federico II University of Naples, Italy **Title: *Octopus' Suckers a Multitasking Sensor***
11:10 – 12:00	**BREAK**
12:00 – 16:00	**Track 1: Biology and Environment** **Invited lectures:** **12:00-12:20** Mladen Horvatović, University of Novi Sad, Faculty of Sciences, Serbia: "*Serbian Stick Grasshopper – Pyrgomorphula serbica (Pančić, 1882) The Most Striking but Little-Known Endemic of Serbian Fauna*" **12:25-12:45** Jaruwan Mayakun, Prince of Songkla University, Faculty of Science, Thailand: "*Underappreciated Roles of Calcareous Green Alga Halimeda*" **12:50-13:10** Sara Bumrungsri, Prince of Songkla University, Faculty of Science, Thailand: "*Local and Landscape Compositions Influence Stingless Bee Communities and Pollination Networks in Tropical Mixed Fruit Orchards, Thailand*"

12:00 – 16:00	**Track 2: Biotechnology and Bioengineering** **Invited lecture:** **13:15-13:35** Komwit Surachat, Prince of Songkla University, Faculty of Science, Thailand: "*In Silico Safety Assessment of Probiotics for Human Use Using Genomics and Bioinformatics Analysis Approach*"
	Oral presentations (T1/T2) **13:40-13:50** Saowalak Malawa, Faculty of Natural Resources, Prince of Songkla University, Thailand: "*Efficiency of indian-almond leaf (Terminalia catappa Linnaeus, 1767) extracts in rearing Siamese fighting fish (Betta splendens Regan, 1910)*" **13:55-14:05** Jirapan Satjarak, Faculty of Natural Resources, Prince of Songkla University, Thailand: "*Post-prandial changes in digestive enzymes and chyme characteristics in bigfin reef squid (Sepioteuthis lessoniana)*" **14:10-14:20** Paweena Sanpradit, Faculty of Science, Prince of Songkla University, Thailand: "*Alterations of growth, oxidative stress and energy reserves in Daphnia magna after ZnO exposure under thermal stress*" **14:25-14:35** Aleksandar Bajić, University of Novi Sad Faculty of Sciences, Serbia: "*Signal crayfish, Pacifastacus leniusculus (Dana, 1852) new invasive species in the waters of Serbia*" **14:40-14:50** Serena Ducoli, University of Brescia, Department of Information Engineering, Italy, and INSTM: "*Nanoplastics characterization at the biological interface*" **14:55-15:05** Payal Patel, School of Science, Indrashil University, India: "*Characterization and purification of a novel halothermotolerant l-Asparaginase from Bacillus licheniformis ppd37 and its anti-proliferative activity against cancer cell lines*" **15:10-15:20** Dimitrije Stefanović, University of Novi Sad, BioSense Institute, Serbia: "*Suppression of ring artifacts in reconstructed holographic images using graph signal processing*"

12:00 – 16:00	**Track 3: Biochemistry, Molecular Biology and Biomedicine** **Invited lectures:** **12:00-12:20** Ioannis Mourtzinos, Aristotle University of Thessaloniki, Greece: "*Stability and Color Evolution of Anthocyanins from Cornelian Cherry Extracts in Different Food Systems*" **12:25-12:45** Pimonsri Mittraparp-arthorn, Prince of Songkla University, Faculty of Science, Thailand: "*Impact of climate change in the adaptation and virulence of marine luminous bacterium Vibrio campbellii*" **12:50-13:10** Nebojša Kladar, University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Serbia: "*The current status of Cannabis sativa L. therapeutic potential*"
	Track 2: Biotechnology and Bioengineering **Invited lecture:** **13:15-13:35** Patamarerk Engsontia, Prince of Songkla University, Faculty of Science, Thailand: "*From Butterfly Diversity to Peptide Drug Discovery*" **Oral presentations (T3)** **13:40-13:50** Tatjana Majkić, University of Novi Sad Faculty of Sciences, Serbia: "*Wine against obesity – Cabernet Sauvignon wine as inhibitor of pancreatic lipase*" **13:55-14:05** Jakkrit Nukitram, Faculty of Science, Prince of Songkla University, Thailand: "*Medial prefrontal cortex local field potential oscillations and attenuated craving behaviors in methamphetamine-induced addictive-like behaviors mice in response to Mitragyna speciosa (Korth.) Havil. leaves extract treatment*"
12:00 – 15:00	**Track 4: Agri-food and Biosensing** **Invited lectures:** **12:00-12:20** Oskar Marko, University of Novi Sad, BioSense Institute, Serbia: "*Digital Services for Farmers Based on Sentinel-2 Satelllite Images and Advanced Machine Learning*" **12:25-12:45** Pissared Muangnil, Prince of Songkla University, Faculty of Science, Thailand: "*Prebiotic Oligosaccharides: Dietary Strategies for Improving Gut Health*" **12:50-13:10** Chongdee Buranachai, Prince of Songkla University, Faculty of Science, Thailand: "*A 3D Gelatin Aerogel Sorbent for the Extraction of Polycyclic Aromatic Hydrocarbons in Tea Drinks*" **13:15-13:35** Alena Stupar, Institute for Food Technology, University of Novi Sad, Serbia: "Natural Deep Eutectic Solvents for Green Agri-Food Solutions"

12:00 – 15:00	**Oral presentations (T4)** **13:40-13:50** Ivana Dimić, University of Novi Sad, Faculty of Technology: "*Cherry seed oil: supercritical fluid extraction of lipophilic bioactive compounds*" **13:55-14:05** Sofia Lalou, School of Chemistry, Aristotle University of Thessaloniki, Greece: "*Natural carotenoids and pectin from the juice by-product of microwave-heated persimmon fruits (cv. Jiro)*" **14:10-14:20** Dragana Miladinović, Institute of Field and Vegetable Crops, National Institute of Republic of Serbia: "*Oil crops breeding at IFVCNS – new tools for tackling changing environment and market demands*" **14:25-14:35** Anastasia Kyriakoudi, School of Agriculture, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, Greece: "*Screening of hydrophobic deep eutectic solvents for the extraction of tomato carotenoids*" **14:40-14:50** Anastasia Loukri, School of Agriculture, Aristotle University of Thessaloniki, Greece: "*Recovery of bioactive compounds using green extraction solvents*" **14:55-15:05** Stamatia Christaki, School of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, Greece: "*Incorporation of bioactive compounds from oregano plant to Greek whey cheeses*"
15:00 – 17:00	**PARALLEL POSTER SESSIONS** **T1, T2, T3 & T4**
17:00 – 17:30	**CLOSING REMARKS**

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ABSTRACTS

PLENARY LECTURES

PL-1 Carotenoids and derivatives: versatile compounds for nature and the agro-food industry

Meléndez-Martínez, A.J.[1]

Carotenoids are isoprenoids widely distributed in Nature. They are biosynthesized by all photosynthetic organisms and some fungi, bacteria and arthropods. They appeared early in life history on Earth in cyanobacteria to intervene in photosynthesis and protect them from photooxidation. Curiously, millions of years later some carotenoids are selectively transported to the human macula lutea, the location of the retina receiving the highest intensity of light radiation. In between, carotenoids can be found in many organisms adapted to live in the most diverse environments, where they intervene in different actions. Additionally, carotenoids can be converted enzymatically or non-enzymatically into a myriad of compounds that expand the diversity of actions carotenoids are involved in. Examples of derivatives are retinoids with vitamin A activity. Considering that carotenoids are essential in photosynthesis (the engine of life on Earth), for the pollination and the dispersal of seeds (as they attract pollinators and seed dispersers through their colours and aroma-derived compounds) and for the regulation of key processes in plants (through phytohormones and other signalling molecules) and that plants are essential to feed animals and humans, it can be stated that the importance of carotenoids in food security is undeniable. Beyond these facts, carotenoids are versatile components of foods as they are colorants , precursors of vitamin A and they are involved in health-promoting biological actions. Due to their versatility, carotenoids and their derivatives can be used for different applications for the agro-food and other industries. While there are well-established commercial applications, others are emerging and many more can be envisaged.

PL-2 Emerging technologies and consumer perception for sustainable meat processing

Declan J Troy[2]

KEYWORDS: Meat, sustainability, meat processing, consumer

INTRODUCTION:

The responsibility to produce high quality, sustainable and cost effective meat products rests with producers, manufacturers, distributors and retailers to ensure that consumer demands are met. New and emerging robust technologies can play an important role in ensuring a more resilient meat value chain and satisfying consumer demands and needs.

OBJECTIVES:

The objective of this presentation is to outline various novel thermal and non-thermal technologies which have shown potential for meat processing applications. A number of process analytical techniques which have shown potential for rapid, real-time assessment of meat quality will be discussed.

RESULTS:

New and emerging robust technologies can play an important role in ensuring a more resilient meat value chain and satisfy-

1 *Food Colour & Quality Laboratory, Area of Nutrition & Food Science, Universidad de Sevilla, 41012 Seville, Spain*

2 *Teagasc Food Research Centre, Dublin, Ireland; Corresponding author: declan.troy@teagasc.ie*

ing consumer demands and needs. Novel thermal and non-thermal food processing and preservation technologies, including radiofrequency (RF), microwave, infrared, ohmic heating, high pressure processing (HPP), pulsed-UV light, pulse electric field (PEF), power ultrasound, cold atmospheric plasma and ozone processing have gained much attention in recent years. These technologies can offer several benefits, including increased process efficiency, improved product safety, enhanced quality attributes and extended shelf-life stability of products.

The commercial uptake and consumer acceptance of novel technologies in meat processing have been subjects of great interest over the past decade. Consumer focus group studies have shown that consumer expectations and liking for novel technologies, applicable to meat processing applications, vary significantly. New processing and meat quality assessment techniques have shown potential benefits to various stakeholders involved in meat production and supply chain. These technologies have demonstrated the potential in achieving various consumer demands associated with the meat product. Nonetheless, the path from the introduction of a new technology on the production line to consumer acceptance is not straight forward.

CONCLUSIONS:

The necessity for meat processors to address consumer risk-benefit perceptions, knowledge and trust in order to be commercially successful in the application of novel technologies within the meat sector.

PL-3 Evolutionary and functional links between neuropilins and VEGF

Khushboo Matwani, Andrew Martin, Snezana Djordjevic[3]

KEYWORDS: VEGF; angiogenesis; phylogeny; sequence conservation

INTRODUCTION:

Neuropilins are single-pass transmembrane proteins that are implicated in a range of processes including vasculogenesis, angiogenesis, cell adhesion and migration, immunomodulation, and axon guidance. Molecular mechanisms of regulation of these processes involve neuropilins' interactions with the range of diverse ligands that are supporting neuropilins' pleiotropic functions. One of the best-characterized group of ligands of neuropilins includes vascular endothelial growth factors (VEGFs). In this context, it was shown that neuropilin-1 interacts with VEGF-A165 form via its b1 domain. At the same time, VEGF-A165 also binds to its cognate receptor (VEGF-R2) forming a ternary complex that is essential for regulating angiogenesis. While the functions of VEGF-A165 and its main tyrosine kinase receptor are well-understood independently of neuropilins, the specific role of neuropilin-1 in supporting angiogenesis and other processes remains to be fully explained. The presence of neuropilins and the formation of the ternary complex is indispensable in the development, with neuropilin-1 gene deletion in mice resulting in embryonic lethality. In adult humans, overexpression of neuropilins is however linked to tumor development and the levels of expression inversely correlate with tumor prognosis.

OBJECTIVES:

In this project, we aimed to identify the evolutionary origin of neuropilins and to correlate their evolutionary emergence with a lifestyle niche or developmental stage that would be dependent on the neuropilins' function. Furthermore, we aimed to infer mechanistic and functional details of neuropilins, VEGF and VEGF receptor interactions, by comparing their phylogenic trees and protein sequence conservation.

3 *Structural and Molecular Biology, Division of Biosciences, University College London, Gower Street, London WC1E 6BT, UK;*

Corresponding author: s.djordjevic@ucl.ac.uk

METHOD / DESIGN:

We designed the workflow which included BLAST searches with the functionally coherent domain fragments as queries, followed by sequence filtering and name conversion. These sequences were then aligned using MAFFT protocol based on fast Fourier transform. The well-aligned sequences were identified and selected with BMGE tool, and a phylogeny tree was generated using FastTree tool, all with the NG phylogeny server (https://ngphylogeny.fr). Analysis and annotation of trees were carried out in iTOL (https://itol.embl.de) and the sequence conservation was calculated using the Scorecons Server.

RESULTS:

After the filtering of the initial BLAST hits, more than 3000 neuropilin sequences were used to generate trees which showed that neuropilin-1 and neuropilin-2 diverged from an ancestral sequence at an early stage. From the VEGF-like sequences that were identified and examined only a subset contained exons 7-8 that are known to be required for interaction with neuropilin protein as described for VEGF-A165 isoform. The phylogenetic tree demonstrated early separation between members of VEGF family with the placental growth factor and VEGFA, separated earlier from the clades containing VEGFB, VEGFC and VEGFD. While VEGF homologues are also identified in non-chordate invertebrates, full-length neuropilin proteins are only found in vertebrates.

CONCLUSIONS:

The earliest full-length neuropilins would have been present in cartilaginous fish, which are considered to have descended from some of the most ancient vertebrates, while VEGF homologues arose much earlier as they are found in various invertebrates such as insects but also the lancelets. As lancelets also contain some neuropilin-like sequences, it is speculated that gene fusion events from proteins in these 'fish-like' species could have led to the evolution of neuropilin. Conservation analysis of the b1 domain indicates that the early neuropilins could have been capable of mediating angiogenesis. Our findings suggest that the emergence of neuropilins correlates with the evolution of endothelial cells and that VEGF-dependent endothelial angiogenesis was necessary for the survival of the earliest jawed/jawless vertebrates. Further analysis should also include phylogenetic analysis of semaphorins that interact with neuropilins in a process of axon guidance as well as consideration of neuropilin coreceptors.

PL-4 Octopus' suckers a multitasking sensor

Gianluca Polese, Al-Sayed Al-Soudy, Valeria Maselli, Tania Russo, Heethaka K. S. de Zoysa, Anna Di Cosmo[4]

KEYWORDS: Octopus vulgaris, Suckers, Tactile Sense, Chemoreception, Light sensing

INTRODUCTION:

Octopus' arms are a fascinating and evolutionarily unique sensory organ, with hundreds of motile suckers, each with thousands of sensory cells, lining eight highly flexible arms. Scientifically, there are many open questions regarding the sensory capabilities of the arms and specifically the highly innervated suckers.

OBJECTIVES:

Our main aim is to fully characterize Octopus vulgaris' suckers to fully understand how they can use their arms.

METHOD / DESIGN:

We use a multidisciplinary approach, ranging from behavioral, morphological, and molecular techniques.

RESULTS and CONCLUSIONS:

Our findings, together with the scientific literature available, indicate that octopus suckers have many abilities and can function as tactile, chemical, and light sensors.

4 *Dipartimento di Biologia – Università degli Studi di Napoli Federico II; Corresponding author: gianluca.polese@unina.it*

PL-5 Seagrass biodiversity and conservation for sustainable development goals: a case study from Thailand

Anchana Prathep, Milica Stankovic, Piyalap Tuntiprapas, Ratchanee Keawsrikhaw, Ekkalak Rattanachot and Janmanee Panyawai[5]

KEYWORDS: Seagrass; Ecosystem Services; Climate change; Blue carbon; Sustainable goals

Seagrasses are marine flowering plants that occur along coastlines, forming large meadows, which provide nursery, shelter and food for marine life. Thailand, South East Asia, is situated in a seagrass hotspot biodiversity, providing a great opportunity for research as well as their roles as an important coastal habitat. We reviewed our research throughout these years (~ 20 years) together within the SE Asian region to capture the current status of seagrass research in the region. We emphasized on our findings, when we first focused on biology and ecology of some common species, such as *Enhalus acoroides, Thalassia hemprichii, Cymodocea rotundata* and *Halophila ovalis*; we discovered an additional new record of seagrass in Thailand, *Halophila major*. We examined the ecological roles of seagrass including the habitat complexity, which they provided for marine life and ability to trap sediment and eco-engineering the sediment through their root complexity. Their roles as a home for marine life, where there are various economic species such as fishes, crabs and sea cumbers associated within the seagrass meadows. We also deployed some new technologies such as drone and remote sensing which allow us to work in a larger spatial scale including understand the history of the sites. The tools, which could help us understand, past, present and also the future of the seagrass meadows. Furthermore, these technologies together with some model analyses will allow us to estimate how much carbon accumulation within the seagrass meadows, known as a blue carbon. We understand that not only local livelihoods and dugongs, an endangered species, depend on healthy seagrass meadows but our passions on seagrass research as well. As to help answering the sustainable develop goals, our conservation efforts would be a straight forward answer, but we know that seagrass could do more. We map our research throughout these years and examine what seagrass biodiversity and conservation could contribute to those sustainable goals. We understand that there is still a long road ahead but these are what seagrass and we could do to help contributing to this sustainable world.

5 Seaweed and Seagrass Research Unit, Excellence Center for Biodiversity of Peninsular Thailand, Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand; Corresponding author: anchana.p@psu.ac.th

PL-6 Encapsulated bioactives for the food industry

Viktor A. Nedović[7], Ana Salević[7], Ana Kalušević[6] Steva Lević[7],
Bojana Balanč[8], Verica Đorđević[8], Branko Bugarski[8]

The modern food industry is facing the challenges associated with the production of high-quality food with enhanced safety, improvement of process efficiency and reduction of environmental pollution. The inclusion of bioactive compounds with potential health benefits, such as vitamins, probiotics, minerals, polyphenols, omega-3-fatty acids, and phytosterols into the foodstuff became the regular practice in contemporary industrial production of food. Most of these compounds are sensitive to the external conditions and influences that might cause the loss of functionality or degradation of an ingredient before it has time to act. In this sense, encapsulation, a process to entrap an active compound within a carrier material, gained great interest as a way to overcome the poor stability of bioactives and their susceptibility to adverse external factors during food processing, storage, and consumption. It provides a physical barrier between active compounds and the environment and can prevent reaction with other components in food products such as oxygen or water. Further on, it can be used to mask unpleasant feelings during eating, such as bitter taste and astringency of polyphenols. Encapsulation is also a useful tool to make delivery of bioactive molecules (e.g., antioxidants, minerals, vitamins, phytosterols) and living cells (e.g., probiotics) at the desired place or within an appropriate time possible.
The paper gives an overview of different techniques and carrier materials commonly used in the food industry for encapsulation of bioactive molecules and presents several examples of encapsulated bioactives and cells developed in our laboratories to be used for the production of value-added food.

KEYWORDS: encapsulation, bioactives, value-added food

6 *Academy of Applied Studies Belgrade, Belgrade, Serbia*

7 *Faculty of Agriculture, University of Belgrade, Serbia*

8 *Faculty of Technology and Metallurgy, University of Belgrade, Serbia*

PL-7 Application of plant phenotyping methods in research and breeding

Vass Imre[9], Kondić-Špika Ankica[9], Pauk János[10], Sass László[9], Hovári Miklós[11], Kertész Attila[12]

KEYWORDS: Plant phynotyping; drought stress; salt stress; crops; wheat

INTRODUCTION:

One of the important recent global challenges is to provide sufficient amount of food and plant derived raw materials for the growing population. This task requires new approaches in plant research and breeding. A very important development in this field has been the development and wide spread application of plant phenotyping methods during the last 15 years. The main goal of this approach is the quantitative characterization of environmental stress effect on the growth and physiological response of plants under controlled greenhouse- and field conditions, as well as the clarification of how the genetic and molecular background determines the phenotypic characteristics of plants.

OBJECTIVES:

The lecture will cover the basic methods and approaches of plant phenotyping, as well as recent results from the Szeged group.

RESULTS:

The presented results deal with: (i) The applicability and limitation of image based shoot phenotyping approaches to estimate grain yield in wheat. (ii) The interaction of drought and salt stress in crop plants. (iii) The development of affordable phonetyping tools, which could promote more wide spread applications of phenotyping approaches in everyday research and breeding tasks.

CONCLUSIONS:

Plant phenotyping is a rapidly growing field, both in basic research in plant biology and in applied agricultural sciences, which is still under rapid development at the moment and expected to continue prospering in the coming years.

9 *Institute of Field and Vegetable Crops, Novi Sad, Serbia*

10 *Cereal Research Non-profit Limited Company, Szeged, Hungary*

11 *Institute of Plant Biology, Biological Research Centre, Szeged, Hungary; Corresponding author: vass.imre@brc.hu*

12 *Department of Software Development, Szeged University, Hungary*

T1-IL-1 Plants in extreme environments: focus on space

Carmen Arena[13]

KEYWORDS: abiotic stress; higher plants, photosynthesis; plant radioresistance; space factors.

INTRODUCTION: Plants are vital organisms on Earth since they provide oxygen and food, maintaining life on our planet. For their essential ecosystem role, plants could also be important in environments other than Earth. The idea to use plants to support life in Space is increasing in interest in the last decades because they could serve as crucial elements in Controlled Ecological Life Support Systems (CELSS) conceived to sustain human wellbeing in long-duration space missions. The role of plants in CELSS would be crucial for food production and resource regeneration (i.e., carbon dioxide removal, oxygen production, water purification) directly on board. However, plants' engagement on the Space platform requires deep knowledge of the functioning of the vegetal systems under the effect of Space factors, mainly altered gravity and ionizing radiation. There is a mutual opinion that plants may survive in Space, but it is uncertain their performance in such an environment. The extreme high plasticity and adaptation capacity of plants to the changing environment allowed their colonization in many habitats on Earth. From an evolutionary perspective, space radiation and altered gravity might be considered the primary factors driving the evolution of plants in Space. Therefore, in the view of the potential colonization of extraterrestrial environment by higher plants, it is reasonable to hypothesize a sort of "back to the origin" because plants would face ecological conditions similar to those of remote past on Earth: microgravity and increased doses of ionizing radiation.

OBJECTIVES:
The main objective of this study was to evaluate plant's response to different doses and types of ionizing radiation in crop model species, such as tomato, soybean, bean and chard, through a multidisciplinary approach, analyzing different molecular, eco-physiological and biochemical aspects in order to identify the mechanisms associated with plant radioresistance.

METHOD / DESIGN:
The study has been conducted on different crops by integrating non-destructive gas exchange and chlorophyll fluorescence emission measurements, for the quantitative estimations of photosynthesis, with enzymatic and molecular essays to assess photosystems' functionality and overall plant health status.

RESULTS:
A selection of results demonstrated that ionizing radiation might exert opposite effects on plant growth and photosynthesis, ranging from detrimental outcomes at high doses, harmful consequences at intermediate levels, and stimulatory effects at low doses. The severity of the effects depends on several factors, including radiation-related parameters (e.g., dose, Linear Energy Transfer - LET) and organism-related traits (e.g., species, plant physiological status, plant developmental stage at the time of irradiation). Our studies demonstrate that the seed is the most resistant stage to IR, which does not prevent the achievement of the seed-to-seed cycle. From a physiological point of view, plants irradiated at the vegetative stage are the most sensitive showing a reduction of photosynthetic efficiency and the occurrence of DNA polymorphisms. A relevant aspect is that low doses of ionizing radiation exert a stimulatory effect on photosynthesis and the production of bioactive compounds in leaves and fruits, revealing a tool to improve the nutraceutical properties of tissues.

13 Department of Biology, University of Naples Federico II, via Cinthia, 80126, Napoli, Italy; Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, Portici (NA), Italy. Corresponding author: c.arena@unina.it

CONCLUSIONS:

Our experiments support the studies that demonstrated that plants tolerate ionizing radiation, maintaining vital functions also at high doses. Plant radioresistance is a fundamental requirement for achieving good photosynthetic performance, which guarantees an adequate resource regeneration on CELSS. In addition, plants enduring ionizing radiation increase the synthesis of secondary metabolites as a defense mechanism, producing fresh food richer in functional compounds. This aspect represents an attractive perspective to supplement the astronaut diet directly onboard.

T1-IL-2 Local and landscape compositions influence stingless bee communities and pollination networks in tropical mixed fruit orchards, Thailand

Kanuengnit Wayo[14], Tuanjit Sritongchuay[14],
Bajaree Chuttong[15], Korrawat Attasopa[15], Sara Bumrungsri[16]

KEYWORDS: agriculture; floral resources; forest proximity; land-use change; meliponine; plant–pollinator interaction; urbanization.

INTRODUCTION:

Stingless bees are vital pollinators for both wild and crop plants, yet their communities have been altered by anthropogenic land-use change. Additionally, few studies have directly addressed the consequences of land-use change for meliponines, and knowledge on how their communities change across gradients in surrounding landscape cover remains scarce.

OBJECTIVES:

Here, we examine both how local and landscape-level compositions as well as forest proximity affect both meliponine species richness and abundance together with pollination networks across 30 mixed fruit orchards in Southern Thailand.

RESULTS:

The results reveal that most landscape-level factors significantly influenced both stingless bee richness and abundance. Surrounding forest cover has a strong positive direct e_ect on both factors, while agricultural and urbanized cover generally reduced both bee abundance and diversity. In the local habitat, there is a significant interaction between orchard size and floral richness with stingless bee richness. We also found that pollinator specialization in pollination networks decreased when the distance to the forest patch increased.

CONCLUSIONS:

Both local and landscape factors thus influenced meliponine assemblages, particularly the forest patches surrounding an orchard, which potentially act as a key reservoir for stingless bees and other pollinator taxa.

14 Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla 666303, China

15 Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

16 Department of Biology, Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand;

T1-IL-3 Underappreciated roles of calcareous green alga "*Halimeda*"

Jaruwan Mayakun[17]

KEYWORDS: Biology; Calcium carbonate producer; Ecological roles; *Halimeda*; Potential use

INTRODUCTION:

Genus *Halimeda* consists of a single, multinucleate, siphons cell and its thallus is composed of calcified segments. *Halimeda* inhabits both hard and soft substrata from intertidal zone to subtidal zone. It is widely distributed across the tropics and subtropics such as in the Pacific, Atlantic, Indian Ocean, and Thai waters. The ecological importance of *Halimeda* is well documented; playing vital roles in marine ecosystem serve as a primary producer, refuges, nursery grounds and food for marine organisms, reef builder, and carbonate sediment generation. Recent research has suggested that *Halimeda* is a very important player in the global carbonate budget. In Thailand, *Halimeda* is common and abundant alga from both the Gulf of Thailand and the Andaman Sea and has the highest abundance compared with other regions. Its density varies from 24-200 thalli m-2. However, little is known of its population dynamics and actual calcium carbonate contribution. Therefore, understanding the population of Halimeda and estimated $CaCO_3$ production of *Halimeda* would provide a valuable data for the carbonate contribution in the carbon budget and the potential role of *Halimeda* as a carbonate contributor. In this study, population of *Halimeda* was monitored by looking at the standing stock, growth rate, calcification rate, and content. *Halimeda* quickly produced one to two new segments daily or thalli grew by 0.021 g dry weight thallus^{-1} day^{-1}. Total $CaCO_3$ production was 291.94 to 908.11 g m^{-2} year^{-1}. This alga is a significant contributor to carbonate budgets due to its high growth rate and calcium carbonate productivity.

T1-IL-4 Acclimation and adaptation capacity of northern Silver birch (*Betula pendula*) populations to climate change

Sari Kontunen-Soppela[18]

KEYWORDS: acclimation, adaptation, Betula pendula, climate change, photoperiod

Boreal regions are currently undergoing extensive climate change. For Finland, climate change scenarios for the end of this century project an about 1-7 °C increase in average temperature which is estimated to be higher during winter and fastest in the Northern Finland. The growing season will become longer and warmer. At this pace, climate zones will shift northwards at a greater speed than long-living trees can migrate. To be able to avoid the adverse effects of these changes, plants will have to either acclimate (in the short term) or adapt (in the long term) to the new conditions.

While temperature, CO_2 concentration, air humidity and many other factors change due to global warming, photoperiod remains the same. Plants that cover a wide geographic range need to cope with the different photoperiods. Silver birch (*Betula pendula Roth*) is a widely distributed pioneer tree species in boreal forests in Europe and Asia. In Finland, its latitudinal and longitudinal distribution is spanning almost the whole country, which makes silver birch an excellent model tree for studying local adaptation and acclimation capacity to different environments.

17 *Molecular Evolution and Computational Biology Research Unit, Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand. Corresponding author: jaruwan.may@psu.ac.th*

18 *Department of Environmental and Biological Sciences, University of Eastern Finland, Joensuu, Finland. Corresponding author: sari.kontunen-soppela@uef.fi*

We have studied Finnish silver birch provenances from latitudes 60°C to 67°N for their geographical variation in common garden experiment and in controlled experiments in response to temperature and photoperiods. The provenances can be separated into two groups, northern and southern, by their genetic background and by differences in leaf traits, photosynthesis (gas exchange and chlorophyll a fluorescence), phenology, growth, and biomass allocation patterns. In general, the northern provenances show higher photosynthetic capacity per leaf area (higher rates of net photosynthesis, higher maximum quantum yield of photosystem II photochemistry, Fv/Fm), higher stomatal conductance and lower water use efficiency than the southern provenances. The provenances show similar temperature response of photosynthesis, but the northern provenances require a longer photoperiod for the higher growth rate and biomass allocation than the southern provenances. The biomass allocation pattern also differs among the provenances; northern provenances invest relatively more to the below-ground fraction whereas southern provenances have more leaves, branches, and stem biomass.

In conclusion, the provenances seem to be able to acclimate to a common growth temperature and thus the carbon assimilation of the birch trees may not be significantly affected by rising temperatures alone. The requirement for long day conditions in the northern provenance suggests adaptation to local environment.

T1-IL-5 Serbian stick grasshopper - *Pyrgomorphula serbica* (Pančić, 1882) The most striking but little-known endemic of Sebian fauna

Mladen Horvatović, Ivo M. Karaman[19]

KEYWORDS: Tara Mt.; Balkan; critically endangered; black pine

Pyrgomorphidae is an ancient grasshoppers family within the monotypic superfamily Pyrgomorphoidea of the order Orthoptera. A typical genus of the family *Pyrgomorpha* Serville, 1838 and a monotypic genus *Pyrgomorphula* Kevan and Akbar, 1963 were merged into the tribe Pyrgomorphini in the subfamily Pyrgomorphinae. Representatives of the mentioned family form a separate, old phyletic line of grasshoppers with a pan-tropical distribution. Only the species *Pyrgomorphula serbica* (Pančić, 1882) is present exclusively in the European continental fauna. Unlike most representatives of the Pyrgomorphini tribe, the Serbian stick grasshopper lives in a small area, which includes several Balkan mountains: Tara, Mokra Gora and Zlatibor in Serbia, and Varda in Bosnia. The habitat of this stenoendemic species is actually much smaller due to the pronounced disjunctivity, with small and isolated populations, which makes the species very rare and extremely endangered. Hence it's IUCN status is listed as critically endangered (CR).

Although the research of the *Pyrgomorphula serbica* began in 1882, when it was found and described by Josif Pančić, there were only few and very sporadic. Our research on Tara Mt. in the period 2019-2021 is the first systematic and detailed research of the condition and number of populations of this species. We have also gained important new knowledge related to the biology and ecology of the species. Over the course of three years, we searched more than 300 seemingly suitable locations (most of them two or more times) and registered the presence of the Serbian stick grasshopper at 72 of them. The presence of several individuals or individual specimens at the site was mostly confirmed. It is extremely worrying that we could register only 6 populations numbering more than ten individuals and who's area covers more than a few hundred square meters. Only two populations contained more than fifty individuals and could be conditionally marked as stable.

The limited data from the middle of the 20th century mention numerous populations of this species exclusively in the relict

19 *Department for Biology and Ecology, Trg D. Obradovića 2, 21000 Novi Sad, Serbia. Corresponding author: mladen.horvatovic@dbe.uns.ac.rs*

black pine (*Pinus nigra*) forests with a sparse structure and virgin forest type, with winter heath (*Erica carnea*) on the ground floor and numerous stumps and rotten trunks. Presently, there are no such forests at Tara Mountain. We concluded that the presence of the Serbian stick grasshopper is not related to the winter heath or any other specific plant species. Its survival is related to a specific set of optimal habitat conditions: southwestern terrain exposure, semi-open and sunny habitats enabled by sparse forest with older trees, areas with sparse ground vegetation, well-drained or skeletoid soil and proximity to a permanent water source. The species is distinctly thermophilic and moderately mesophilic, but it inhabits habitats that become extremely xerothermic during the spring. Hence, the presence of water sources within the habitat is crucial (especially during late spring and early summer) for the possibility of choosing a location with an optimal humidity regime. In addition to the great negative anthropogenic impact (total deforestation of old pine forests, urbanization, disturbance and destruction of habitats), we also found a favorable effect of human activity for the survival of this species. Steep sections of land above the roads, created by the road construction, have replaced the missing sunny clearings of old pine forests. We noticed a number of individuals of the Serbian stick grasshopper right on the slopes with southwestern exposure, above the old roads, which are overgrown with sparse vegetation of perennial herbaceous plants, individual shrubs and rare trees, with the existence of moisture sources. Such habitats also serve as corridors that enable communication and expansion of populations of the Serbian stick grasshopper.

Today's populations of the *Pyrgomorphula serbica* on Tara Mt. are just few remains previously widespread and possibly larger populations. We believe that the situation could be significantly improved if the remaining habitats were strictly protected, the existing young forests were adequately managed with the potential to become suitable habitats and the urbanization of potentially important areas was stopped.

T1-IL-6 Biomarkers of heavy metal pollution of bryophyta

Adriana Basile[20]

KEYWORDS: Heavy metal; Ultrastructural alterations; Oxidative stress; DNA; Phytochelatins

INTRODUCTION:

Bryophyta show a high metal storage capacity due to the high surface / volume ratio and the presence of a thin cuticle. It is known that these organisms have no roots and that their rhizoids do not contribute mainly to the absorption of substances from the substrate; therefore, most of the elements absorbed by bryophytes come from atmospheric deposition or aquatic uptake, so the levels of specific elements in bryophytes reflect total deposition or their presence in aquatic systems and can be used to monitor air or aquatic pollution in space and time. Many studies have used bryophytes to study environmental pollution levels (Tyler 1990; Harmens et al. 2012) and pollution tolerant species have been used to study environmental pollution in highly contaminated sites (Basile et al. 2001, 2008, 2012, 2013, 2017; Maresca et al. 2018, 2020; Esposito et al 2018). Some examples are: the notorious "Land of fires", , so called because of the burning of waste and fraudulent dumping, that show an alarming increase in the incidence of chronic-degenerative diseases and tumors and Regi lagni and Fiume Sarno, considered among the most polluted watercourses in Europe. These areas are located in the Campania Region (Southern Italy).

OBJECTIVES:

Mosses such as *Leptodictyum riparium* and *Scorpiurium circinatum* and liverworts such as *Lunularia cruciata* and *Conoceph-*

20 *Department of Biology University Federico II Napoli Italy. Corresponding author: adbasile©unina.it*

alum conicom, known to be pollution tolerant species, have been used to investigate the rate of accumulation of heavy metals from air or water in systems characterized by strong risk to human health. Furthermore, the cytophysio-ecological effects due to the presence of pollutants were have been studied with the aim of use them as a biomarker of environmental pollution.

METHOD / DESIGN:

The response chain to environmental pollution was investigated considering: ultrastructure, vitality, photosynthetic efficiency, chlorophyll degradation, Reactive Oxygen Species production and localization, activity of antioxidant enzymes, DNA damage, heat shock protein 70 (Hsp70) induction and gene expression levels, presence of chelating molecules (phytochelatins).

RESULTS:

The biological modifications studied generally show a good correlation with the degree of heavy metal pollution considered and can be proposed as biomarkers of environmental stress.

CONCLUSIONS:

We discuss the data considering the possibility of using these biological changes as environmental pollution biomarkers. Finally, it is underlined the importance of phytochelatins due to of their specificity for metal pollution.

Harmens, H et al. 2012.. Environ. Pollut. 166, 1e9
Basile A. et al. 2008. Environmental Pollution 151: 401-407
Basile, A et al. 2009. Environ. Pollut. 157, 2255e2260. https:// doi.org/10.1016/j.envpol.2009.04.001.
Basile A. et al. 2013. Environmental Pollution 182 209e216
Basile, A., et al. 2017. Environ. Sci. Pollut. Res. 24, 26185e26193. https://doi.org/10.1007/s11356-017- 9304-y.
Esposito S. et al. 2018. 2018 PLOS ONE 13(4),0195717 doi:10.1371/journal.pone.0195717
Maresca, V., et al. 2018. Ecotoxicol. Environ. Saf. 163, 665e673. https://doi.org/10.1016/j.ecoenv.2018.07.122.
Tyler G. 1990. Botanical Journal of the Linnean Society 104: 231-253.

T1-IL-7 Detailed analysis of extreme heatwaves in Serbia, south-east Europe

Biljana Basarin[21], Tin Lukić[22], Tanja Micić Ponjiger[21],
Dragoslav Pavić[21], Minučer Mesaroš[22]

KEYWORDS: HWMId, biometeorology, extreme temperature, heat wave

INTRODUCTION:

During the last 20 years, the magnitude and impact of heatwaves in Europe have increased substantially, thus raising concerns not only in the European continent but worldwide. Many studies have emphasized how important is the understanding of present changes, along with the prediction of future occurrences. Heatwaves have devastating impacts on different

21 Scientific-Technological Center NIS Naftagas D.O.O., Narodnog fronta 12, 21000, Novi Sad, Serbia

22 University of Novi Sad, Faculty of Science, Department of Geography, Tourism and Hotel Management, Trg Dostieja Obradovića 3, 21000 Novi Sad, Serbia.

Corresponding professor: biljana.basarin@gmail.com

systems and the first one to think of is the impact on human health. A detailed analysis of extreme heatwave events in Serbia from the biometeorological point of view is presented in this study. For this purpose, the newly developed Heat Wave Magnitude Index daily (HWMId), was used on Physiologically equivalent temperature (PET) for Serbia.

OBJECTIVES:

The main target of this paper is to describe and analyze the meteorological conditions and the consequences of this phenomenon.

METHOD / DESIGN:

For the identification of the most extreme heat waves over South East Europe (SEE) the E-OBS 20.0e dataset in 0.1° horizontal grid spacing was used, which is analogous to that used in the ERA5 reanalysis and CORDEX regional climate models. The HWMId was calculated using daily maximum temperatures for SEE and the events with the higest scores were used for further detailed analysis. The focus was then shifted on biometeorological conditions of heat waves in Serbia. A series of daily maximum air temperature, relative humidity, the wind was used to calculate PET for the iperiod 1979–2019. HWMId is defined as the maximum magnitude of the heatwaves in a year. Here, the heatwave is characterized as 3 consecutive days with maximum PET above the daily threshold for the reference period 1981–2010.

RESULTS:

The analysis revealed that during the investigated period the most intensive heat waves in SEE occurred in 2007, 2012 and 2015. HWMId values for 2007 were as high as 37 indicating extreme heat stress, while for the other two events the values were not as high. In Serbia biometeorological heat waves was very intense during 2007 with HWMId ranging between of 8 to 23. Hourly temperatures revealed that the PET values during the day were as high as 55°C. The nighttime temperatures were very high as well, above 22°C. These high nighttime temperatures are very dangerous as they do not allow people to recover from daytime heat. When warm low temperatures are combined with high humidity, conditions can become dangerous, if not deadly, even in the middle of the night. Without relief from the heat at night, heat stress can continue to build and increase the risk of heat illnesses and death.

CONCLUSIONS:

Thus, the mitigation and adaptation to extreme temperature events are of vital importance for humans and their everyday activities. Future investigation should be oriented towards a way to deal with the oppressive heat. Additionally, more research is needed in order to explain and predict these catastrophic events. The main focus of future activities will be on determining the physical causes which lead to the occurrence of extreme heatwaves.

T1-P-1 Program R in mapping of National Parks

Milena Lakićević[23], Duško Ćirović[23], Mia Vicković[23], Radenka Kolarov[24]

KEYWORDS: map; protected areas; R package "leaflet"

INTRODUCTION:

Program R and its interface RStudio have become excessively applied in ecology and environmental sciences. The program offers full support for diverse statistical and numerical analysis, but also provides a wide range of possibilities for mapping and spatial analysis, and spatial metrics. Some of the most commonly applied R packages suited for mapping are "leaflet", "map", "mapview", "ggmap", "RgoogleMaps", etc. These packages produce either interactive or static maps, and selecting the specific package depends on the type of mapping tasks, as well as on the user's preference and fluency in R programming.

OBJECTIVES:

The main objective of this research is the mapping of national parks in Serbia using the R package "leaflet", as one of the packages that do not require high fluency in R. The package produces interactive maps that could be additionally zoomed in or zoomed out. Mastering this package can be a useful introduction to more advanced R packages. Therefore, the secondary objective of the paper is to provide an overview of other R packages that could be suitable for mapping and spatial analysis of protected areas. The mapping of protected areas is crucial from the perspective of defining adaptive management plans and conserving natural values within, and this paper aims to show a procedure that supports the mapping process in these terms.

METHOD / DESIGN:

For mapping purposes, we have used the "leaflet" package within the R program (version 3.5.1) and RStudio (version 1.1.463). R is free software, as well as RStudio and the "leaflet" package, and that gives this procedure a fair advantage in comparison to the other mapping methods and techniques. Creating maps of five national parks in Serbia: NP "Fruska gora", NP "Djerdap", NP "Tara", NP "Kopaonik", and NP "Sar planina" has been set as a mapping assignment.

RESULTS:

There were six maps created in total: five maps for each of the national parks separately (NP "Fruska gora", NP "Djerdap", NP "Tara", NP "Kopaonik" and NP "Sar planina") and one map containing previews of all national parks together. Maps presenting a single national park are being set in a narrow spatial context – encompassing a small portion of the surrounding area, while a final map presents all national parks on the map of the Republic of Serbia. This section provides a brief explanation below each map, related to the size of the protected area, year of the proclamation of the zone as a national park, characteristics of local flora, etc.

CONCLUSIONS:

Contemporary computer tools allow easy procedure of creating maps. Nowadays, program R with its supporting packages is one of the most prominent computer tools. When the coordinates of a certain area are being known, it is an easy task to import them in the R environment and work over them using one (or more) available packages. For users encountering the R for the first time, we recommend starting with the "leaflet" package. The syntax necessary for this package is easily under-

23 University of Belgrade, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia

24 University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia. Corresponding author: milenal@polj.uns.ac.rs

standable and straightforward and allows creating different types of maps in a short time notice. This procedure is relevant for the mapping of protected natural areas, such as national parks in Serbia presented in the paper. Creating different types of maps in R is very useful when defining adaptive management plans and conservation strategies for natural assets within protected areas.

T1-P-2 The responce and tolerance mechanisms of lettuce (*Latuca Sativa L.*) exposed to increased concentrations of manganese in aquatic cultures

Marija Marinković[25], Jelena Vulinović[25],
Danijela Arsenov[26], Milan Župunski[26],
Nenad Popov[27], Milica Živkov Balaš[26], Milan Borišev[27]

KEYWORDS: Mn; accumulation; antioxidative activity; tolerance.

INTRODUCTION:

Manganese (Mn) is a microelement that has an important metabolic role within different plant cell compartments. The essential amount of Mn in plants should be in the range of 20-40 mg Mn / kg of dry weight for its various functions. Mn is an essential cofactor for the oxygen evolving complex of the photosynthetic machinery, catalyzing the water-splitting reaction in photosystem II. Manganese is involved in chlorophyll biosynthesis and is therefore vital for the photosynthesis process, as it plays an important role in electron transport in this process. Due to different degrees of valence, Mn is a regulator of many oxido-reduction processes. It is part of many enzymes and is involved in protein synthesis. In addition, it has a significant impact on building resistance to abiotic and biotic stresses, which is important for this experiment.

OBJECTIVES:

The main goal of this paper is to monitor the reaction of lettuce *Lactuca sativa L.* to elevated Mn content, as well as the potential of Mn uptake and the development of plant tolerance to stress.

METHOD / DESIGN:

The plants used for this experiment were grown by the method of static water cultures with aeration, using the Hoagland nutrient solution of full strength. After 40 days of growth, the plants were exposed to Mn treatment. For seven days, the control plants were grown on a pure nutrient solution, whereas half of plants where placed on tenfold higher concentrations of manganese in a nutrient solution. After sampling, the parameters of photosynthesis and water regime were measured, as well as the enzymes activity and biochemical indicators.

RESULTS:

Conducted analyses showed that Mn treatment did not significantly affect the intensity of photosynthesis, indicating stable bioproduction in spite of Mn treatment. Mn treatment significantly reduced stomatal conductivity, although the transpiration rate was not affected accordingly. Intrinsic water use efficiency was elevated under the influence of Mn treatment. These

25 *Faculty of Technology, University of East Sarajevo, Zvornik, Republic of Srpska, Bosnia and Hercegovina.*

Corresponding author: marija.marinkovic197@gmail.com

26 *Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia*

27 *Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia*

results lead to the conclusion that Mn to some extent affected the function of the stomatal apparatus. Among the examined stress indicators, only the concentration of proline increased, indicating the metabolic acclimatization of plants to Mn exposure. Since Mn did not affect the concentration of glutathione and thiobarbituric acid-reactive substances, it implies that the stress was relatively moderate and successfully managed by the treated plants. Mn treatment of *Lactuca sativa L.* plants did not significantly affect the activity of antioxidant enzymes except for the decrease in catalase activity. This indicates that the antioxidant system of cells was not under intense oxidative pressure coming from the treatment. Respiration enzymes where also not affected. Treatment of plants with Mn resulted in significantly higher Mn accumulation in treated plants (788,1 µg/g in leaves and 5775,2 µg/g in roots), compared to the control (122, 6 µg/g in leaves and 1641,0 µg/g in roots). The accumulation was significantly higher in the roots of plants than in the leaves.

CONCLUSIONS:

The results of the analysis lead to the conclusion that *Lactuca sativa L.* is a good candidate species for phytoextraction at moderate Mn load. The tenfold increase of Mn levels resulted in negligible and very moderate stress, and at the same time balanced bioproduction as an important factor for successful phytoremediation trials.

T1-P-3 New Species Discovery Using Dna Barcoding Approach - A Case Study In *Merodon aureus* Group (Diptera, Syrphidae)

Iva Gorše[28], Ivana Matić[28],
Ljiljana Šašić Zorić[29],
Mihajla Djan[28], Ante Vujić[28]

KEYWORDS: DNA barcode; Georgia; *Merodon cinereus* subgroup; pupa

INTRODUCTION:

Hoverflies (Diptera, Syrphidae) are recognized as insect pollinators of exceptional importance in both natural and agricultural ecosystems. Considering the accelerating pollinator's decline, the accurate taxa identification within this insect family is prudent. Nevertheless, due to the presence of multiple cryptic taxa, resolving the *Merodon aureus* group of hoverfly species has been challenging. The traditional taxonomy based on the morphological approach has its limitations, especially in the cases of the cryptic taxa and morphological differences between developmental stages and/or sexes. However, the implementation of the integrative taxonomy approach involving molecular markers, geometric morphometry, and ecological data helps to resolve species of the *M. aureus* group into subgroups and species complexes.

OBJECTIVES:

In the present study, we analyzed both the 3' and 5' ends of the mitochondrial cytochrome c oxidase I gene (COI), with the aim to delineate and identify hoverfly specimens collected in Georgia to a species level within the *M. aureus* group.

METHOD / DESIGN:

The genomic DNA was extracted from the insect mid and hind legs following SDS DNA extraction protocol. The 3' and 5' ends of the COI gene were amplified and commercially sequenced in the forward direction by the Sequencing Laboratory of the Finnish Institute for Molecular Medicine (Helsinki, Finland) and Macrogen Europe (Amsterdam, Netherlands). The edited and

28 *University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia*

29 *University of Novi Sad, BioSense Institute, Dr Zorana Đinđića 1, 21000 Novi Sad, Serbia. Corresponding author: ljsasic@biosense.rs*

aligned 3' and 5' COI gene sequences were concatenated and combined into a single sequence matrix with the final length of 1260 bp. The COI matrix was used as input for the maximum parsimony and the maximum likelihood tree construction. The COI haplotypes were compared with the sequence database of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST).

RESULTS:

Specimens from Georgia are resolved as monophyletic on both maximum parsimony and maximum likelihood trees with high bootstrap support values (99). The BLAST search of the NCBI nucleotide database confirmed the belonging of these specimens to the *M. aureus* species group, while the tree topologies indicate that they were genetically most similar to the *M. aff. bessarabicus* from Turkey. Furthermore, this clade comprises the specimen in the juvenile stage from Georgia, that has not been successfully identified to a species level in the previous studies.

CONCLUSIONS:

In this study, we discovered one new candidate species within the *M. aureus* group and defined the species status of the juvenile specimen from Georgia. The new species morphologically belong to the *M. cinereus* subgroup. However, our study showed a high genetic similarity between this species and morphologically different *M. aff. bessarabicus* from Turkey which belongs to the *M. bessarabicus* subgroup. Such high discordance between molecular and morphological divergence has already been noticed within the *M. aureus* group, and in this particular case, it is probably a result of the geographical proximity, as well as the introgression during the evolutionary past of the two species. In order to fully address causes of observed discordance additional morphological and molecular analyses will be needed.

T1-P-4 Diversity of *branchipus* populations (branchipoda, crustacea) on the territory of Serbia - could the body size be an indicator of geographical and environmental distinctness?

Dragana Miličić[30], Sofija Pavković-Lučić[30], Jelena Trajković[30],Tatjana Savić[31]

KEYWORDS: Branchiopoda; morphology; diversity

INTRODUCTION:

Large branchiopod crustaceans (Class Branchiopoda) usually inhabit small and ephemeral inland water bodies. Some species have been included into national red lists, and some are strictly protected in many European countries. Both the abundance of their specimens in natural populations and their body size can be used as bioindicators of geographical and environmental differences. For the reasons stated above, this group can be used for defining the ephemeral wetland habitats and their possible functions and values.

OBJECTIVES:

The objective of this study was to determine whether the morphological analysis can be used to differentiate *Branchipus* populations from several areas within a certain territory. We used populations from the northern, Pannonian parts (Srem, Banat, and Bačka Districts), and from the southern, mountainous region of Serbia, whose habitats are particularly different in their origin, and physical/chemical features.

30 *Universety of Belgrade – Faculty of Biology, Belgrade, Serbia, 2Institute for Biological Research. Corresponding author: jelena.trajkovic@bio.bg.ac.rs*

31 *"Siniša Stanković" - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia*

METHOD / DESIGN:

After selecting the most representative locations, body parts that are common to both sexes were measured in all examined populations: a total body length, thorax, abdomen, and cercopod lengths, ratio of total body to thorax length and ratio of total body to the abdomen length. The measurements were performed with the accuracy of 0.1 cm. Data were analyzed using descriptive statistics.

RESULTS:

According to the linear body measurements common for both sexes, results of descriptive statistical analysis showed that two geographical groups of individuals can be distinguished: *Branchipus* populations from the northern habitats (plain landscape of the country), and populations from localities in the southern (hilly part of the country). Morphological parameters that mostly affected differentiation of samples were: abdominal length, the contribution of thorax in total body length, and the contribution of abdomen in total body length.

CONCLUSIONS:

Large branchiopod crustaceans are the flagship animal group for some inland water ecosystems and temporary pools. Results of this study show that two geographical groups of individuals stood out from very different types of habitats, occurring in the northern lands and in the southern hilly and mountainous areas of the country. Present results indicate that linear morphological data obtained by applying the method of discriminate morphological analysis can be an auxiliary method in taxonomic determination of populations and assessment of the regional biodiversity.

T1-P-5 Food choice in *Drosophila melanogaster*: the role of diet type, sex and social environment

Jelena Trajković[32], Sofija Pavković-Lučić[32], Dragana Miličić[32], Tatjana Savić[33]

KEYWORDS: nutrition; food choice; fruit fly

INTRODUCTION:

Insect nutrition is a widely researched topic, since diet affects many biological processes, which can be monitored from cellular to behavioral level. As one of the most important environmental factors, food quality and balance in the amount of key nutrients, strongly affect fruit fly *Drosophila melanogaster* fitness. A number of genes involved in multiple sensory pathways and complex physiological systems are consequently included in the regulation of feeding behavior. The fruit fly food-related behavior is primarily influenced by nutritional needs, and may change throughout life. Also, food choice may be determined by sex and the social environment, i.e. by the presence of other individuals.

OBJECTIVES:

The aim of this study was to determine whether there were differences in food choice between *D. melanogaster* strains reared on two different substrates for more than 20 years. For that purpose, five food items were offered. Further, it was examined whether sex and social environment influenced food choice in these two strains.

32 *Universety of Belgrade – Faculty of Biology, Belgrade, Serbia, 2Institute for Biological Research. Corresponding author: jelena.trajkovic@bio.bg.ac.rs*

33 *Siniša Stanković" - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia*

METHOD / DESIGN:

D. melanogaster strains used in this experiment were maintained for more than 450 generations on two different substrates, standard cornmeal substrate and substrate modified by adding apple. Transparent plastic boxes, dimensions: 220 × 140 × 90mm, which contained five Petri dishes (r = 30mm) were used for monitoring food choice. Petri dishes were filled with five different substrates: standard cornmeal substrate and substrates that contain tomato, banana, carrot and apple. Flies were starved for 18h before being placed into each box. Virgin females and males, 3 - 5 days old, were separated and tested individually and in groups of five individuals, and foraging flies were sampled every 3 min for 1h. Four-way ANOVA was applied in order to determine difference in time that flies spent on different diets, between individuals and groups, and between sexes.

RESULTS:

Results pointed out significant differences in the time that flies spent by occupying different food items. On the other hand, sex, strain and social environment revealed no significant influence on *D. melanogaster* food choice. However, significant interaction between strain and food choice was observed. In both strains, the preference toward standard cornmeal substrate was noticed. Even more, flies reared on apple substrate spent significantly more time on Petri dish filled with standard cornmeal diet, compared to flies reared on standard substrate.

CONCLUSIONS:

These results indicated that flies chose nutritionally richer food (standard cornmeal substrate, rich in sugar and yeast), especially if they were reared on poor diet (the apple substrate). According to data from our previous surveys, substrates that we offered to flies differ in protein content and in the proportion of protein relative to the total content of organic carbon (C/N ratio), which accurately reflected the protein/carbohydrate ratio. Contrary to standard substrate, apple substrate contained smaller amount of protein and higher C/N ratio. The fact that food choice was not influenced by sex or social environment might suggest the same nutritional requirements for the best available food in both sexes, regardless of whether flies were tested individually or in a group.

T1-P-6 Proline-based deep eutectic solvents as greener alternative for obtaining ployphenol rich extracts of *Satureja Kitaibelii*

Jelena Arsenijević[34], Nada Kovačević[34], Milica Drobac[34], Slavica Ražić[35],
Fathi Emhemmed[36], Christian Muller[36], Christophe Marcic[36], Eric Marchioni[36]

KEYWORDS: DES; cytotoxicity; extraction; caffeic acid oligomers; *Satureja L.*

INTRODUCTION:

Aerial parts of *Satureja kitaibelii* Wierzb. ex Heuff. (Lamiaceae), in Serbia known as Rtanj's tea, are traditionally used to treat various respiratory, urinary and other health disorders. Extracts of this herb exhibit a significant bioactivity as well[37]. Using deep eutectic solvents (DESs) for extraction of certain phenolic compounds is in line with the principles of green chemistry[38].

34 *University of Belgrade – Faculty of Pharmacy, Department of Pharmacognosy, Belgrade, Serbia. Corresponding author: jelena.arsenijevic@pharmacy.bg.ac.rs*

35 *University of Belgrade – Faculty of Pharmacy, Department of Analytical Chemistry, Belgrade, Serbia*

36 *Institut Pluridisciplinaire Hubert Curien, UMR 7178 CNRS, Université de Strasbourg, Illkirch, France*

37 *Gopčević et al., 2019. Plant Foods Hum Nutr 74:179.*

38 *Jakovljević et al., 2020. Plants 9(2): 153.*

However, the toxicity of DESs must be considered[39].

OBJECTIVES:

The main objectives of this work are to assess the polyphenol-extracting ability of proline (Pro) and sugar/sugar alcohol based natural DESs from commercially available Rtanj's tea, as well as to evaluate cytotoxicity of these solvents against AsPC-1 cells.

METHOD / DESIGN:

Accurately weighed Pro and sugar/sugar alcohol, were dissolved in water, frozen, and freeze-dried. The obtained seven DESs (i.e. Pro with: glucose 1:1 and 5:3, fructose 1:1 and 5:3, sorbitol 1:2, and sucrose 2:1 and 3:2) were mixed with water (30%, *m/m*). Polyphenol extraction was examined using commercial sample of Rtanj's tea (manufacturer Bojan Radosavljević, Boljevac). Powdered herb (particle diameter 100-200 μm) was extracted by sonication during 30 min at room temperature with the obtained aqueous DESs (herb-to-solvent ratio 1:20), as well as with water, absolute ethanol, or 50% (*v/v*) ethanol under the same conditions. The qualitative analysis of extracts was conducted by both HPLC and LC-MS. The content of the identified marker compounds in the extracts, i.e. rosmarinic acid (RA) and clinopodic acid O (CAO), was determined by external calibration using RA as the standard compound. Cytotoxicity of the aqueous DESs against human pancreatic adenocarcinoma cells AsPC-1 was tested at four concentration levels (5-25%), after 48 h of incubation and propidium iodide staining. The analysis on Guava® easyCyte 12HT Benchtop flow microcapillary cytometer, was performed afterwards, using InCyte® software package.

RESULTS:

Upon freeze-drying, mixtures had glassy appearance and transformed into liquids after mild heating. All obtained DESs were highly viscous, and therefore mixed with water. Qualitative LC-MS analysis of 50% ethanol extract revealed the presence of phenolic acids, flavonoids, and jasmonic acid derivatives. Among phenolic acids, the dominant compounds were caffeic acid oligomers RA and CAO. Among conventional solvents, 50% ethanol was better extracting agent than absolute ethanol or water for both RA (88.2 μg/mL) and CAO (116.8 μg/mL). Water extract was also abundant with CAO (106.7 μg/mL), but contained moderate amount of RA (21.7 μg/mL). It is noteworthy to mention that the extraction with ethanol resulted in very low yield of both phenolics, with CAO concentration, being even below detection limit in the absolute ethanol extract. Concentration of RA in the tested DES extracts was higher than corresponding one in the water extract, but lower than in the 50% ethanol extract, and varied in the range from 61.6 μg/mL (in Pro-fructose 1:1 extract) to 85.6 μg/mL (Pro-glucose 1:1 extract). The extraction of CAO, with six out of seven aqueous DESs, was more efficient than with 50% ethanol, resulting in CAO concentration range from 119.6 μg/mL, in Pro-sucrose 3:2 extract, to 172.4 μg/mL, in Pro-glucose 1:1 extract. Pro-fructose 1:1 extract had the lowest content of CAO (86.7 μg/mL) among the tested DESs. At the lowest tested concentration (5%), aqueous DESs did not significantly affect survival of AsPC-1 cells in comparison to the untreated cells (83.1-90.0% and 86.6% of cells remained viable, respectively). Both proline-glucose DESs demonstrated the lowest toxicity. However, at the highest concentration (25%) all aqueous DESs caused death of more than 70% of AsPC-1 cells.

CONCLUSIONS:

The obtained results indicate that proline and sugar/sugar alcohol based deep eutectic solvents are good extracting agents for phenolic compounds, especially for higher caffeic acid oligomers such as clinopodic acid O. Additionally, low cytotoxicity of tested DESs is a good starting predictor of their safety and potential usage.

39 *Hayyan et al., 2013. Chemosphere 90(7): 2193.*

T1-P-7 Assessment of litter decomposition in temperate deciduous forest: A case study in Fruška Gora, Serbia

Andrijana Andrić[40], Dušanka Vujanović[40],
Snežana Popov[41], Zlata Markov Ristić[41]

KEYWORDS: TeaComposition initiative; litter decomposition; litter quality

INTRODUCTION:

Our study has been conducted as a part of the large scale decomposition experiment within the global collaborative network "TeaComposition initiative". The aim of this initiative was to estimate short- to long-term plant litter decomposition rates by using standard protocols and substrates—commercially available Green tea and Rooibos tea with different decomposition rates—for comparison of the litter mass loss at numerous sites across various ecosystems worldwide.

OBJECTIVES:

The aim of our study was to test the effects of both litter type and land-use on litter decomposition in 3, 12, 24 and 36 months of incubation, by comparing the percentages of the tea mass lost.

METHOD / DESIGN:

The TeaComposition method (modified Tea bag method) involves measuring a tea bag before and after incubation in the field, and using the difference in weight as a measure of the organic material decomposed. The three localities chosen for our experiment corresponded to the three levels of protection regime established for the National park Fruška gora, with different management and treatments within the temperate deciduous forest. The guidelines of the standardized protocol of the "TeaComposition initiative" were followed throughout. Two homogenous plots were selected at each of the three sub-sites; two replicates of the two tea types were buried in the topsoil layer in each of the two blocks, resulting in four bags of each tea type per sub-site and sampling time.

RESULTS:

The values of the tea mass lost during all four incubation periods were higher for the Green tea than for the Rooibos tea. This pattern was expected because of the faster decomposition rate of Green tea due to higher content of non-lignified cellulose and of water-soluble compounds. Furthermore, the difference of the two tea types' mass loss was the highest in three-months incubation. Our study has also shown no clear pattern regarding the values variation of the tea mass loss among three different plots; however, the highest level of variation was found for the Green tea in the longer incubation periods (24 and 36 months).

CONCLUSIONS:

These conclusions are in accordance with the results of previous research showing that, in the early stage of litter decomposition, the litter quality had the strongest influence on mass loss, whereas there was no significant effect of land-use or management practices. The microbial decomposition is carried out by many groups of microorganisms and is not limited by nutrients during the growing season. Still, differences in the litter mass loss among the land-use types increase in the later

40 University of Novi Sad, BioSense Institute, Dr Zorana Đinđića 1, 21000 Novi Sad, Serbia. Corresponding author: andrijana.andric@biosense.rs

41 University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

phases—as decomposition progresses—because of the decomposer groups being more selected, since fewer microbes possess the degradation enzymes for the remaining organic compounds. This pattern is clearer in Green tea, because Rooibos tea has much slower plant litter decomposition rate, due to high lignin content.

T1-P-8 First record of the endemic earthworm *Allolobophora (sensu lato) strumicae* (Šapkarev, 1973) (*Clitellata: Lumbricidae*) in Serbia, with comments on its ecology and distribution

Filip Popović[42], Mirjana Stojanović[42], Tanja Trakić[42], Jovana Sekulić[43]

KEYWORDS: earthworm; new record; *Allolobophora (sensu lato) strumicae*; Serbia;

INTRODUCTION:

The earthworm fauna of Serbia is quite well-known. It is worth mentioning that most of the earlier research focused mainly on northern, central and eastern Serbia, and not many collecting expeditions were led to the areas of Kopaonik Mountain. The Kopaonik Mt. (43°16'N, 20°49'E) is situated between the central and southern part of Serbia and belongs to the Dinaric Mountain range.

OBJECTIVES:

The aims of the present study are to provide information on the distribution of *Allolobophora (sensu lato) strumicae* (Šapkarev, 1973) in the country and in the adjacent areas of the Balkan Peninsula. In addition, we comment on the ecological preferences.

METHOD / DESIGN:

The specimens for this study were collected during the period from 2018 to 2021, in the southwestern and southern slopes of Kopaonik Mt. They were collected using the diluted formaldehyde method complemented with digging (0.4 x 0.4 m^2). The earthworms were killed in 70% ethanol, immediately fixed in 4% formalin solution and transferred and stored in 90% ethanol. Earthworms were identified to species level and only mature individuals were counted. All of the specimens collected and examined are permanently archived at either and the Earthworm Collection of the University of Kragujevac, Serbia (CEKUS).

RESULTS:

Identified the earthworm material recently collected from this mountain range, resulting in a new record of the endemic species *All. (s.l.) strumicae*. The endemic earthworm species *All. (s.l.) strumicae* previously known only from the Strumica region in North Macedonia (Šapkarev, 1973; Mršić, 1991), is reported from the Serbia for the first time. Further, the new localities from Kopaonik Mt. represent the northernmost limit of the species' natural range for now. The distribution of this species is hill meadows, pastures and oak forests at altitudes of 600 to 800 m a.s.l. The most represented period of All. (s.l.) strumicae is identified as April–May. Regarding ecological categories, it belongs to the deep-burrowing-endogeic species. Actually, this species has a remarkable adaptation to life in the deep soil and strong development of the capacities of displacement in the soil.

42 University of Kragujevac Faculty of Science, Institute of Biology and Ecology, Radoja Domanovića 12, 34000 Kragujevac, Serbia.

Corresponding author: filip.popovic@pmf.kg.ac.rs

43 University of Kragujevac, Institute for Information Technologies Kragujevac, Department of Science, Jovana Cvijića bb, 34000 Kragujevac, Serbia

CONCLUSIONS:

The results of our study provide new faunistic data about expand the knowledge about the distribution of *All. (s.l.) strumicae* on the Balkan Peninsula. The finding endemic species in the Serbia confirms the rich and remarkable biodiversity in this country as well the importance of defining mitigation measures for minimizing the negative anthropogenic impacts towards the habitats of this species. Also, currently this species has an uncertain status within the genus *Allolobophora*. Our future research will try to solve the taxonomic status of this species by applying integrative systematics.

T1-P-9 Comparison of the earthworm fauna of oak and spruce forest on western slopes Kopaonik mountain in Serbia

Filip Popović[44], Mirjana Stojanović[44], Tanja Trakić[44], Jovana Sekulić[45]

KEYWORDS: earthworm; diversity; oak forest; spruce forests; Kopaonik Mountain;

INTRODUCTION:

In this paper, we compare the earthworm fauna of coniferous and deciduous forests with different environments in the western slopes of Kopaonik Mountain.

OBJECTIVES:

Our objectives were: a) to determine earthworm fauna in oak and spruce forests b) to determine which forest is richer in earthworm fauna c) to examine the impact of vegetation cover, soil and altitude on the diversity of earthworm fauna in the studied forests.

METHOD / DESIGN:

Earthworm communities were sampled within two months in both 2018 and 2020 (from May and June, coinciding with the rainy season) in the western slopes of Kopaonik Mt. In the hilly belt up to 1.000 m, sampled three plots of the oak forest (OF1 411 m, OF2 670 m and OF3 800 m a.s.l.) which is where sierozem on serpentines and humus silicate soil on serpentines occurs. Also, the mountain belt from 1.500 to 1.950 m, sampled three plots of the spruce forest (SF1 1.750 m, SF2 1.830 m and SF3 1950 m a.s.l.), types of soil present are brown podzolic soil and acidic humus-silicate soils. We were sampled eight samples 50x50 cm from each of the sampling units (plots). Earthworms were identified to species level and only mature individuals were counted. Paleontological statistics software (PAST) was utilized for calculating the alpha diversity index (Shannon-Wiener, Evenness and Berger-Parker) in the studied localities.

RESULTS:

A total of 195 earthworm individuals (113 individuals in oak forest/82 individuals in a spruce forest), of which 9 species *Allolobophora chlorotica, Allolobophora leoni, Allolobophora (s.l.) strumicae, Apporectodea rosea, Dendrobaena vejdovskyi, Dendrobaena octaedra, Eisenia fetida, Lumbricus rubellus* and *Proctodrilus antipai* were recorded in oak forest, while species *Dendrobaena attemsi attemsi, Dendrobaena alpina alpina, Dendrobaena byblica byblica, Dendrobaena illyrica and D. octaedra* were recorded in a spruce forest. Oak forests were the richer in earthworm species (average 6 species). Upper montane spruce forests were the poorer in earthworm species (average 4 species). to the Shannon-Wiener and Evenness index, it was indicat-

44 University of Kragujevac Faculty of Science, Institute of Biology and Ecology, Radoja Domanovića 12, 34000 Kragujevac, Serbia.

Corresponding author: filip.popovic@pmf.kg.ac.rs

45 University of Kragujevac, Institute for Information Technologies Kragujevac, Department of Science, Jovana Cvijića bb, 34000 Kragujevac, Serbia

ed that oak forest had a higher species diversity than the spruce forest. In contrast, the Berger-Parker index showed higher dominance species in spruce forests (*Table 1*).

	Studied locations					
Alpha diversity index	OF	OF	OF	SF	SF	SF
	1	2	3	1	2	3
Taxa_S	6	6	6	3	5	4
Individuals	44	40	29	23	31	28
	1,6	1,7	1,6	0,8	0,9	0,6
Shannon_H	9	1	6	2	1	6
Evenness_e^H/S	0,9	0,9	0,8	0,7	0,5	0,4
	0	2	7	6	0	8
	0,3	0,2	0,2	0,7	0,7	0,8
Berger-Parker	0	8	8	0	4	2

Table 1. Alpha diversity index of earthworm fauna in the studied locations

This study indicates that oak forests are richer in the earthworm taxa relative to the spruce forests. This is because of the optimum environmental factors in oak forests and they can also provide suitable habitats for earthworms (i.e., more humidity and food). However, dominance was higher in spruce forests, because mostly epigeic species has freeze-hardiness and tolerate acid soils. Overall, our results support that climatic factors, vegetation cover, soil characteristics and altitude is impacting the diversity of earthworm fauna in studied forests.

T1-P-10 Available vs used prey – combined methods reveal breeding diet of the European Roller (*Coracias Garrulus*) in Serbia

Lea Milinski[46], Maja Arok[46], Ivana Matić[46], Tijana Nikolić[46], Dimitrije Radišić[47]

KEYWORDS: Ivlev index; sweep net; pitfall trap; arthropod

INTRODUCTION:

Population recovery of the threatened European roller (*Coracias garrulus*) was achieved during the last few decades by installation of nest-boxes as a substitute for lost natural nesting places. Lack of available food acts as the second limiting factor for the roller.

OBJECTIVES:

Data about roller food preference and prey availability are lacking in Serbia. Therefore, the diet study was essential to understand roller needs in order to achieve conservation measures which would provide returns to breeding sites in the future.

METHOD / DESIGN:

Field work was carried out within the breeding territories in the Central Banat region (Serbia). Prey availability and preference were determined by sampling available (pitfall traps and sweep net catches) and used prey (food remains from nest-boxes) during five breeding seasons.

46 *University of Novi Sad, Biosense Institute – Research Institute for Information Technologies in Biosystems, Doktora Zorana Đinđića 1, Novi Sad, Serbia*

47 *University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 3, Novi Sad, Serbia.*

Corresponding author: leav@dbe.uns.ac.rs

RESULTS:

Ivlev index was used to assess compatibility of used vs available prey for coleopterans and vertebrates. Avoidance of orthopterans and the rest of invertebrate prey (arthropods other than Coleoptera and Orthoptera) was misleading due to the methods used. Orthopterans are consumable to a higher degree, so over-estimation of coleopterans due to higher amounts of chitinous remains found in nest-boxes doesn't surprise. Apiaries were found near the research plots, causing higher proportions of hymenopterans within available invertebrate prey. Contrary to this, we only found one bee specimen in the nest-box. This confirms that roller avoids hunting fast flying insects. Low percentage of orthopterans within the pitfall traps has been complemented by sweep net catches. Therefore, combining methods resulted with a composition of available prey that corresponds to the food remains. Variety of prey groups have taken part in the diet composition. Most of them were arthropods, while 5% of vertebrates were detected (amphibians, reptiles and small mammals).

CONCLUSIONS:

Based on our findings, we recognize the European roller as an opportunistic predator that can survive upon poor, overgrazed, and dry pastures.

T1-P-11 Integration of data from the *In vitro* long-term exposure study on human endothelial cells and the *In silico* analysis: A case of dibutyl phthalate-induced vascular dysfunction

Bojana Stanic, Dunja Kokai, Kristina Pogrmic-Majkic, Dragana Samardzija Nenadov, Biljana Tesic, Svetlana Fa, Nebojsa Andric[48]

KEYWORDS: dibutyl phthalate; human endothelial cells; vascular dysfunction; *in vitro*; *in silico*

INTRODUCTION:

Dibutyl phthalate (DBP), a synthetic chemical used in many industrial and consumer products, exhibits its effects mainly on the endocrine and reproductive system, but it can also affect the function of the vasculature, thereby contributing to the incidence of cardiovascular diseases (CVDs) in the human population; however, the underlying mechanisms behind the DBP-induced vascular dysfunction are not fully understood. Aside from CVDs, endothelial dysfunction has also been associated with other human diseases such as diabetes, insulin resistance, chronic kidney failure, tumor growth, venous disease, and several viral infection diseases, thus widening the potential target organs affected by DBP and human diseases that DBP can contribute to.

OBJECTIVES:

Here, we sought to integrate the experimental data obtained from the long-term exposure of human vascular endothelial cells (ECs) to DBP and the *in silico* analysis to infer the molecular mechanism of DBP's action in vascular ECs and provide a possible association between DBP exposure and human diseases with vascular etiology.

METHOD / DESIGN:

EA.hy926 cells originating from three different cryopreserved stock vials were exposed to either control conditions (vehicle, 0.05% DMSO) or three human exposure relevant concentrations of DBP (10^{-9}, 10^{-8} and 10^{-7} M), and cultured independently in cell culture flasks during 4 weeks. The cells were passaged and counted twice a week, whereas the media containing differ-

48 *University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Serbia. Corresponding author: nebojsa.andric@dbe.uns.ac.rs*

ent treatments were replaced four times a week. The mRNA expression analysis was performed using quantitative RT-PCR. Cell viability, cell adhesion to gelatin, cell migration, endothelial permeability, monocyte adhesion to the endothelial monolayer, nitric oxide (NO) production, and protein expression were examined using standard protocols. The list of enriched diseases was obtained by entering the DBP-affected genes in the Comparative Toxicogenomic Database's (CTD) Set Analyzer tool. Statistical analysis was performed using the two-sided hypergeometric test with Bonferroni step-down correction and a k score of 0.4. Cytoscape analysis was used for network visualization, whereas the Gene Ontology and the KEGG pathway analysis were performed to identify biological processes, molecular functions, and specific signaling pathways for the DBP-affected genes. Count 2 and the EASE score of 0.05 were used for the threshold, whereas the Benjamini method was applied to adjust the p value.

RESULTS:

Since vascular endothelium represents the first place of contact with blood and a semi-permeable barrier between the blood and the interstitial space that controls the extravasation of fluids, macromolecules, hormones, and blood cells, we have investigated the genes involved in these processes, namely cell adhesion, cell migration, leukocyte/monocyte adhesion, and NO production. The genes with altered expression after 2 and/or 4 weeks of exposure were considered to be affected by DBP. Prolonged exposure to DBP alters the expression of six genes encoding the integrin family of proteins, $VCAM^{-1}$, $ICAM^{-1}$, and MMP^{-2} in EA.hy926 cells, whereas the mRNA expression of the gene encoding eNOS, *NOS3*, was not affected by DBP exposure in any investigated time point. The in silico analysis has identified 21 functional annotation pathways of KEGG associated with the affected genes. The top molecular functions include integrin binding, cell adhesion molecule binding, signal receptor binding, and chemokine binding, whereas the top biological processes include extracellular matrix (ECM) organization, extracellular structure organization, and cell adhesion mediated by integrins. The role of the ECM-receptor interactions, cell adhesion molecules, and focal adhesions in the DBP-exposed human vascular ECs was further confirmed by the results from the experimental study showing that cell adhesion and migration were altered during the four-week-long *in vitro* exposure of EA.hy926 cells to DBP. In addition, the CTD analysis has shown that the top three human disease categories associated with DBP exposure and vascular dysfunction include CVDs, CVDs/nervous system diseases, and immune system diseases.

CONCLUSIONS:

Integration of experimental and *in silico* approaches used in this study may offer a better understanding of the potential human health risks associated with DBP exposure. Our results provide novel information regarding the molecular mechanism of DBP's action in vascular ECs and contribute to the evidence that exposure to DBP may constitute a risk factor for the occurrence of different diseases with vascular etiology. Additional *in vivo* and *in vitro* studies are needed to improve the confidence in the chain of molecular events involved in DBP's action in vascular ECs and the association with human vascular diseases.

T1-P-12 Constructing the mode of action for di-(2-ethylhexyl) phthalate-induced ovarian toxicity

Kristina Pogrmic-Majkic, Dragana Samardzija Nenadov, Biljana Tesic,
Svetlana Fa, Dunja Kokai, Bojana Stanic, Nebojsa Andric[49]

KEYWORDS: DEHP; mode of action; ovary; bioinformatics analyses; quantitative weight of evidence

49 University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, ENDOS Laboratory, Serbia.

Corresponding author: nebojsa.andric@dbe.uns.ac.rs

INTRODUCTION:

European Commission for Endocrine Disruptors categorizes di-(2-ethylhexyl) phthalate (DEHP), a widely used synthetic chemical of high concern, in Group I – Evidence of endocrine disrupting activity. Human exposure to DEHP is inevitable and arises mainly from ingestion, inhalation, and dermal absorption. DEHP affects the synthesis and regulation of thyroid hormones, causes changes in testosterone level, disrupts testicular morphology, induces sperm damage and genotoxicity. The adverse effects of DEHP on the female reproductive system are also well described. Data from animal studies indicate that DEHP can affect steroidogenesis in the ovary and interfere with oocyte maturation and ovulation. The mechanism of DEHP action in the ovaries involves oxidative stress, expression of peroxisome proliferator-activated receptors, DNA damage, and apoptosis. However, identification of the sequential series of molecular and biological events in DEHP-induced ovarian toxicity remains to be established. The mode of action (MOA)/adverse outcome pathway is a useful tool in linking key molecular and biological events with adverse outcomes of chemical exposure. Identification of MOA could also be valuable in the risk assessment of DEHP exposure.

OBJECTIVES:

We aimed to assess the potential MOA of DEHP-induced ovarian toxicity by using the Comparative Toxicogenomic Database (CTD)-based bioinformatics analysis and the qualitative and quantitative weight of evidence (QWOE).

METHOD / DESIGN:

The CTD (http://ctdbase.org/) literature search was conducted in May 2021. The word "*DEHP*" was input in the "*Chemicals*" search box and the references were retrieved and downloaded for further evaluation. Only experimental research papers on DEHP-induced toxicity were selected for the study. The exclusion criteria were: 1) co-exposure; 2) epidemiological studies; 3) without available full text; 4) non-relevant to DEHP-induced toxicity; and 5) environmental monitoring. The filtered references were further marked and selected based on the ovaries as the target organ. All genes retrieved from the literature search were manually inspected, deduplicated, and pooled. The genes were used as an input for bioinformatics analysis in the Database for Annotation, Visualization and Integrated Discovery (DAVID v6.8). The functional annotation pathways of the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the enriched Gene Ontology (GO) term "*Biological Processes*" (BP) were extracted. The data were presented using the "*ggplot2*" package in the R software. All evidence was used for the QWOE method to assess the potential MOA of DEHP, which included five steps: 1) MOA hypothesis; 2) qualitative evaluation of the evidence for each key event (KE); 3) quantitative rating of each KE by using the Bradford Hill causal considerations; and 4) composite score derivation for each KE.

RESULTS:

The CTD analysis revealed a total of 538 studies investigating DEHP effects in different organs and organ systems. After classification and screening, we have selected 9 studies that describe the effect of DEHP on the ovaries. Some of the main target genes of DEHP exposure in the ovaries are aromatase, steroidogenic acute regulatory protein, cytochrome P450 family 11 subfamily A member 1, cytochrome P450 family 17 subfamily A member 1, cyclin D2, cyclin B1, caspase 3, caspase 8, apoptosis regulator BCL2, luteinizing hormone/choriogonadotropin receptor, inhibin B beta subunit, and others. The results of the bioinformatics analysis show that ovarian steroidogenesis, p53 signaling pathway, PI3K-Akt signaling pathway, steroid hormone biosynthesis, and apoptosis pathway might be involved in DEHP-induced effects on the ovaries. The results of the GO enrichment analysis in DAVID demonstrated that BPs such as activation of cysteine-type endopeptidase, the activity involved in the apoptotic process, steroid biosynthetic process, intrinsic apoptotic signaling pathway in response to DNA damage, positive regulation of intrinsic apoptotic signaling pathway, and estrogen biosynthetic process could be altered in DEHP-exposed ovaries. Two MOAs for DEHP-induced ovarian toxicity were proposed after QWOA: DNA damage and steroidogenic MOA; however, the confidence for these two MOAs was moderate.

CONCLUSIONS:

Based on the CTD-based bioinformatics analysis and QWOE evaluation, we have constructed two MOAs for DEHP-induced ovarian toxicity. QWOE showed moderate confidence for both MOAs, suggesting that the data gap still exists in some KEs. Additional studies are needed to improve the MOA and risk assessment for DEHP-induced ovarian toxicity.

SUPPORT:

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T1-P-13 Nanoplastics Characterization At The Biological Interface

Serena Ducoli[50], Stefania Federici[51], Andrea Zendrini[52], Paolo Bergese[52], Laura E. Depero[51]

KEYWORDS: Nanoplastics; Nanobeads; Biological systems; Interface; Protein corona

INTRODUCTION:

Environmental plastic pollution is a great issue affecting our Planet, especially marine ecosystems. Once enter the environment, plastics undergo degradation processes and fragment into smaller pieces up to the nanoscale. The need to understand the possible implications of microplastics and nanoplastics pollution on the environment and living organisms is becoming increasingly pressing. Given the complexity of separating nanoplastics from environmental samples, studies have been so far conducted using synthetic polystyrene nanobeads. There is an urgent need to create nanomaterials that better reflect the real characteristics of nanoplastics naturally formed, viz. true-to-life nanoplastics (T2LNPs), to close the gap between the laboratory parameters and the rules of nature, and to provide more realistic understandings of the characteristics of nanoplastics.

OBJECTIVES:

In this paper, we present a study on T2LNPs production and characterization and the protein corona formation with respect to synthetic polystyrene nanobeads (nanobeads).

METHOD / DESIGN:

T2LNPs samples were produced from daily life plastic items subjected to a mechanical fragmentation through an ultra-centrifugal mill operating in cryogenic conditions. The produced T2LNPs were characterized by Fourier transform Infrared (FT-IR) spectroscopy to investigate their chemical nature and check the absence of induced chemical modifications. Morphology and size distribution analyses were performed through Atomic Force Microscope (AFM). Finally, the protein corona formation from human plasma on T2LNPs and nanobeads were examined by SDS-PAGE electrophoresis.

RESULTS:

FT-IR analysis highlights the presence of the typical polystyrene peaks in fragmented T2LNPs samples, confirming chemical fingerprint and the presence of chemically intact polymer after fragmentation procedure without induced degradations. The features recorded in AFM topography images show spheroidal nanoparticles, with a strong predominance of tiny particles of few tens of nanometers; aggregates and bigger particles are also present.

50 University of Brescia, Department of Information Engineering, Italy, and INSTM

51 University of Brescia, Department of Mechanical and Industrial Engineering, Italy, and INSTM

52 University of Brescia, Department of Molecular and Translational Medicine, Italy, and CSGI

The bio-nanointerface, investigated through the formation of the protein corona from human plasma both on the T2LNPs and nanobeads surface, reveals different protein adsorption for the two nanomaterials, as highlighted by SDS-PAGE analysis an protein profiles comparison, suggesting different biological behaviors.

CONCLUSIONS:

The differences detected in the two protein corona profiles confirm the gap between controlled models and the complexity in real-life scenarios, supporting the need to develop true-to-life materials as reasonable models for environmental nanoplastics. The broad heterogeneity in size and shape shown by fragmented T2LNPs gives the nanomaterial a peculiar and different behavior compared to the defined pristine nature of nanobeads, nominating T2LNPs as a more faithful material for naturally-occurring nanoplastics and opening the possibility to new and unexpected results in biological interactions.

T1-P-14 Nitric oxide mediates the effects of chronic stress on purinergic P2x7 and adenosine A2b receptore xpression in bone marrow cells

Sanja Momčilović[53], Andrija Bogdanović[53], Maja Milošević[53], Dragana Marković[53], Aleksandra Jauković[53], Diana Bugarski[53], Sanja Vignjević Petrinović[54]

KEYWORDS: chronic stress; hypoxia; stress erythropoiesis; purinergic signaling; nitric oxide

INTRODUCTION:

Insufficient tissue oxygenation under chronic stress conditions causes anemia and triggers a process called stress erythropoiesis (SE). However, despite a marked increase in the number of erythroid progenitors, anemia remains persistent during chronic stress. We have recently demonstrated that chronic stress induces SE by altering local production of nitric oxide (NO) in the bone marrow. Purinergic P2X7R and adenosine A2B (ADORA2B) receptors are expressed on hematopoietic progenitors and activation of ADORA2B regulates erythroid lineage commitment, while P2X7R activation leads to erythroid progenitor cell apoptosis in the hypoxic microenvironment. Both purinergic and NO signaling mediate cellular adaptation to hypoxia and influence cell fate decisions, but their roles in SE remain unknown.

OBJECTIVES:

This study was aimed to examine 1) the effects of chronic psychological stress on the expression of P2X7R and ADORA2B receptors in the bone marrow, and 2) a potential role for NO in obtained stress-induced changes.

METHOD / DESIGN:

Adult male mice were subjected to 2h daily restraint stress for 7 consecutive days. The expression levels of P2X7R and ADORA2B genes in the bone marrow were determined by quantitative real-time PCR analysis. The role of NO was assessed by NO biosynthesis blockade, and mice were randomly assigned to following groups: (1) R - restraint group exposed to daily restraint stress for 7 days; (2) L-NAME + R group, received a subcutaneous injection of L-NAME (non-selective nitric oxide synthase inhibitor) 30 min prior daily restraint; (3) L-NAME group, treated with L-NAME only; or (4) control group.

53 Clinic of Haematology, University Clinical Centre of Serbia, Belgrade, Serbia

54 Institute for Medical Research, National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia.

Corresponding author: sanja.momcilovic@imi.bg.ac.rs

RESULTS:

Chronic exposure to restraint stress significantly increased the expression of P2X7R ($P<0.01$) and ADORA2B ($p<0.05$) genes in the bone marrow. The subcutaneous injection with NO synthase inhibitor L-NAME for 7 consecutive days under basal conditions did not alter the expression of these receptors ($p>0.05$) in the bone marrow. However, blockade of NO biosynthesis prior to daily stress completely prevented stress-induced increase in P2X7R and ADORA2B mRNA levels within bone marrow microenvironment.

CONCLUSIONS:

Taken together, obtained results demonstrate an interplay between NO and purinergic signaling in bone marrow of chronically stressed animals, indicating a physiological significance of this interaction in the regulation of either cellular adaptation or apoptosis under chronic stress conditions.

T1-P-15 Amperometric determination of H_2O_2 by carbon paste electrode surface modified with self-assembled iridium complex particles

Jasmina Anojčić[55], Keerati Kullawanichaiyanan[55], Sanja Mutić[55], Valéria Guzsvány[55], Nararak Leesakul[56], Neda Mimica Dukić[57]

KEYWORDS: hydrogen-peroxide; carbon paste electrode; iridium(III) complex; electrode activation; amperometry

INTRODUCTION:

Hydrogen-peroxide (H_2O_2) has widespread use in many fields including chemical, pharmaceutical, textile and food industries, environmental protection, medicine, etc. It is also a by-product of classical biochemical reactions catalyzed by enzymes from the group of oxidases[58]. Since H_2O_2 itself is an electro-active molecule, nowadays voltammetry and amperometry are among the most convenient methods for its determination including the use of carbon based working electrodes, especially in their modified forms[54].

OBJECTIVES:

In present work, previously synthesized photoactive iridium(III) complex with 3-methyl-2-phenyl pyridine and 1,1-bis(diphenylphosphino)methane i.e. [Ir(3m-ppy)2(dppm)Cl], which showed potential anticancer activity[59], was investigated as surface modifier of carbon paste electrode (Ir/CPE) for novel H_2O_2 sensor design.

55 Division of Physical Science and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla, 90112, Thailand

56 Division of Physical Science and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla, 90112, Thailand

57 University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg D. Obradovića 3, 21000 Novi Sad, Serbia. Corresponding author: jasmina.anojcic@dh.uns.ac.rs

58 W. Chen, S. Cai, Q-Q. Ren, W. Wen, Y-D. Zhao, Analyst 2012, 137. 49–58; J. Anojčić, V. Guzsvány, O. Vajdle, D. Madarász, A. Rónavári, Z. Kónya, K. Kalcher, Sens. Actuat. B 2016, 233. 83–92; V. Guzsvány, J. Anojčić, E. Radulović, O. Vajdle, I. Stanković, D. Madarász, Z. Kónya, K. Kalcher, Microchim. Acta 2017, 184. 1987–1996

59 N. Leesakul, K. Kullawanichaiyanan, S. Mutić, V. Guzsvány, T. Nhukeaw, A. Ratanaphan, S. Saithong, T. Konno, U. Sirimahachai, V. Promarak, J. Coord. Chem. 2021, DOI: 10.1080/00958972.2021.1949585

METHOD / DESIGN:

The surface morphology of Ir/CPE was studied by scanning electron microscopy in combination with energy dispersive spectrometry (SEM-EDS), the electrochemical characterization of the prepared electrodes was performed by cyclic voltammetry (CV), afterwards the amperometry was used for the determination of H_2O_2.

RESULTS:

The characterization of Ir/CPE by SEM-EDS confirmed the presence of densely populated self-assembled Ir based spherical particles of micrometer diameter on the electrode surface. It was found that after appropriate electrochemical activation, the Ir/CPE showed good electrocatalytic activity towards H_2O_2, as well as good stability, selectivity and reproducibility. Designed working electrode offers the possibility for the analysis of H_2O_2 at different working potentials in anodic range from 0.30 to 0.60 V and in cathodic range from -0.05 to -0.30 V vs saturated calomel electrode in phosphate buffer pH 7.50 (0.1 M) as supporting electrolyte. At 0.60 V, the linearity of the calibration curve was acceptable from 1.75 to 17.28 μg mL-1 H_2O_2, with RSD lower than 3% and the evaluated LOQ as 0.245 μg mL^{-1}. Additionally, Ir/CPE based method was successfully applied for determination of H_2O_2 content in commercially available color cream developers.

CONCLUSIONS:

Based on the obtained results, it can be concluded that developed amperometric method is simple, reliable and suitable for obtaining fast information in terms of quality control of commercially available products that contain H_2O_2. Also, the possibility for application of Ir(III) complex for biosensor design and its use in other complex matrices such as biological samples was opened.

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T1-P-16-ORAL Signal crayfish, *Pacifastacus Leniusculus* (Dana, 1852) new invasive species in the waters of Serbia

Mladen Horvatović, Aleksandar Bajić, Nemanja Pankov, Branko Miljanović[60]

KEYWORDS: non-indigenous crayfish; astacofauna; Danube.

INTRODUCTION:

Non-indigenous species of macroinvertebrates are potentially the greatest threat to the indigenous biodiversity of freshwater ecosystems in Europe and Serbia. Twenty-nine non-indigenous species of macroinvertebrates have been recorded in Serbian waters up today. Of particular concern are the indigenous crayfish species (ICS), which are extremely endangered. The main cause in generally is not competition with non-indigenous crayfish species (NICS), but disease "crayfish plague" transmitted by invasive crayfish species native to North America. The cause of this disease is the fungus *Aphanomyces astaci* Schikora, 1906 to which NICS are highly resistant, while for ICS it is deadly. The presence of one NICS, Faxonius limosus (Rafinesque, 1817) has been recorded in Serbia so far. In many waters where this species appeared, there was a drastic decline

60 Department for Biology and Ecology, Faculty of Sciences, Trg D. Obradovića 2, 21000 Novi Sad, Serbia.

Corresponding author: mladen.horvatovic@dbe.uns.ac.rs

in the number of ICS populations. The signal crayfish, *Pacifastacus leniusculus* (Dana, 1852) is currently the most widespread NICS in Europe. The presence of this species has been registered in 30 European countries so far, in Croatia and Bosnia and Herzegovina, among the others. In Serbia it has not been recorded so far. The signal crayfish is considered to be the most successful crayfish invader in Europe. Given its large growth, high reproductive potential, aggressiveness and possible transmission of crayfish plague, this species is considered the greatest threat to the ICS fauna in Europe and Serbia as well.

OBJECTIVES:

The aim of our research was to determine whether the signal crayfish already reached the waters in Serbia. The most probable direction of entering the Serbian waters would be through the Drava River and its confluence with the Danube. First records of *Pacifastacus leniusculus* from the Mura-Drava river basin in Croatia was in 2008. In 2019 it was found in the Drava River, only 50 km upstream from the confluence with the Danube. Calculated downstream dispersal rate of the signal crayfish in the Drava River was 21.3 km/yr which is among the highest in Europe. If the dispersal rate remains at the mentioned level, according to prediction *P. leniusculus* would reach the Danube and the waters of Serbia in 2-3 years.

METHOD:

Our research was part of the periodic hidrobiological monitoring of Danube River. The crayfish was caught by net which was set on a hard rocky bottom in a strong current at a depth of around 3m. During research basic physico-chemical parameters of water were recorded (temperature, conductivity, pH – value, dissolved oxygen, oxygen saturation, water depth and transparency).

RESULTS:

During our research we recorded for the first time the presence of *Pacifastacus leniusculus*, the second NICS in the Serbian waters. At the locality Koruška at 1280 km of the Danube watercourse (102 km downstream of the Drava confluence) on the November 26, 2020, an adult male of signal crayfish was caught. Since only one individual was found, it is to be assumed that this is the initial settlement of the species and not the established population in this area.

CONCLUSIONS:

Significantly faster occurrence of *Pacifastacus leniusculus* in the Danube than predicted, showed that the dispersal rate in the last 50 km of the Drava River before the confluence with the Danube was significantly higher than the dispersal rate in the entire previous course through Croatia. To explain the fact that the species was found in the Danube 102 km downstream from the confluence of the Drava River "so early" we can offer only several assumptions. 1) Its spread through the Drava was much faster, but it was not registered; 2) In the lower course of the Drava River and in the Danube, its spread was rapid; 3) The spread of the species was accelerated in another way, e.g. ballast water of ships. We can rule out with great certainty the possibility that such a fast spread in the Danube was the result of human translocations. Without going into the causes of such a fast expansion, it is reasonable to expect that the dispersal rate of the signal crayfish in Serbia will be the highest in Europe. Its spread in other river systems of the Danube basin seems inevitable.

It is necessary to conduct the most urgent monitoring of the progression and intensity of spread of signal crayfish on the territory of Serbia. At the same time, it is necessary to start with management actions aimed at its control. Given that this species is considered the greatest threat to the indigenous crayfish fauna in Europe, it is extremely important to monitor the impact that *P. leniusculus* will have on the native Serbian astacofauna.

T1-P-17-ORAL Influence of monsoon-driven oceanographic variability on recruitment of Rocky intertidal dominant Sessile species

Kringpaka Wangkulangkul[61], Phuripong Meksuwan[62], Milica Satnkovic[63]

KEYWORDS: Rock oyster; Barnacle; Recruitment; Monsoon

INTRODUCTION:

Most marine benthic invertebrates have a complex life cycle including a planktonic larval phase and sessile adult phase. In a life history, settlement of larvae and survival through to recruitment, are the most crucial points determining success of population establishment and regulating population dynamics. Mechanisms responsible for variation in recruitment of coastal species, such as reproduction and oceanographic processes, can operate on a range of spatial and temporal scales. In tropical Andaman Sea coast of Thailand, the Indochina monsoon is the main driver influencing climate and hydrodynamics of the areas. However, how the monsoon has impacts on coastal species is poorly known.

OBJECTIVES:

Influence of monsoon-driven oceanographic variability on recruitment pattern of intertidal rock oyster *Saccostrea cucullata* (Born, 1778) and barnacle *Chthamalus malayensis* Pilsbry, 1916 was investigated.

METHOD / DESIGN:

Spatial and temporal variation in recruitment and environmental parameters, such as salinity, temperature, wind direction, wind velocity and chlorophyll a, were examined from monthly sampling at northern (Phuket: Kalim and Surin) and southern regions (Satun: Hat Sai Yao and Tanyong po) on the Andaman Sea coast of Thailand. Relationships between recruitment and these environmental parameters was evaluated. Sampling was carried out from September 2019 to September 2020, except the period of provincial lockdown due to COVID-19 from March to May 2020. The environmental parameters were measured by both in situ measurement and remote-sensing.

RESULTS:

Variation in recruitment of both species and most environmental parameters were significant at small spatial and temporal scales (among locations and months), while variation among regions and among seasons (southwest and northeast monsoon) was not detected. However, it is worth noting that the highest recruitment values of both species were recorded during the southwest monsoon. The highest correlation coefficient was found between barnacle recruitment and sea surface temperature. In oyster, the highest coefficient was with wind velocity. Multiple regression suggests that only sea surface temperature was significantly related to barnacle recruitment, whereas there was no environmental parameter related to oyster recruitment. When effect of wind type (northeasterly-offshore predominant wind vs southwesterly-onshore predominant wind) on recruitment was analyzed. Barnacles had a higher recruitment rate when southwesterly-onshore wind predominated; while oyster shows no difference.

CONCLUSIONS:

Recruitment of *S. cucullata* and *C. malayensis* was generally higher in southwest monsoon than northeast monsoon but it

61 *Division of Biological Science, Faculty of Science, Prince of Songkla University. Corresponding author: kringpaka.w@psu.ac.th*

62 *Faculty of Science and Technology, Phuket Rajabhat University*

63 *Excellence Centre for Biodiversity of Peninsular Thailand, Faculty of Science, Prince of Songkla University*

is a context-dependent. High sea surface temperature found during the southwest monsoon might be a factor influencing breeding activity, leading to greater larval production. A key determinant of onshore larval delivery was wind direction and velocities. During southwest monsoon, southwesterly predominant wind could bring larvae onshore, which enhanced recruitment of both species.

T1-P-18 Influence of false mussel *Mytilopsis sallei* (Récluz, 1849) on benthic macroinvertebrate community in Pawong canal, Songhkla lagoon system, Thailand

Lutfee Hayeewachi[64], Eknarin Rodcharoen[65], Kringpaka Wangkulangkul[64]

KEYWORDS: Invasive species; *Mytilopsis sallei*; Benthic invertebrate community; Habitat complexity; Songkhla Lagoon System.

INTRODUCTION:

Invasive bivalves can act ecosystem engineers. Their aggregations and filter-feeding activity may provide habitat complexity and resources for benthic organisms. False mussel *Mytilopsis sallei* is one of the most widespread aliens invasive mussel in the Indo-Pacific and has been reported to be introduced to the south of the Gulf of Thailand. However, there were no available data on the effect of false mussel invasion on benthic macroinvertebrate, organic matter in sediment and sediment particle which will be used as a basis to understand the ecological effect of *M. sallei*.

OBJECTIVES:

To investigate the influence of mussel-modified habitat on benthic macroinvertebrate community, amount of organic matter and sediment particle, as well as examine how these biological and physical parameters vary seasonally. To assess the relationship between habitat complexity and benthic macroinvertebrate community.

METHOD / DESIGN:

We compare benthic macroinvertebrate community (in term of community composition, species richness, total number of individuals, and biodiversity) amount of organic matter, and sediment particle between mussel-modified habitat and outside, moreover, compare in three seasons. We then use data obtained from mussel-modified habitat to assess the relationship between habitat complexity and benthic macroinvertebrate community (in term of number of species and number of individuals). The habitat complexity index, we use false mussel biomass as an indicator. In addition, we collected water parameters including salinity, pH, and water temperature from the field.

RESULTS:

We found 29 taxa and detected the difference in community composition between mussel-modified habitat and outside. Species richness, total number of individuals and biodiversity in mussel-modified habitat was greater than outside. While organic matter and sediment particle were not different. We found the relationship between habitat complexity and number of individuals, while number of species was not related.

64 Coastal Ecology Laboratory, Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand 90110. Corresponding author: 6210220061@email.psu.ac.th

65 3Discipline of Excellence for Sustainable Aquaculture, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, Thailand 90110

CONCLUSIONS:

This study demonstrates that mussel-modified habitat had a positive effect on benthic macroinvertebrate community. We propose that deposition by *M. sallei* did not influence organic matter in sediment. In addition, we suggested that habitat complexity might not be sufficient to determined number of species in Pawong Canal.

T1-P-19-ORAL The roles of dragon fruit oligosaccharide on immunity of freshwater crustacean, *Daphnia magna*

Thanwarat Sangkuanun, Saranya Peerakietkhajorn[66]

KEYWORDS: *Daphnia magna*; immunity; prebiotic

INTRODUCTION:

Dragon fruit oligosaccharide (DFO) is a prebiotic which cannot be digested and absorbed in the digestive tract of animal. DFO can be digested and absorbed by gut microbiota in the intestine of animals. The beneficial bacteria have potential to resist the growth of harmful bacteria and stimulate host's immunity. Innate immunity is a non-specific immunity which is divided into humoral and cellular immune responses in invertebrate. Cellular defenses refer to haemocyte-mediated responses like phagocytosis and encapsulation. Humoral defenses include antimicrobial peptides, coagulation cascades and melanization. An essential innate immunity in crustacean is the prophenoloxidase activating system (Pro-POAS) which is against a wide range of pathogens. Phenoloxidase (PO) is the key enzyme of melanization which plays an important role in bactericidal process. Melanization start when recognition proteins on heamocyte recognize specific molecules of pathogen (e.g. lipopolysaccharide, peptidoglycan). Then, heamocyte surround the pathogen and form a thick wall and produce melanin inside. Melanin prevent the reproduction and growth of invader. Then, invaders are starved and eventually die. In addition, nitric oxide (NO) is non-specific reactive nitrogen molecules that has been reported to play an important role in immune defense. NO is produced by the conversion of L-arginine into L-citrulline by nitric oxide synthase (NOS). NO has been shown their function in host defense by inhibiting enzymes in cellular respiration processes of pathogens. In this study, *Daphnia magna*, a freshwater crustacean zooplankton, was used as a model organism to study the activation of crustacean immunity by using of DFO.

OBJECTIVES:

The purpose of this study is to illustrate the effects of DFO on innate immunity of *Daphnia magna*.

METHOD / DESIGN:

In this study, we observed haemocyte number, PO activity, and NO level and localization. To investigate haemocyte number, ten-day-old D. magna were treated with 0 and 9 mg/l DFO for 24 and 85 h. Two microliters of haemolymph was collected from *D. magna*'s heart and mixed with 8 µl of Trypan blue. The mixture was placed on hemocytometer and haemocyte number were counted under compound light microscope.

For evaluation of PO activity, ten-day-old *D. magna* were treated with 0 and 9 mg/l DFO for 24 and 85 h. Three microliters of haemolymph was mixed with 450 µl of PBS and 1350 µl of 15 mM L-dopa. Then, the absorbance was immediately measured at 475 nm at 0 h and 4.5 h. PO activity was calculated. One unit of PO activity was defined as an increase in absorbance by 0.001/min/µL heamocyte.

66 *Division of Biological Science, Faculty of Science, Prince of Songkla University, Songkhla, Thailand. Corresponding author: Thanwarat1995@gmail.com*

Gut Biology and Microbiota Research Unit, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

To observe the NO level and localization, ten-day-old *D. magna* were treated with 0 and 9 mg/l DFO for 24 and 85 h. Twenty microliters of 10 mM diacetylaminofluorocene (DAF) was added to 10 ml of culturing COMBO medium, then cultured 5 D. magna for 24 h before observing under fluorescence microscope.

RESULTS:

Heamocyte number in *D. magna* treated with 9 and 27 mg/l DFO for 24 h and 85 h tended to increase when compared to control group. Moreover, PO activity in *D. magna* treated with 9 and 27 mg/l DFO for 24 h significantly decreased when compared to control group ($p<0.05$). PO activity in *D. magna* treated with 27 mg/l DFO for 85 h significantly decreased when compared to control group ($p<0.05$). In addition, NO was detected at all parts of *D. magna*'s gut. NO level was increased with increasing of DFO concentration in *D. magna* treated with DFO for 24 h, in contrast, NO level was decreased with increasing of DFO concentration in *D. magna* treated with DFO for 85 h.

CONCLUSIONS:

DFO has potential to stimulate innate immunity of *D. magna* by increasing of haemocyte number. Therefore, DFO might activate the innate immune responses and induce the production of haemocyte in *D. magna*. Moreover, NO showed higher level in *D. magna* after 24 h of DFO exposure but NO level was lower after 85 h of DFO exposure. This might be because DFO stimulated NO production in short burst for physiological processes, especially in immune responses. The results also suggested that exposure of DFO for long time might reduce oxidative stress by decreasing of NO level in *D. magna*. In addition, PO activity were reduced in *D. magna* treated DFO. This might be because *D. magna* did not challenge with pathogen in this study, therefore PO was not activated. Our study might be useful for the further studies related to immunity in crustacean and improvement of aquaculture.

T1-P-20-ORAL Nanoplastics characterization at the biological interface

Serena Ducoli[67], Stefania Federici[68], Laura E. Depero[68], Andrea Zendrini[69], Paolo Bergese[69]

KEYWORDS: Nanoplastics; Nanobeads; Biological systems; Interface; Protein corona

INTRODUCTION:

Environmental plastic pollution is a great issue affecting our Planet, especially marine ecosystems. Once enter the environment, plastics undergo degradation processes and fragment into smaller pieces up to the nanoscale. The need to understand the possible implications of microplastics and nanoplastics pollution on the environment and living organisms is becoming increasingly pressing. Given the complexity of separating nanoplastics from environmental samples, studies have been so far conducted using synthetic polystyrene nanobeads. There is an urgent need to create nanomaterials that better reflect the real characteristics of nanoplastics naturally formed, viz. true-to-life nanoplastics (T2LNPs), to close the gap between the laboratory parameters and the rules of nature, and to provide more realistic understandings of the characteristics of nanoplastics.

OBJECTIVES:

In this paper, we present a study on T2LNPs production and characterization and the protein corona formation with respect

67 *University of Brescia, Department of Information Engineering, Italy, and INSTM. Corresponding author: serena.ducoli@unibs.it*

68 *University of Brescia, Department of Mechanical and Industrial Engineering, Italy, and INSTM*

69 *University of Brescia, Department of Molecular and Translational Medicine, Italy, and CSGI*

to synthetic polystyrene nanobeads (nanobeads).

METHOD / DESIGN:

T2LNPs samples were produced from daily life plastic items subjected to a mechanical fragmentation through an ultra-centrifugal mill operating in cryogenic conditions. The produced T2LNPs were characterized by Fourier transform Infrared (FT-IR) spectroscopy to investigate their chemical nature and check the absence of induced chemical modifications. Morphology and size distribution analyses were performed through Atomic Force Microscope (AFM). Finally, the protein corona formation from human plasma on T2LNPs and nanobeads were examined by SDS-PAGE electrophoresis.

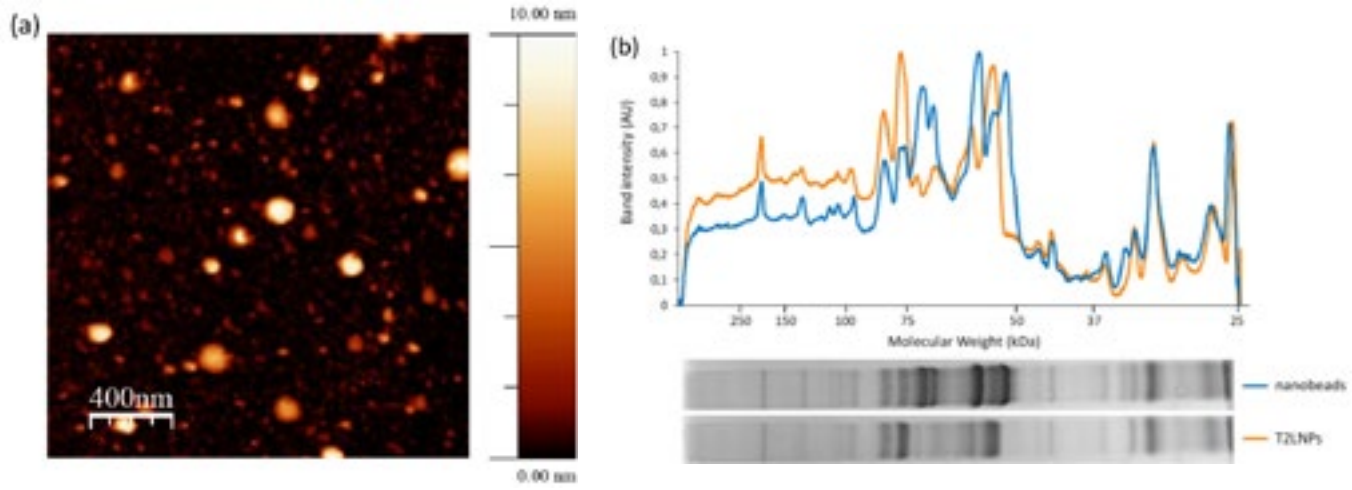


Figure 1: (a) AFM image of T2LNPs; (b) Protein corona profiles on T2LNPs and PS nanobeads.

RESULTS:

FT-IR analysis highlights the presence of the typical polystyrene peaks in fragmented T2LNPs samples, confirming chemical fingerprint and the presence of chemically intact polymer after fragmentation procedure without induced degradations.

The features recorded in AFM topography images show spheroidal nanoparticles, with a strong predominance of tiny particles of few tens of nanometers; aggregates and bigger particles are also present.

The bio-nanointerface, investigated through the formation of the protein corona from human plasma both on the T2LNPs and nanobeads surface, reveals different protein adsorption for the two nanomaterials, as highlighted by SDS-PAGE analysis an protein profiles comparison, suggesting different biological behaviors.

CONCLUSIONS:

The differences detected in the two protein corona profiles confirm the gap between controlled models and the complexity in real-life scenarios, supporting the need to develop true-to-life materials as reasonable models for environmental nanoplastics. The broad heterogeneity in size and shape shown by fragmented T2LNPs gives the nanomaterial a peculiar and different behavior compared to the defined pristine nature of nanobeads, nominating T2LNPs as a more faithful material for naturally-occurring nanoplastics and opening the possibility to new and unexpected results in biological interactions.

T1-P-21-ORAL Post-prandial changes in digestive enzymes and chyme characteristics in bigfin reef squid (*Sepioteuthis lessoniana*)

Jirapan Satjarak[70], Karun Thongprajukaew[71], Chantana Kaewtapee[69], Naraid Suanyuk[72], Sappasith Klomklao[73], Aekkaraj Nualla-ong[72], Hirun Saelim[74], Kannika Preedaphol[75]

KEYWORDS: Digestion; Digestosomatic index; Harvesting time; Loliginid squid; Protein pattern

INTRODUCTION:

Bigfin reef squid (*Sepioteuthis lessoniana*) is an important cephalopod for the commercial fisheries market of Thailand, and is distributed in the Gulf of Thailand and the Andaman Sea. This species is one of the candidate squid species that has been developed for culture in commercial scale because of having large sized hatchlings, short life cycle, rapid growth rate, and tolerating captivity for a long period. Although this species has been successfully cultured in multiple generations but the information about digestive physiology and feeding management are scarcely known.

OBJECTIVES:

The present study was focused on the post-prandial pattern of digestive enzymes and chyme biochemical characteristic of this species.

METHOD / DESIGN:

The squids (1.78 ± 0.05 g body wet weight) were serially collected at different post-prandial times (0, 0.5, 1, 2, 4, 6, 8, 12, 18, and 24 h) and the specimens were used for analysis of digestive enzymes (including total proteinases, trypsin, chymotrypsin, lipase, and amylase; n = 10) and chyme characteristics (including thermal properties, microstructure, and molecular weight pattern of protein; n = 5).

RESULTS:

The food substantially transited to gastrointestinal tract and peaked at 1 h after feeding and then dramatically decreased with post-prandial time ($P < 0.05$). The digestion process occurred shortly after the food arrived and exhibited the same pattern over 24 h observations. The highest activities of protein-, carbohydrate- and lipid-digesting enzymes were observed within a range of 0.5 to 4 h after feeding and reached a basal level as observed in fasted squids within 8 h post- prandially. These responses matched with chyme characteristics, in terms of thermal properties of nutrients, microstructure, and mo-

70 Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90112, Thailand. Corresponding author: 5910630003@psu.ac.th

71 Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand, Center of Excellence in Agricultural and Natural Resources Biotechnology Phase 3, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90112, Thailand

72 Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90112, Thailand, Kidchakan Supamattaya Aquatic Animal Health Research Center, Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90112, Thailand

73 Department of Food Science and Technology, Faculty of Agro- and Bio-Industry, Thaksin University, Phattalung Campus, Phattalung 93110, Thailand

74 Center of Genomics and Bioinformatics Research, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand, Division of Biological Science, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand

75 Phang-Nga Coastal Fisheries Research and Development Center, Phang-Nga 82120, Thailand,

lecular weight pattern of proteins.

CONCLUSIONS:

The results in term of digestosomatic index, specific activities of digestive enzymes, and chyme biochemical characteristics physiologically responded with post-prandial changes in bigfin reef squids. The food transited rapidly and was maximally digested by enzymes within 0.5 to 4 h after feeding. Findings from the current study provide basic information on digestive physiology of bigfin reef squids that can be applied when designing the specimen harvesting time for biological research.

T1-P-22 Acute toxicity assessment of defense secretions of *Megaphyllum bosniense* (Verhoeff, 1897) and *M. Unilineatum* (C. L. Koch, 1838) (Diplopoda, Julida) on *Artemia Salina*

Jelena Milovanović[76], Jelena Đorđević[77/78], Aleksandra Stevanović[78], Anastasija Malešević[78], Bojan Ilić[76], Branka Vuković-Gačić[78], Slobodan Makarov[76]

KEYWORDS: millipedes; Julidae; allomones; biological activities; ARC test

INTRODUCTION:

Different orders within the class Diplopoda possess a variety of chemical compounds in their defense secretions: quinones, phenolics, alcohols, aldehydes, ketones, esters, alkaloids, cyanogenic compounds. Defensive secretions of species from the order Julida are regarded as the most complex within Diplopoda, and they are blends of several classes of chemical compounds: quinones, esters and ketones. Numerous biological activities of these secretions have been reported: antimicrobial, antioxidative, antineurodegenerative, cytotoxic and embryotoxic on zebrafish. Besides zebrafish embryos, *Artemia salina* is one of the common model organisms in toxicity assessment which has not been used for screening of toxicity of millipedes' defensive secretions.

OBJECTIVES:

The main goal of this study was to examine the toxic effects of defensive secretions of two species from the order Julida [*Megaphyllum bosniense* (MBO) and *M. unilineatum* (MUN)] using *Artemia salina* (ARC test).

METHOD / DESIGN:

Adult individuals of *M. bosniense* were collected during April and May of 2021 on Mt. Avala, near Belgrade, while adults of *M. unilineatum* were collected during the same period in the Krnjača, suburb of Belgrade. After the capture, millipedes were kept in plastic boxes containing ground cover from the collecting site. The boxes were regularly sprayed with water to maintain high humidity. Due to the fact that the sample was female-biased, defensive secretions of female specimens were used for further analyses. Excretion of defensive secretions was elicited from glands of mentioned species via mechanical stress in closed glass vials. Secretions collected from both species were dissolved in 10 ml of hexane, concentrated under reduced pressure in a rotary evaporator (Rotavapor R-210, Buchi) at 40°C to a dry residue, and redissolved in 50% dimethyl

76 University of Belgrade, Faculty of Biology, Institute of Zoology, Chair of Animal Development, Belgrade, Serbia. Corresponding author: jelena.milovanovic@bio.bg.ac.rs

77 University of Belgrade, Institute for Multidisciplinary Research, Belgrade, Serbia

78 University of Belgrade, Institute of Botany and Botanical Garden "Jevremovac", Chair of Microbiology, Centre for Genotoxicology and Ecogenotoxicology, Faculty of Biology, Belgrade, Serbia

sulphoxide (DMSO). The stock concentration of extracts used in ARC test was 20 mg/mL. Before treatments, eggs of *A. salina* were incubated for 72h with constant lighting and aeration. For the purposes of the experiment, stage II and III larvae were used (separated by phototaxis in 300 ml of seawater). In a plate with 24 wells, 900 µl of seawater with larvae (10-15 per well) was placed and then 100 µl of tested extracts (range of concentrations 0,1 mg/mL - 0,003125 mg/mL) was added. Potassium dichromate ($K_2Cr_2O_7$) was used as a positive control and DMSO was used as solvent control. The total number of individuals per well was counted after 24h and 48h, as well as the number of living and dead individuals. These data were used for estimation of survival rate and determination of LC_{50} value. The experiment was done in triplicate.

RESULTS:

Our results show that secretions from both species exhibit a toxic effect on the survival of the chosen model organism, with the MBO extract showing weaker activity in comparison with MUN extract. The LC_{50} value after 24h was about the same for both species (LC_{50}=73,23 µg/mL for MBO and LC_{50}=68,56 µg/mL for MUN). The LC_{50} value for MBO after 48h was 47,18 µg/mL, while LC_{50} value in the same period for MUN was 29,12 µg/mL. Positive control (LC_{50}= 13,5 µg/mL) showed three times stronger effects in relation to MBO and twice as strong when compared to MUN extract. It has also been shown that the number of surviving individuals decreases with increasing concentration of tested extracts and the increasing incubation time.

CONCLUSIONS:

The defense secretions of both tested millipede species show toxic effects in the ARC test. It is shown that MBO extract has a weaker toxic effect than the MUN extract. This result can be linked with the fact that esters of long-chain fatty acids are dominant compounds in MBO, while MUN is almost exclusively benzoquinone-based. Esters detected in MBO are generally regarded as low-toxic compounds, but with the potential to interact with compounds from other chemical classes. However, as MBO achieved toxic effects and many esters that are detected in MBO are new natural products and their biological potential is unknown, further extensive studies are needed to determine their toxicological potential.

T1-P-23 Preliminary modification of the Eshippo Crayfish model

Simona Đuretanović[79], Tijana Veličković[79], Aleksandra Milošković[80], Nataša Kojadinović[79], Marija Jakovljević[79], Ivana Maguire[81], Vladica Simić[79]

KEYWORDS: *Astacus astacus*; Balkan Peninsula; Species conservation; Conservation models; Multidisciplinary approach

INTRODUCTION:

The extinction of species and the decline of biodiversity are the most severe global consequences of environmental threats. The decline of biodiversity is far greater in freshwater ecosystems than in the most threatened terrestrial ecosystems, and the most vulnerable are invertebrates, such as freshwater crayfish. Even one-third of freshwater crayfish worldwide are at risk of extinction. Natural subpopulations of the noble crayfish have been declining by 50-70%, and it is classified as a "vulnerable species" in the IUCN Red List of Threatened Species, with a decreasing trend of populations and subpopulations and decreasing distribution areas.

We used our published morphometric, phylogenetic, and population genetic data of the noble crayfish populations from aquatic ecosystems of Serbia, Slovenia, and Albania in order to upgrade the existing ESHIPPO crayfish model, and in this way

79 *Faculty of Science, University of Kragujevac, Serbia. Corresponding author: simona.djuretanovic@pmf.kg.ac.rs*

80 *Institute for Information Technologies, University of Kragujevac, Serbia*

81 *Faculty of Science, Department of Biology, University of Zagreb, Croatia*

to contribute to conservation plans and management strategies for protection of this threatened species.

OBJECTIVES:

The main objectives of this study were:

1. to modify the ESHIPPO crayfish model,
2. to assess the risk of extinction, and
3. to determine the priority of protection in the study area.

METHOD/DESIGN:

In this study, we upgraded ESHIPPO crayfish model by adding population genetic component of the studied populations. This model is designed to assess the risk of extinction and define the priorities of species conservation in aquatic ecosystems at the local and national levels since frequent differences exist in the assessment of the risk of extinction at the local and global levels.

RESULTS:

According to the obtained results, the populations from Lake Prespa (68 points), and the Gazivode reservoir (62 points) are defined as populations with a high level of extinction risk at the national level, i.e., the degree of protection priority 1. On the other hand, a moderate risk of extinction, i.e., the degree of protection priority 2, was determined for the populations from the reservoirs Grlište (58 points), Korenica (56 points), and Bloke (56 points), and for the Kočevska River (52 points).

CONCLUSIONS:

We determined six populations as the priority of protection. This kind of model can help identify and preserve the diversity of the species and the integrity of local populations.

T1-P-24 Estimation of the wastewater impact on the Krka River by Daphnid Acute toxicity testing

Vlatka Filipović Marijić[82], Želimira Cvetković[83], Camille Boucaud[84], Ivana Karamatić[82], Damir Valić[82], Tomislav Kralj[82], Tatjana Mijošek[82], Zuzana Redžović[82], Vlatka Mikulec[83], Dušica Ivanković[82], Zrinka Dragun[82]

KEYWORDS: *Daphnia magna*; industrial and municipal wastewaters; Krka National Park; toxic impact

INTRODUCTION:

In addition to chemical water analyses, assessment of water quality might involve toxicity testing, as a biological tool that reflects toxic impact on aquatic organisms. The commonly used testing organism is crustacean *Daphnia magna* Straus, 1820, which is sensitive to a wide range of contaminants and important consumer in the food chain. Water quality was assessed in the karst Krka River (Croatia), whose lower part was proclaimed national park due to its exceptional natural beauty. Only 2 km upstream from the northern border of the park industrial and municipal wastewaters from the Town of Knin are released in the Krka River without proper purification. Their impact on the river water was assessed at five sites, Krka River source (KRS) as reference location and at four locations downstream of the wastewater impact (industrial (IWW) and municipal (MWW)

82 *Ruđer Bošković Institute. Corresponding author: vfilip@irb.hr*

83 *Andrija Štampar Teaching Institute of Public Health*

84 *University of Côte d'Azur*

wastewaters, Orašnica (TOR) and Butišnica (TBU) rivers near outlets) in April 2021.

OBJECTIVES:

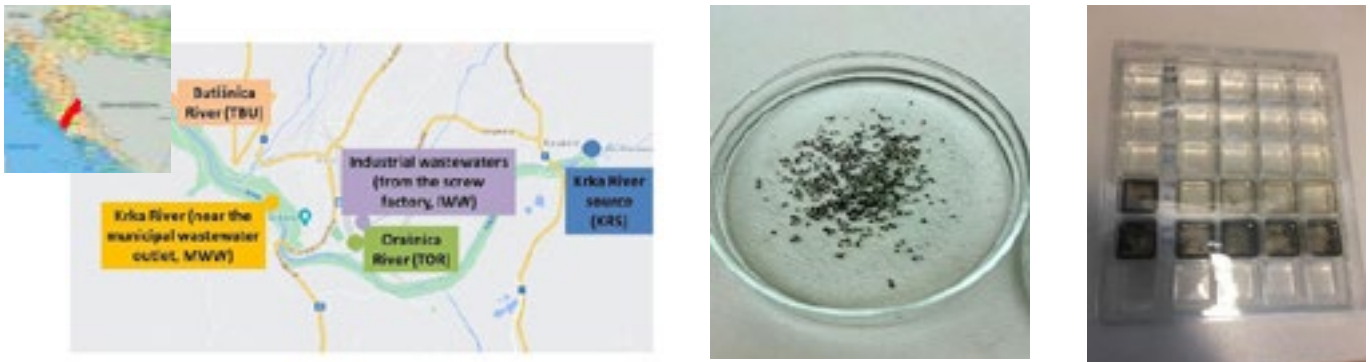
The potential toxicity of industrial and municipal wastewaters and nearby Krka River water was assessed by an acute toxicity test in order to estimate pollution impact on aquatic organisms and establish protection plans.

METHOD / DESIGN:

Acute toxicity was evaluated after hatching of the ephippia in a specific media supplemented with salt nutrient (Fig. 1a). The obtained neonates were used immediately for ecotoxicity testing of water samples from five sites, which was manifested as an immobilization (meaning death) of the crustacean. Probit analysis was used to calculate 50% toxic effect thresholds (EC50) for 24-48h exposure and the lowest ineffective dilution (LID) for which at least 90% of Daphnids are mobile. Potassium dichromate ($K_2Cr_2O_7$) was applied as a reference toxicant.

RESULTS:

The percentage of mobile daphnids was up or equal to 90% for all freshwaters except IWW in the first 24h in both seasons. After 48h all daphnids remained alive in water from KRS and TBU, justifying good ecological status of these locations. For TOR and MWW, 5% of daphnids were immobile in the 50% diluted and 20% were immobile in the non-diluted freshwater, resulting in LID= 2, which confirmed moderate pollution in sites nearby wastewater outlets. The highest impact on daphnids was found for IWW, in which a decreasing vitality rate in increasing concentration of the sample was observed. Moreover, the lowest ineffective dilution calculated by Probit analysis was significantly higher compared to the other samples (LID= 18, 48h). Such higher mortality was in agreement with the poor physico-chemical water parameters and a blackish color, high viscosity and strong odor related to fuel oil (Fig. 1b) at site IWW.



a) *b)* *c)*

Fig. 1 Toxicity testing using Daphnia magna: a) map of the sampled area in the Krka River (Croatia); b) hatching of the ephippia; c) testing with industrial wastewaters (IWW)

CONCLUSIONS:

A statistically significant increase, meaning a high LID value, was observed for IWW, pointing to toxic influence of the industrial wastewater from the screw factory and the importance of proper purification before discharging it in the environment. Therefore, strict and continuous biomonitoring plans must be established if serious consequences want to be avoided on the whole ecosystem, biota and the national park itself.

T1-P-25-ORAL Habitat use by the cryptic sea slug *Elysia pusilla* (Bergh, 1872) (Sacoglossa) in a tropical *Halimeda macroloba* Decaisne, 1841 Meadow

Apisara Nakpan, Jaruwan Mayakun, Kringpaka Wangkulangkul[85]

KEYWORDS: Plant-animal interactions; Sacoglossa; algal host; symbiosis; *Elysia; Halimeda*

INTRODUCTION:

Elysia pusilla is a species of sap-sucking sea slug of the superorder Sacoglossa which is stenophagous, eating only a few species of *Halimeda*, and these algae also provide the animal's habitat. In this study area, Lidee Island, Satun Province, southern Thailand, only *Halimeda macroloba* was found. In this algal species, there are variations in number of calcium carbonate accumulation and chemical composition in different ages and different position of segments. The higher amount of toxin was contained in younger thalli and younger, terminal segments of the older thalli. More calcium carbonate was accumulated in older thalli. Thus, the variation in characteristics of the algal host might affect the selection for living, feeding or spawning behaviour of *E. pusilla*.

OBJECTIVES:

The relationship between *H. macroloba* and *E. pusilla* in term of habitat use was studied. The algal life-history stage influences the presence of slugs, their egg masses and grazing marks were investigated and the position of egg masses and grazing marks on thalli of *H. macroloba* were also examined.

METHOD / DESIGN:

E. pusilla and *H. macroloba* were collected by random sampling. The quadrats (20 × 20 cm) were placed randomly in *Halimeda* meadow. Each thallus in the quadrats was collected and put in a plastic bag. Samples were brought back to the laboratory and left undisturbed for 1-2 hours to let the slugs emerge. Numbers of slugs, egg masses and *H. maroloba* thalli found in each quadrat were counted to obtain the density of slugs, egg masses and *H. macroloba*. To evaluate the surface area of a thallus, photographs were taken of the thallus flattened on a tray and the area was calculated from the image, using the ImageJ © program. Photographs of thalli with grazing marks were taken in the field. The life-history stage of thallus and position of marks on grazed segments were noted. We counted the numbers of thalli and segments which presented grazing marks. In this study, the life history stages of *H. macroloba* were categorized in 6 stages, following Sinutok (2008) and Mayakun and Prathep (2019). To determine the position of segments along the algal thalli, the longest branch of a thallus was used as the reference. The terminal segment on this branch was classified as the 1st segment and the preceding segments were classified as 2nd, 3rd, 4th and so on.

RESULTS:

The density of *E. pusilla* was positively correlated with the density of *H. macroloba* and the total surface area of the alga. The density of egg masses was not positively correlated with algal density but was positively correlated with the total surface area of the alga. However, mean values of monthly data did not indicate correlations between either *E. pusilla* density or egg mass density and *H. macroloba* density or *H. macroloba* surface area. There was a different population density of *H. macroloba* between months but the population density of *E. pusilla* was not different. Egg masses were found in two months. The density of slugs and egg masses were very low. The highest number of slugs and grazed *H. macroloba* were found on mature

85 *Division of Biological Science, Faculty of Science, Prince of Songkla University, Thailand. Corresponding author: Apisara.A.Nakpan@outlook.co.th*

thalli which presented the largest surface area but the number of egg masses was slightly higher on younger thalli than mature thalli. The highest number of egg masses and grazing marks were found on terminal algal segments.

CONCLUSIONS:

E. pusilla and *E. pusilla* egg masses occurred in the study area at higher densities where the surface area of *H. macroloba* provided a large habitat space. Therefore, in this *Halimeda* meadow, there was a higher chance that *E. pusilla* and the egg masses would be found in areas populated by a high density of larger mature plants. Since the number of individuals found in each month was low, we could not detect covariation between the abundance of *E. pusilla* and the area of its habitat over time. The majority of *E. pusilla* and their grazing marks were found on mature thalli. This finding suggests that larger, mature thalli were used as shelter and feeding grounds. Although younger plants contain higher amounts of toxin that the slugs can utilize. The amount of space might be more important in determining habitat use than the chemical properties of the algal host. The slugs might choose the younger thalli to lay eggs because ensurance of the thallus is still alive when their larvae hatch. This hypothesis has yet to be tested. The highest number of egg masses and grazing marks occurred on the youngest, terminal segments which contains a higher amount of secondary metabolites that the slugs can utilize and has little accumulated calcium carbonate, which makes grazing easier. Our results suggested that habitat use by *E. pusilla* is possibly determined largely by the availability of space or surface area of *H. macroloba* rather than the chemical or anatomical characteristics of the alga.

T1-P-26 Recycling of green plant waste – production of iron nanoparticles

Andrea Rónavári[86], Árpád Szilágyi[87], Margit Balázs[88], Mónika Kiricsi, Zoltán Kónya[86/89]

KEYWORDS: iron nanoparticles; green synthesis; sustainability; recycling

INTRODUCTION:

Zero-valent iron nanoparticles (nZVI) have already proven their efficacy in the reductive degradation of a wide range of environmental contaminants. However, their large-scale application in remediation applications is hindered by the high costs and the environmental and legislative issues associated with the conventional nZVI synthesis method as it places a massive burden on the environment by utilizing toxic chemicals for the production process and leaving hazardous waste materials behind. On these grounds, green synthetic approaches have emerged, offering eco-friendly, sustainable, nature-derived alternative production methods, thus attenuating the ecological footprint of the nanomaterial industry.

OBJECTIVES:

In this connection, the aim of our present work was to further develop green syntheses from an innovative, economic and environmental point of view, primarily by involving plants that are widely available and easily accessible, and through their use, the synthesis can be carried out on an industrial scale. We investigated the possibility of recycling green plant waste materials, namely whether these waste materials can be used multiple times for particle synthesis.

86 Department of Applied and Environmental Chemistry, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary.

Corresponding author: ronavari@chem.u-szeged.hu

87 Department of Biochemistry and Molecular Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

88 Bay Zoltán Nonprofit Ltd. for Applied Research, BAY-BIO Division for Biotechnology

89 MTA-SZTE Reaction Kinetics and Surface Chemistry Research Group, Szeged, Hungary

METHOD / DESIGN:

The green material extracts were analyzed with analytical methods to determine the main components involved in nanoparticle synthesis. The formed nZVIs were subjected to detailed material science characterization (e.g., transmission electron microscopy, surface area, and reactivity test).

RESULTS:

We successfully carried out the synthesis of iron nanoparticles using multiple times the coffee and green tea extracts during the procedure. Based on our comprehensive screening, we delineated major differences in the characteristics of the obtained materials. Moreover, we analyzed the changes in main components involved in nanoparticle synthesis (e.g., proteins, sugars, polyphenols).

CONCLUSIONS:

We showed that the various green waste materials could be recycled multiple times for the generation of iron nanoparticles. However, we proved that the importance of properly selected green waste materials and synthesis methods must be emphasized as they profoundly influence the properties of materials and therefore their chemical and biological activity.

This research was supported by the New National Excellence Program of the Ministry for Innovation and Technology from the National Research, Development, and Innovation Fund (ÚNKP-21-5-SZTE-576 for A.R) and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00384/21/7 for A.R.).

T1-P-27-ORAL Adsorption mechanism of magenta printing dye on polyethylene microplastics

Aleksandra Tubić[90], Maja Lončarski[90], Vesna Gvoić[91], Jasmina Agbaba[90], Dejan Krčmar[90], Živko Pavlović[91], Miljana Prica[91]

KEYWORDS: microplastics; printing dye; Magenta; kinetics; adsorption mechanism

INTRODUCTION:

Polymers which can be classified as microplastics are ingredients used in the manufacture process of certain printing dyes. Therefore, microplastics and printing dyes can be found in wastewater. The presence of synthetic water-based dyes and microplastics in industrial wastewater poses a threat to aquatic ecosystems, as well as a source of indirect negative effects on human health and the knowledge about their behavior need to be expanded.

OBJECTIVES:

In this paper, kinetics and adsorption mechanism of printing Magenta dye on polyethylene (powdered - PEp and granulated - PEg), as one of the most common types of microplastics, were investigated.

METHOD / DESIGN:

The experiments were performed in a batch mode, in laboratory conditions. Adsorption kinetics was followed during 312 h, using 30 mg/l of PE and 100 mg/l of Magenta. The adsorption mechanism was evaluated after 72 h and 144 h contact time

90 Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia. Corresponding author: aleksandra.tubic@dh.uns.ac.rs

91 Department of Graphic Engineering and Design, Faculty of Technical Sciences, University of Novi Sad, Trg Dositeja Obradovića 6, 21000 Novi Sad, Serbia

for PEp and PEg, respectively.

RESULTS:

Obtained results indicate that a higher adsorption rate degree of Magenta dye was determined on powdered PE (max adsorbed amount was 321 µg/g), compared to granulated PE (max adsorbed amount was 273 µg/g). The adsorption data were fitted well by pseudo-second-order kinetics. Langmuir equation showed slightly better performances to fit the solid/liquid distribution of Magenta dye on powdered polyethylene (R^2 = 0.937) and granulated polyethylene (R^2 = 0.953) than Freundlich equation (R^2 = 0.908 for PEp and R^2 = 0.934 for PEg).

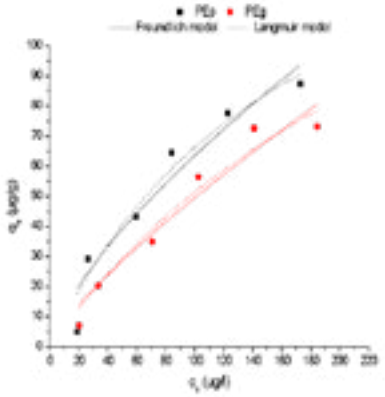


Figure 1. Kinetic study results (n = 3, mean value ± SD) of printing Magenta dye adsorption on PEp and PEg in the synthetic water matrix

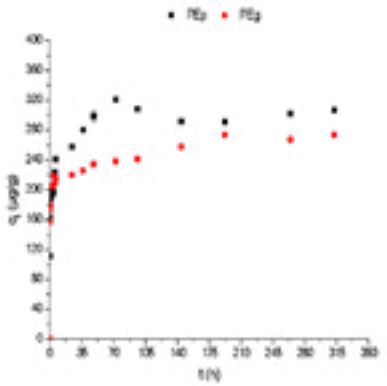


Figure 2. Adsorption isotherms plots (n = 3, mean value ±SD) for printing Magenta dye on PEp and PEg in synthetic water matrix

CONCLUSIONS:

The adsorption process of Magenta dye on polyethylene is multistage process which follows the Langmuir isotherm model and the pseudo-second-order kinetics, indicating that monolayer adsorption is mainly controlled by chemical process. Based on the obtained results, polyethylene can serve as a carrier for Magenta printing dye, and affect the behavior of this dye in the environment.

ACKNOWLEDGMENT: This research has been supported by the Provincial Secretariat for Science and Technological Development, Autonomous Province of Vojvodina (Grant No. 142-451-3186/2020-03).

T1-P-28 Antioxidant response to heavy metal pollution in *Conocephalum Conicum L.* (Dum.)

Piergiorgio Cianciullo[92], Viviana Maresca[92], Giovanna Salbitani[92], Federica Moccia[93], Sergio Sorbo[94], Marilena Insolvibile[95], Simona Carfagna[92], Lucia Panzella[93], Adriana Basile[92]

KEYWORDS: *Conocephalum conicum*; Bryophyta; heavy metal pollution; oxidative stress; polyphenol.

92 *Department of Biology, University of Naples Federico II, Naples, Italy. Corresponding author: piergiorgio.cianciullo@unina.it*

93 *Department of Chemical Sciences, University of Naples Federico II, Naples, Italy*

94 *CeSMA, Section of Microscopy, University of Naples Federico II, Naples, Italy*

95 *ISPRA, Italian National Institute for Environmental Protection and Research, Rome, Italy*

INTRODUCTION:

The Regi Lagni consists of a network of straight channels that, collecting meteoric, spring and also waste waters, carry them from the plain north of Naples to the Tyrrhenian Sea, covering a length of about 56 km. Nowadays the Regi Lagni channels are in a completely careless condition and are affected by severe contamination caused by heavy urbanization and industrialization. Apart from the classical use of bryophytes in biomonitoring (i.e. atmospheric pollution), it is important to measure the biological responses of these plants to pollution stressor. *Conocephalum conicum* is a cosmopolitan liverwort species able to respond to local environmental pollution by changing its biological features. Biological responses in *C. conicum* can be used as a tool to monitor biological pressure in the environment.

OBJECTIVES:

The objective of our study is the evaluation of the bioaccumulation of metals in the gametophyte *Conocephalum conicum* during the exposure period both in the field and *in vitro*, and the subsequent evaluation of the enrichment factor, the production and localization of ROS, the activation of the enzymatic antioxidant response, and the production of phenolic compounds.

METHOD / DESIGN:

Collected samples of *Conocephalum conicum* were exposed both in-field and in-vitro for 7 days. All samples were collected from a pristine area in the Botanical Garden of Naples. In the field experiment, samples were exposed through moss bags technique in three stations along the Regi Lagni Channel. In the *in vitro* experiment, exposed samples were treated with the same heavy metal concentrations detected at the three stations in the Regi Lagni Channel. Antioxidant enzymes activity (SOD, CAT and GST) as well as ROS and GSH contents were measured spectrophotometrically. At the same time, ROS and GSH were localized by confocal laser microscopy technique. HPLC-UV VIS and LC-ESI MS were used to identify and measure the polyphenol profile extracted from the sample.

RESULTS:

ROS contents, antioxidant enzymes activity and phenolic compounds content were increased, showing an enhancement of the antioxidant defense both by enzymatic way and the synthesis of antioxidant phenolic compounds. Specifically, ROS contents, antioxidant enzyme activities, as well as GSH and total gluthatione contents showed a dose response correlation in both experiments (field and in-vitro). Moreover, HPLC UV VIS and LC-ESI MS analysis revealed the induction of the biosynthesis of the lunularic acid in the heavy metal treated samples.

CONCLUSIONS:

This study confirms the ability of the liverwort *C. conicum* to respond to heavy metal pollution, as all the biological responses considered show a trend consistent with the pollution degree of the sites. Moreover, this study reports for the first time the induction of lunularic acid biosynthesis in liverworts following the exposure to heavy metal. This finding opens up further investigations on the contribution of secondary metabolites to metal tolerance and antioxidant defense in bryophythes.

T1-P-29 Influence of low temperatures on *Pseudomonas stutzeri* biomass stability

Teodora Cvanić, Ana Tomić, Olja Šovljanski, Siniša Markov[96]

KEYWORDS: denitrification; biomass stability; *Pseudomonas stutzeri*; low temperature;

INTRODUCTION:

Environmental parameters are known to have a strong impact on bacterial activity and stability. Usually, several variables interact to produce a complex response in view of bacterial stability. In extreme environments, however, a single factor such as salinity, temperature, pH, or intense radiation usually predominates, and monitoring the influence of targeted condition parameter may have significant effects on the survival capacity of bacteria. As bacterial biotechnology progresses, there is a growing necessity to preserve cultures without the high costs and time-consuming processes, but with high genetic stability. This protocol implicates conventional serial transfers, which include issues regarding potential loss by failure to regrow, contamination on transfer, decreasing stability, etc. Furthermore, it is vital to ensure viability and functionality are retained by stored stock cultures. Low-temperature storage, such as temperatures between -80 and 4 °C, is widely being used, but the implication to stability rarely investigated.

OBJECTIVES:

The aim of this study was to examine the stability of the produced biomass of *Pseudomonas stutzeri* strains during storage on low temperatures to maintain satisfactory vitality and viability of cells for further bioremediation processes.

METHOD / DESIGN:

For this study, *Pseudomonas stutzeri* ATTC 17588 and *P. stutzeri* D1 (isolate from the Danube river water) were used. A precipitated biomass obtained after centrifugation of the cultivation fluid at the end of bioprocess (after 32 h at 37 °C) is divided into two equal parts. Biomass was resuspended in 8.5% NaCl solution. Further, the gained suspensions were distributed in sterile vials (2 ml) and were stored in the refrigerator at 4 °C and freezer at -20 ° C. The same procedure was applied to vials with biomass resuspended in sterile distilled water. At defined time intervals (0, 7, 14, 21, 28, 35 and 50 days), the number of viable cells was determined using the indirect method of streaking suspension on nutrient agar.

RESULTS:

During storage period of 35 days, significant difference in cell concentrations at 4 °C did not observe for both tested strains, regardless of the used diluent. At the end of the incubation, bacterial concentration was decreased by approximately one log unit in both suspensions. Contrary, the effect of freezer temperature (-20 °C) had significantly different influence of bacterial stability. Briefly, biomass concentration decreased by approx. one log unit after only one week and remains constant until the 14th day. In the case of the reference strain, cell concentration in sterile saline decreased from approximately 9.7 to 6 log CFU/mL after three weeks, and maintained constant until one month, but at the end of the incubation period, growth of colonies did not observe.

In contrast, a temperature of -20 ° C has been shown to have a lethal effect on cells of the reference strain contained in sterile distilled leads after 3 weeks of storage. Relatively rapid cell death at temperatures below 0 ° C may be associated with the formation of ice crystals that disrupt the integrity of the cell membrane, resulting in cell death. An effective way to solve this problem is to use cryoprotectant, such as glycerol, which are added in a certain volume to the cell suspension before stor-

96 *University of Novi Sad, Faculty of Novi Sad, Bulevar cara Lazara 1, Novi Sad, Serbia. Corresponding author: cvanic35@gmail.com*

age. Glycerol creates a thin film around the cell membrane and protects the cell from the destructive effects of ice crystals, allowing the cell to survive in such an environment. It can be concluded that the liquid for suspension preparation (saline and distilled water) has almost no effect on the vitality and viability of *P. stutzeri* D1 cells during storage at -20 ° C. After the initial reduction of the biomass content by approximately one log unit after one week of storage, the number of cells remains approximately constant until the 35th day.

CONCLUSIONS:

The presented results of testing the effects of low storage temperatures on the viability of selected denitrifiers indicate the possibility of storing freshly prepared denitrifier suspensions at refrigerator temperature in sterile distilled water and sterile saline for a month while fully preserving cell viability and vitality. This is very important from the point of view of the application of denitrifiers in the process of biocleaning of building materials, because the storage of denitrifiers in distilled water allows their direct application inadequate carriers for the biocleaning process, without introducing additional ions that can cause further damage to the material. Additionally, the process of cell lyophilization can be avoided, which reduces the complexity of the preparation of the cell suspension and the cost of a biocleaning procedure.

T1-P-30 Natura 2000 habitats in the Zasavica special nature reserve

Mihajlo Stanković[97]

KEY WORDS: Zasavica SNR, Natura 2000, priority habitats, conservation status.

INTRODUCTION:

Zasavica is a wetland-peat complex that has been placed under protection as a Special Nature Reserve since 1997 and is located in the municipalities of Sremska Mitrovica and Bogatić, with a total area of over 3400 ha. The area is dominated by a lowland river ecosystem with different types of wetlands, consisting of water surfaces of the Jovača and Prekopac canals, and the river Zasavica with the tributary Batar with a total length of 33.1 km. Within the reserve there are three protection regimes (I, II and III) where there are 332 ha of forest land, 348 ha of pastures, 134 ha, 115 ha of reeds, 102 ha of rivers, 65 ha of canals, 12 ha of meadows and 19 ha of other land.

OBJECTIVES:

The aim of this paper is to present the types of Natura 2000 habitats in the Zasavica reserve.

METHOD / DESIGN:

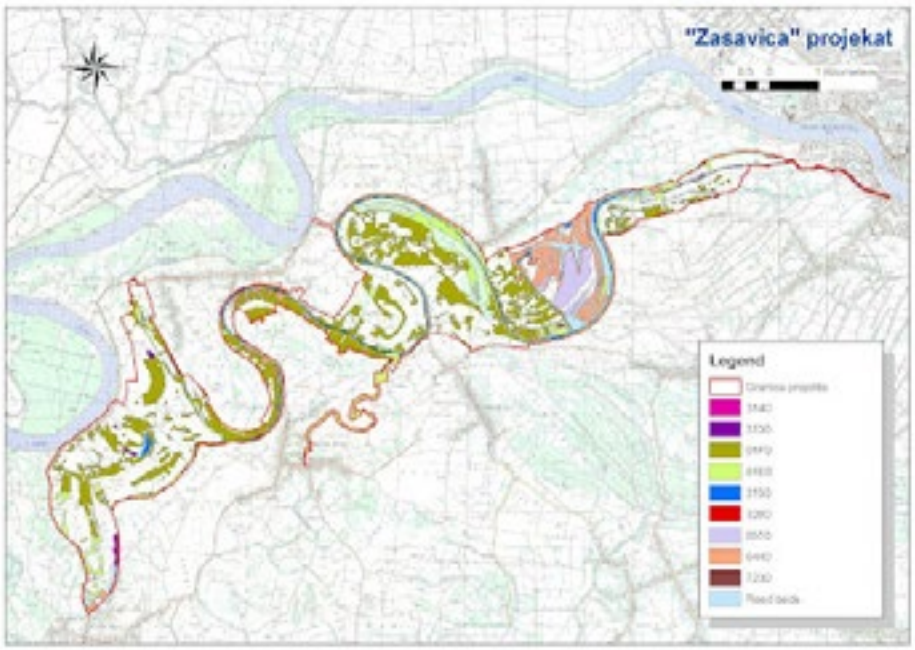
Identification of important Natura 2000 habitat types was performed on the basis of Natura 2000 criteria and in accordance with the national habitat classification (Lakušić et al., 2005) and habitat types defined within the LIFE project "Protection of Sava River Flood Biodiversity" (Kitnaes et al. 2010, Plavac et al. 2009. For each habitat type, the percentage of the area it occupies within the investigated area is given and the conservation status of the habitat is assessed using the following criteria: the degree of preservation of the habitat structure; assessment of habitat maintenance in the future and the possibility of habitat renewal (restoration). Overall assessment of conservation status (A, B, C) for each identified habitat type A: excellent conservation status (excellent or well-preserved structure and excellent conservation prospects, regardless of the assessment of habitat restoration); B: good conservation status, well-preserved structure and good conservation prospects,

97 *Pokret gorana Sremska Mitrovica, Svetog Save 19, 22000 Sremska Mitrovica , zasavica@zasavica.org.rs*

regardless of the assessment of habitat restoration (restoration), well-preserved structure and average / unfavorable conservation prospects, with easy restoration or feasibility with average effort; average structure / partially degraded, excellent conservation prospects, with easy restoration or feasibility with average effort; average structure / partially degraded, good prospects for preservation and easy restoration) and C: reduced (reduced) conservation status (all other combinations of conservation status).

RESULTS:

In the area of SNR Zasavica and its surroundings, based on Natura 2000 criteria, 10 types of habitats priority for protection in Zasavica were identified. The percentage of area ocupied witih the investigated area is determined. Conservation status was assesed based on the conservation degree of habitat structure, assesment of the maintenance perscpective and posibility of habitat renewal (restoration), respectfully: 3130 which were given the percentage of area occupied within the investigated area and conservation status was assessed through the degree of habitat structure conservation; assessment of habitat maintenance in the future and the possibility of renewal (restoration) of habitats and these are: 3130 Oligotrophic to mesotrophic standing water with vegetation of the order *Littorelletea uniflorae* and / or *Isöeto-Nanojuncetea* has an area of 0.30% and conservation status B -70% C-30%; 3140 Hard oligotrophic to mesotrophic water with vertebral carpets (*Chara spp.*), has an area of 0.18% and conservation status A-40% B-40% C-20%; 3150 Natural eutrophic lakes with vegetation of the *Magnopotamion* or *Hydrocharition* type have an area of 2.03% and a conservation status of B -70% C-30%; 3260; Watercourses from the plains to the hills with the vegetation of *Ranunculion fluitantis* and *Callitricho-Batrachion* have an area of 0.02% and a conservation status of B -50% C-50%; 6440 Meadows of alluvial river valleys with vegetation of *Cnidion dubii* have an area of 5.17% and conservation status A-40% B -40% C-20%; 6510 Lowland meadows (*Alopecurus pratensis, Sangisorba officinalis*) have an area of 2.39% and conservation status B-60% C-40%; 7230 Alkaline (lowland) peat has an area of 0.03% and conservation status A-20% B-40% C-40%; 91E0 Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion, Alnion incanae, Salicion albae*) have an area of 9.6% and conservation status B-60% C-40%; 91F0 Lowland forests *Quercus robur, Ulmus laevis, Ulmus minor, Fraxinus excelsior* or *Fraxinus angustifolia* near large rivers (*Ulmenion minoris* connection) have an area of 21.7% and conservation status B -70% C-30% and Reed beds Vegetation of high helophytes (*Phragmition, Magnocaricion*) has an area of 4.36% and conservation status A-10% B -70% C-20%. Habitats with developed lowland peat (7230) occupy 0.03% of the area of the investigated area, of which about 1/5 has an excellent structure (conservation status A) and all habitats with *Thelypteris palustris* are included here. Habitats 3260 and 3140 develop in cold and oligotrophic waters, mainly in the upper course of the Zasavica and its tributaries and are temporary (occurs during high spring waters along depressions and along the coasts) while the habitat; 3130 develops into vegetation of low muddy shores and stagnant oligotrophic to mesotrophic waters, usually in the period August-October during low summer waters on flattened muddy shores. In the lower course of the Zasavica and near larger ponds (Široka bara, spills on the Valjevac pasture) where the flow is slowed down, habitat 3150 is developed in typical eutrophic water conditions. It is often successively followed by the type of habitat Reed beds due to the change of vegetation that accompanies the variation of the water level during the year. This habitat type occupies up to 10% of the area and occurs mostly in the middle and lower course of the Zasavica and around larger ponds (Jovača, Široka bara, Ribnjača pond). It is further connected by a belt of wet meadows and clearings along the coast, where the habitat type is 6440, and as a secondary type of vegetation, lowland meadows 6510 appear, surrounded by arable land, resulting in a pronounced edge effect. Alluvial floodplain forests (91E0) occupy lower positions along watercourses, mostly in the lower and middle course of the Zasavica and its tributaries. Threating factors that negatively affect the maintenance of favorable habitat protection status are defined as protection measures to achieve favorable conservation status. According to the endangerment of habitat types in relation to the need for intervention measures, the most endangered habitats include 3260 and 6440, endangered 3130, 3140, 6510, 7230, 91E0 and less endangered 3150, 91F0 and Reed beds. Based on the valorization of the area and the estimated status of priority habitats and species, the reserve was officially expanded from 1850 ha to over 3400 ha in 2019 with new zoning with three levels (I, II, III) of protection and a protection zone, which reduces the negative influences of favorable factors from the immediate environment on the protected area..



Map distribution of Natura 2000 habitat types in the study area

CONCLUSIONS:

Ten types of Natura 2000 habitats for protection in Zasavica have been identified, having the following codes: 3130, 3140, 3150, 3260, 6440, 6510, 7230, 91E0, 91F0 and Reed beds. The largest area within the investigated area is occupied by habitats 91F0 with 21.7% and 91E0 with 9.6%. In relation to the need for intervention measures, the most endangered habitats include 3260 and 6440, and the least endangered ones include 3150, 91F0 and Reed beds.

T1-P-31 Midgut trypsin and lipase activities, hemolymph protein and lipids levels with integrated biomarker response (Ibr) in Gypsy moth (*Lymantria Dispar*) larvae from clean and polluted forest after chronic exposure to benzo[*a*]pyrene

Anja Grčić, Larisa Ilijin, Aleksandra Filipović, Dragana Matić, Marija Mrdaković, Dajana Todorović, Vesna Perić Mataruga[98]

KEYWORDS: Integrated response of biomarkers; Benzo[*a*]pyrene; Gypsy moth; Forest ecosystems

INTRODUCTION:

Intense anthropogenic influence led to a significant increase in pollution of the biosphere, in which polycyclic aromatic hydrocarbons, especially benzo[*a*]pyrene (B[*a*]P) made a major contribution. Wet and dry deposition gets atmospheric B[*a*]P on the vegetation, an important sink, and a crucial link for B[*a*]P bioaccumulation in animals. The gypsy moth is a phytophagous polyphagous insect that inhabits wide forest areas. Due to its vast appetite, it can pile great amounts of pollutants making it a suitable model system for biomonitoring the adverse effects of B[*a*]P. The larval midgut is the central metabolic place where trypsin and lipases provide efficient digestion of protein and lipids-rich food, showing sensitivity to chemical pollut-

98 *Institute for biological research „Siniša Stanković" National Institute of the Republic of Serbia, University of Belgrade, Despot Stefan Blv. 142, 11060 Belgrade Serbia. Corresponding author: anja.gavrilovic@ibiss.bg.ac.rs*

ants. Molecular parameters can be affected by physiological and environmental factors, so different adaptations of insects to the contaminants should be considered during the assessment of biomarker potential.

OBJECTIVES:

The aim was to investigate chronic effects of dietary treatment with B[*a*]P on midgut enzyme activities of trypsin and lipase, as well as the content of total proteins and lipids in hemolymph in gypsy moth larvae from two populations - one from an unpolluted oak forest and the other from a polluted oak forest. Furthermore, we used the method of Integrated Biomarker Response (IBR) to summarize responses of multiple molecular parameters across different tissues to estimate their sensitivity to B[*a*]P exposure in terms of population origin.

METHOD / DESIGN:

Gypsy moth egg masses were gathered in two mixed oak forests – Đerdap National Park forest, free of industrial pollution (unpolluted population of larvae, UP), and Bor forest contaminated by various byproducts of the mining industry (polluted population of larvae, PP). From hatching until the sacrifice (third day of the 5th instar) larvae were fed with a diet containing 0 ng (UP 0 ng and PP 0 ng), 5 ng (UP 5 ng and PP 5 ng), or 50 ng (UP 50 ng and PP 50 ng) of B[*a*]P in 1 g of dry diet. Spectrophotometric assays were used for the determination of specific enzyme activities of trypsin and lipase in the homogenates of the midgut, as well as for the evaluation of total proteins and lipids in the hemolymph of larvae. Two-way ANOVA followed by Tukey's post-hoc test was used for statistical analyses, conducted in GraphPad Prism 8 (GraphPad Software, Inc., USA). Statistical significance was determined at probability (p)<0.05. Excel software (Microsoft, USA) was used to calculate IBR values and to generate star plots9.

RESULTS:

The specific activity of trypsin has significantly inhibited after the treatment with lower B[*a*]P concentration in UP (F=9.412, *p*=0.0004), while a higher concentration of B[*a*]P significantly induced lipase activity in the same population of larvae (F=8.382, *p*=0.0007). These enzymes showed no statistically significant changes in the PP. Hemolymph protein content was significantly affected by the chronic dietary exposure to the higher concentration of B[*a*]P in both populations of larvae, showing a decrease in the UP, and the elevation in the PP (F=10.16, *p*=0.0002). Lipid concentration was not significantly changed under the B[*a*]P influence regarding the control groups (UP/PP 0 ng) but there was a meaningful difference between B[*a*]P treated larvae among two populations (F=7.16, *p*=0.019). IBR index increased in a concentration-dependent way only in UP after the chronic exposure to B[a]P and the values were higher than the corresponding ones in the PP (IBR index values - UP 0 ng=0; UP 5 ng=1.62; UP 50 ng=4.84; PP 0 ng=2.01; PP 5 ng=1.10; PP 50 ng=3.08).

CONCLUSIONS:

Gypsy moth population from the unpolluted forest showed higher sensitivity to the chronic dietary exposure to B[*a*]P comparing to the population from the polluted forest, especially in terms of trypsin and lipase activity. Hemolymph protein level expressed well correspondence to B[*a*]P concentration in both populations but with inverse trends. The selected set of Gypsy moth larvae molecular parameters possess a good potential for B[*a*]P biomonitoring in the populations from unpolluted forest ecosystems.

This study was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract No. 451-03-9/2021-14/200007.

T1-P-32 Sensitivity of midgut phosphatases to thermal stress in Gypsy moth (*Lymantria Dispar*) caterpillars

Larisa Ilijin, Anja Grčić, Marija Mrdaković, Milena Vlahović, Dragana Matić, Dajana Todorović, Vesna Perić Mataruga[99]

KEYWORDS: gypsy moth, alkaline phosphatase, total acid phosphatase, increased temperature, thermotolerance

INTRODUCTION: Environmental temperature directly affects the development of phytophagous insects, and indirectly through their host plants. Alkaline phosphatases (ALP) and total acid phosphatases (tot ACP) are midgut enzymes included in metabolic processes. The previous contact of the insect populations with various stressors and their ability to overcome the effects of the raised temperature (thermotolerance) can modify the response of these enzymes to increased environmental temperature.

OBJECTIVES: We aimed to compare the differences in responses of midgut ALP and tot ACP, with the expression of their isoforms, to increased environmental temperature with and without induced thermotolerance, in gypsy moth 5th instar caterpillars from unpolluted and polluted habitats.

METHOD / DESIGN: Caterpillars were hatched from egg masses collected in unpolluted (UP population) and polluted forest (PP population). They were reared at 23°C (PP23 and UP 23) and 28°C (PP28 and UP28) until the 3rd day of the 5th instar. In both populations, a group of individuals was exposed to 28°C for 24 h (induced thermotolerance) at the beginning of the 4th instar. Afterward, they were returned to 23°C until the sacrification (PP23In and UP23In) or exposed to 28°C for 72h before sacrification on the 3rd day of the 5th instar (PP28In and UP28In). The activity of enzymes was measured spectrophotometrically, using p-nitrophenyl phosphate (pNPP) as substrate, under alkaline conditions for ALP and acid conditions for tot ACP. Isoforms of both enzymes were detected on 12% polyacrylamide gel native PAGE.

RESULTS: In the UP groups, midgut ALP showed increased activity upon exposure to 28°C, with and without induced thermotolerance, while in PP caterpillars induced thermotolerance was the only factor that elevated ALP activity. Two way ANOVA analysis revealed that the interaction of temperature treatments and population origin (unpolluted vs polluted forest) was extremely significant ($F_{3,67}$=27.6, p<0.0001) for changes in midgut ALP activity, as well as the individual influence of increased temperature ($F_{3,67}$=30.9, p<0.0001) and the origin of the population ($F_{1,67}$=28.6, p<0.0001). Three ALP isoforms were detected. Isoform 1 was present only in PP groups exposed to 28°C, second is present in all experimental groups, and the third showed lower band density in PP treatments in comparison to UP. In UP23In tot ACP activity was elevated, while in PP treatments it was decreased. The interaction of temperature and population origin was extremely significant for tot ACP activity (two-way ANOVA, $F_{3,72}$=10.48, p<0.0001). Four isoforms of tot ACP were detected on the gel. Isoform 1 was present only in PP groups, isoform 2 has higher density in both populations and all treatments in comparison to controls. High band density of isoform 3 is present in all experimental groups, while induced thermotolerance and increased temperature, in both populations, increased band density of isoform 4.

CONCLUSIONS: Increased environmental temperature and induced thermotolerance have different effects on the activity of both enzymes in caterpillars from unpolluted and polluted habitats. ALP activity was more sensitive to thermal treatments in individuals originating from the unpolluted forests, in comparison to those from the polluted habitats, where on the other

99 Institute for biological research „Siniša Stanković", National Institute of the Republic of Serbia, University of Belgrade, Despot Stefan Blv. 142, 11060 Belgrade, Serbia. Corresponding author: lararid@ibiss.bg.ac.rs

hand, a completely new isoform was detected upon exposure to increased temperature. Tot ACP activity was decreased in all treatments in caterpillars from polluted habitats and a new isoform band was detected on native gels, while in those from the unpolluted forest, induced thermotolerance affected the activity of tot ACP. Obtained results indicate the differences in sensitivity to an increased environmental temperature between populations with different histories of exposure to pollution and that they must be considered as well.

This study was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract No. 451-03-9/2021-14/200007.

T1-P-33 Investigations of the presence of anthropogenic marker for wastewater contamination of the Danube

Nevena Grujić[100], Branislava Teofilović[100], Emilia Gligorić[100], Milan Vraneš[101], Katarina Ivanović[100]
Slobodan Gadžurić[101]

KEYWORDS: caffeine; Danube; HPLC

INTRODUCTION:

Caffeine is a purine alkaloid found in more than 60 plant species (coffee seeds, cocoa, and teas). It is mostly used in the production of food (80%), medicines (16%), and cosmetic products (4%). It is an integral part of various drinks (coffee, tea, caffeinated soft drinks), certain food products (chocolate), and medicines where it acts as a cardiac, cerebral, and respiratory stimulant and as a diuretic. Caffeine is found to be a good indicator for human sewage because of its unambiguous anthropogenic origin. The main paths for caffeine to enter the wastewater stream are either from urine or when caffeine-containing products are discharged through household pipelines or sewers.

OBJECTIVES:

The main goal of this study is to determine the presence of caffeine in the Danube samples as an anthropogenic marker for wastewater contamination of the Danube.

METHOD / DESIGN:

Analysis was performed by solid-phase extraction (SPE) followed by reversed-phase high-performance liquid chromatography (HPLC). The chromatography used a Zorbax Eclipse XDB-C8 column (4.6 mm x 150 mm, i.d., 5 µm particle size) at 25°C, with a mobile phase of water/THF (0.1 % THF in water, pH 8) – acetonitrile (85:15, v/v). The flow rate was 0.9 mL/min, and detection by DAD at 273 nm. The samples were collected during September 2019 at ten representative locations of the Danube on the territory of Novi Sad, Serbia, and stored in amber bottles at 4 °C until analysis.

RESULTS:

The caffeine was ubiquitously detected in samples from all ten locations with concentrations ranging from 305,94-375,97 ng/L. Maximum risk indexes (MaxRIs) for resident organisms (fish) in the Danube were calculated for each sampling site and the results showed that all MaxRIs belong to class II (10<MaxRI<100).

100 Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, Novi Sad, Serbia. Corresponding author: branislava.teofilovic@mf.uns.ac.rs

101 Faculty of Science, University of Novi Sad, Trg Dositeja Obradovića 3, Novi Sad, Serbia

CONCLUSIONS:

The presence of caffeine, which has no natural non-human sources, confirmed the existence of human waste in the Danube. The results obtained for MaxRIs indicated that the potential risk for the chronic effects may occur in the aquatic organisms in the long-term period.

T1-P-34 PCR amplification of *ure*C gene of alcaline Bacillus isolates from soil rich in calcite

Čila Holpert, Olja Šovljanski, Ana Tomić, Siniša Markov[102]

KEYWORDS: ureolytic activity; PCR amplification; *Bacillus*; *Sporosarcina*; *ure*C gene;

INTRODUCTION:

Ureolysis is controlled by genes that are responsible and specific for targeted metabolic activity. The urease production and activity are controlled by the structural *ure*A, *ure*B, and *ure*C genes, which encode enzyme subunits (γ, β, α-). Additionally, functional genes (*ure*D, *ure*E, *ure*F, *ure*G) are required to encode proteins that actively influence enzyme maturation. The three basic structural subunits form the $(UreABC)_3$ complex, while the functional genes create the UreDFG complex which is sequentially organized around $(UreABC)_3$ complex. The best confirmation of urease activity in bacteria is considered to be the presence of all three structural genes. However, detection of ureolytic activity is often based on the amplification of the ureC gene, which is a response to the main genetic structural α- subunit. The reason for choosing the *ure*C gene as a genetic indicator is the fact that the expression of the *ure*C gene significantly increases during ureolytic activity, while the level of *ure*A and *ure*B gene activity does not change.

OBJECTIVES:

This study aimed to amplify the *ure*C gene, as a marker for urease presence in bacteria, using the PCR technique. Comparative analysis of PCR amplification was done for five Bacillus isolates from alkaline soils previously described as ureolytic bacteria based on highly efficient urea hydrolysis in laboratory conditions, as well as control ureolytic and non- ureolytic bacteria.

METHOD / DESIGN:

In this study, for test bacteria are chosen *Bacillus strains* (*B. muralis, B. lentus, B. simplex, B. firmus, and B. licheniformis*) which are previously isolated as ureolytic and alkalophilic/alkaloresistant bacteria from alkaline and calcite-rich soils. For positive control reaction, DNA of *Sporosarcina pasteurii* DSM 33 which contains the *ure*C gene was used, while negative control was DNA extracted from non-ureolytic *Bacillus pseudofirmus* DSM 8715. All bacterial strains were grown at 30°C on Columbia blood agar for 18 hours. The genomic DNA was extracted by a DNA Isolation Kit following procedure for Gram-positive bacteria, while the quantity of extracted DNA was determined by NanoDrop™ One Microvolume UV-VIS spectrophotometer. PCR amplification of *ure*C gene in the genome of tested bacteria was conducted using L2F (59-ATHGGYAARGCNGGNAAYCC-39) and L2R (59-GTBSHNCCCCARTCYTCRTG-39) primers. The final PCR mix (a total volume of 20 µl) involved DNA template (100 ng), PCR Master Mix (10 µl), primers (0.5 µmol per primer), DNA polymerase (0.5 µl), and H_2O. The ureC gene was amplified under the following conditions: 5 min at 94.5 °C (initial denaturation); 30 cycles for 1 min at 94 °C, 1.5 min at 55.7 °C (primer annealing), 2 min at 72 °C (primer extension), and 10 min of final extension at 72 °C. All chemicals, as well as a spectrophotometer, are made by Thermo Fisher Scientific, Waltham, MA USA. Visualization of the PCR products (expected length 300-400 bp) was done by capillary Lab-on-a-Chip electrophoresis at 2100 Bioanalyzer (Agilent Technologies, USA).

102 University of Novi Sad, Faculty of Technology Novi Sad, Bulevar cara Lazara 1, Novi Sad, Serbia. Corresponding author: holpert.csilla@gmail.com

RESULTS:

According to the obtained results of PCR amplification, it can be concluded that the targeted gene for the ureolysis process was successfully amplified from the genomes of all selected *Bacillus isolates*. The expected PCR fragment for the *ure*C gene has about 340 bp. In the case of the reference strain *S. pasteurii* DSM 33, the size of the amplified fragment of the *ure*C gene was 342 bp, while the size of the amplified fragment of the same gene in the case of the natural isolates was 334, 341, 343, 341 and 344 bp for *B. muralis, B. lentus, B. simplex, B. firmus and B. licheniformis*, respectively. The obtained sizes of *ure*C gene fragments coincide with the fragment size of 344 bp of the same gene amplified from the genome of *S. pasteurii* and 340 bp amplified from the genome of isolates belonging to the genera *Bacillus, Virgibacillus, and Lysinibacillus*.

CONCLUSIONS:

Detection of the *ure*C gene provides basic information on the existence of a fully functional operon that carries information on the synthesis of the urease enzyme. Furthermore, among the structural genes for urease, the *ure*C gene is the largest coding structural subunit of urease, and more importantly, there are a large number of highly conserved regions suitable for PCR primer binding. According to the gained results, the *ure*C gene confirms the previously mentioned facts, because it is successfully amplified in all tested bacteria during this study.

T1-P-35-ORAL Alterations of growth, oxidative stress and energy reserves in *Daphnia magna* after ZnO exposure under thermal stress

Paweena Sanpradit, Saranya Peerakietkhajorn[103]

KEYWORDS: Temperature; ZnO; *Daphnia magna*; Oxidative stress; Energy reserves

INTRODUCTION:

Currently, climate change and water pollution are the major global issues. Global warming leads to rise the world's temperature. Intergovernmental Panel on Climate Change (IPCC) reported that temperature will be increased about 1-5°C by the end of this century. Temperature strongly influences metabolic rate and physiological processes in organisms and the toxicity of potentially harmful chemicals. Utilization of ZnO has spread out after the industrial revolution. ZnO has been widely used as a catalyst in many industries such as production of rubber tires and latex, and additional ingredient in sunscreen. The widespread industrial use of ZnO causes contamination in aquatic ecosystems. Excess ZnO induces reactive oxygen species (ROS) generation leading to increase lipid peroxidation of cell membrane. Furthermore, malondialdehyde (MDA) is formed by the decomposition of polyunsaturated fatty acids. The organisms have to control and balance the excess of ROS by producing antioxidant enzymes such as superoxide dismutase (SOD). Moreover, it is well known that environmental stressors directly influence total energy reserve to fulfill the metabolic requirements in animals. Therefore, the changes in level of oxidative stress and energy reserves in aquatic animals are the interesting biomarkers to detect metal contaminations in aquatic ecosystem.

OBJECTIVES:

This study aims to investigate the effects of ZnO toxicity under elevated temperature on growth, oxidative stress and energy reserves in *D. magna*.

103 Division of Biological Science, Faculty of Science, Prince of Songkla University, Songkhla, Thailand. Corresponding author: paweena.sanp@gmail.com

Gut Biology and microbiota research unit, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

METHOD / DESIGN:

In this study, the toxicity of ZnO concentrations was assessed at 23°C and 28°C in *D. magna*. The observed parameters: body length, oxidative stress, zinc accumulation and energy reserves were investigated under different exposure times (0, 2, 9 and 21 days). Body length of *D. magna* was measured using imageJ version 1.53e. Oxidative stress (levels of SOD and MDA) and energy reserves (levels of lipid, carbohydrate and protein) were measured using spectrophotometry. The caloric contents were calculated. Furthermore, Zn accumulation was observed using inductively coupled plasma optical emission spectroscopy (ICP-OES). The integrated biomarker response (IBR) was analyzed to consider the potential of ZnO toxicity under thermal stress.

RESULTS:

At 28°C, body length of 2-day-old *D. magna* cultured was increased, on the other hand, body lengths of *D. magna* with the increment of ZnO concentrations at day 9 and 21 were significantly reduced when compared with control ($p<0.05$). SOD activity tended to increase in 2-day-old *D. magna* cultured at higher temperature and ZnO concentration. In contrast, the alteration of temperature and ZnO concentration was not significantly changed SOD activity in *D. magna* cultured for 9 and 21 days. MDA level was not altered in 2-day-old *D. magna*, however, MDA level tended to increase in 9- and 21-day- old *D. magna* exposed to higher concentration of ZnO at 28°C ($p<0.05$). Zinc accumulation was fluctuated in all treatments, except ZnO treatments in 9-day-old *D. magna* showing the increase of zinc accumulation in higher concentration of ZnO and temperature. Furthermore, lipid content in 2-day-old *D. magna* treated with ZnO at 28°C was increased higher than control ($p<0.05$). Nonetheless, lipid content was significantly decreased in 9-day-old *D. magna* cultured at 28°C and treated with ZnO ($p<0.05$). A reduction of protein content was observed in 2- and 9-day-old *D. magna* treated with ZnO and higher temperature ($p<0.05$). Carbohydrate content was decreased in 2-, 9- and 21-days-old *D. magna* treated with ZnO and elevated temperature($p<0.05$). Total caloric content was also significant decreased in 2- and 9-days-old *D. magna* exposed to higher ZnO concentration and temperature ($p<0.05$). Lipid content, protein and total caloric content were not altered in 21-days-old *D. magna*. Additionally, IBR index was significantly increased under ZnO exposure and thermal stress in all exposure periods illustrating that higher toxicity occurred.

CONCLUSIONS:

Our results suggest that the combination of temperature and ZnO concentration impacts the body length of *D. magna*. Furthermore, the increment of temperature also increased the metabolic rate of D. magna leading to a greater reductionof energy reserves. Both elevated temperature and exposure to ZnO induced oxidative stress in *D. magna* leading to increase MDA level and to alter SOD activity. Besides, energy expenditure is more required for controlling the excess of ZnO. These alterations led to affect the body length of *D. magna*. In addition, toxicity of ZnO under thermal stress was age- dependent in the present study. IBR index also demonstratedthat ZnO exposure under thermal stress resulted in stimulating toxicity in *D. magna*. Hence, IBR should be considered as an index for detecting the contamination of ZnO in environment. This study might be useful for the further studies on zinc toxicity in aquatic ecosystems under global warming conditions.

T1-P-36 Satellite assisted mapping of environmental pollutants: a study on burning crop residues

Miloš Pandžić, Mirjana Radulović, Predrag Lugonja, Vladimir Crnojević[104]

KEYWORDS: crop residue; field burning; Sentinel-2; change detection

104 BioSense Institute, University of Novi Sad, 21000 Novi Sad, Serbia. Corresponding author: mirjana.radulovic@biosense.rs

INTRODUCTION:

An increasingly common problem we are facing at the end of the agricultural season are intentional fires on agricultural plots caused by humans. Generally, this is the most common and easiest way of crop residue removal, which is why it is practiced by farmers worldwide. Despite being very harmful to the environment overall, this method can limitedly mitigate some of the flaws in crop production such as presence of weeds, pests, and various diseases, which gives farmers a legitimate excuse to perform this type of action on their fields. According to previous research[105], at least 34% of the total emissions caused by the combustion of biomass is caused by burning of residues on agricultural plots. Considering the above, this procedure has a high negative impact on the environment, hence on humans.

OBJECTIVES:

The objective of this research was to detect burning of the crop residues on a field-scale using freely available Sentinel-2 images which are characterized by high spectral, spatial, and temporal resolution.

METHOD / DESIGN:

The method is based on change detection between two consecutive satellite images, one before the fire occurrence and the other after the fire. The Sentinel-2 satellite, operated by the European Space Agency on behalf of the European Commission, delivers optical images of the Earth's surface every 5 days with the spatial resolution of 10 x 10 m per pixel at no cost. Apart from the blue (B2), green (B3), and red (B4) channels that belong to the visible part of the electromagnetic spectrum, i.e. it is how the human eye works and sees, the Sentinel-2 instrument (optical camera) has another 10 spectral bands suitable for monitoring vegetation. Two of these, namely B11 and B12, were used in our method through a simple ratio[106] (SR) of the two: SR=B11/B12, on a pixel-level. This ratio is characterized by high values in areas where a fire occurred and low values in those where it did not. Hence, if the difference of SR values between the two consecutive satellite images for the same location is small, we conclude that fire did not occur, otherwise it did. We foresee three possibilities: i) the difference lower than 0.35 means no area was burned, ii) the difference between 0.35 and 0.80 means potentially burned area and iii) the difference greater than 0.80 means fire occurrence. Values were determined by varying the thresholds followed by visual inspection. In order to assure the validity of the results, some of the additional constraints were introduced such as water, cloud, intensive growth, and crop masking.

RESULTS:

The method was applied to the Vojvodina province in Serbia. In the test period of three months (September to November 2020) more than 9000 parcels were detected that were subject to crop residues burning. According to spatial analysis, southern parts of the Vojvodina province were more prone to burning crop residues, in particular, the districts of Srem and South Banat. Comparatively, the district of North Bačka was the least affected by this phenomenon.

CONCLUSIONS:

The results showed that Vojvodina is highly affected by the phenomenon of burning crop residues. Despite being relatively simple, the presented method showed its potential and that it can serve as the base for future steps in handling this problem by the local authorities. An obstacle that can prevent the application of the presented workflow is cloud coverage that can obscure satellite images and make them unusable. Usage of various satellite remote sensing sources (including commercial satellites or SAR sensors) with a higher temporal frequency of image acquisition can alleviate this issue.

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105 Streets, D. G., Bond, T. C., Lee, T. & Jang, C. On the future of carbonaceous aerosol emissions. J. Geophys. Res. Atmos.109, (2004)

106 Van Deventer, A. P., Ward, A. D., Gowda, P. H., Lyon, J. G. 1997. Using thematic mapper data to identify contrasting soil plains and tillage practices. Photogrammetric Engineering and Remote Sensing 63, 87-93.

T1-P-37-ORAL Seagrass ecosystems as nature-based solution for climate change mitigation

Milica Stankovic[107], Anchana Prathep[107/108]

KEYWORDS: seagrass; climate change; blue carbon; coastal ecosystems.

Seagrass meadows provide many valuable seagrass ecosystems essential for human well-being, global diversity, climate change adaptation and resilience. These ecosystems have the ability to sequester and store large amounts of organic carbon. Comparing to the terrestrial ecosystems, carbon stored in seagrass meadows remains trapped for centuries, which suggest the potential contribution towards climate change mitigation strategies as a nature-based solution for offsetting CO_2 emissions. Regardless of their importance, these ecosystems are drastically declining in the Southeast Asia, and the stored carbon is at risk of being eroded and released back into the atmosphere. The rate of decline of seagrass ecosystems is much higher than the loss of tropical and mangrove forests, and 102,888 ha of seagrass meadows is lost every year in Southeast Asia. Although this region represents one of the global seagrass hotspots, with 6-12% of the global seagrass coverage, this ecosystem still remains poorly documented, with very few studies focused on blue carbon. The carbon storage in seagrass meadows in Southeast Asian region accounts for up to 5% of the total global carbon stock, with higher stock than in Gulf of Mexico and similar values in West Africa. However, the accumulation capacity of the meadows is estimated to be 21.42–24.90 Mt of CO_2 per year, which accounts for the $21.42–24.91 million USD per year. Their high carbon sequestration and accumulation rates suggest great potential efficacy to mitigate CO_2 emissions through blue carbon strategies and policies. Through conservation of the existing meadows and the proper restoration practices seagrass meadows can contribute up to 94% of the total carbon burial in some countries of the region by 2030. Most of the countries in the region signed the agreements implemented by the United Nations Intergovernmental Panel on Climate Change (IPCC) to decrease yearly CO_2 emissions though nationally determined contributions. However, current commitments are probably insufficient, suggesting that mitigation and adaptations strategies need to include nature-based solutions through restoration, conservation, and avoidance of emissions from the destruction of natural ecosystems. By 2030 in business-as-usual scenario, seagrass meadows can contribute around 1.5% towards CO_2 offset of the total countries' emissions. The inclusion of seagrass ecosystems into the mitigation strategies could contribute up to 7% of the countries' reduction goal for CO_2 emissions by 2030. However, despite their high potential to contribute towards the mitigation measures, financial mechanisms and policies are still poorly developed. The use of these ecosystems as a nature-based solution is appealing, as they provide multiple ecosystem services towards climate change mitigation and adaptation, suggesting their potential to address multiple sustainable goals.

107 Excellence Center for Biodiversity of Peninsular Thailand, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

Corresponding author: milica.s@psu.ac.th

108 Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

T1-P-38 Sessile oak rhizobacteria with plant growth-promoting potential *in vitro*

Sanja Jovanović[109], Olja Stanojević[110], Aleksandra Mesaroš[110],
Vladan Popović[109], Aleksandar Lučić[109], Slaviša Stanković[110], Tanja Berić[110]

KEYWORDS: rhizobacteria; sessile oak; growth promotion; *in vitro*

INTRODUCTION:

The rhizosphere is a complex habitat with great bacterial heterogeneity, and their activity, diversity, and dynamics are dictated primarily by plant root exudates. Plant growth-promoting rhizobacteria (PGPR) can be distinguished as a specific functional group that elevates plant characteristics and performance by direct and indirect mechanisms and are often members of genus *Bacillus* and *Pseudomonas*. PGPR of oak species in Serbia is poorly understood. Sessile oak (Quercus petraea (Matt.) Liebl) is autochthonous and one of Serbia's most significant forest species due to its economical, technical, ecological, and cultural importance. It is one of the most abundant tree species with a percentage of 7.7% in the growing stock (186.179 ha), and within the belt of its stands, there are 23 different forest types. The main problems of present sessile oak forests are continuous intensive decline, the domination of coppice forests (74.1%), age, and physiological susceptibility to (a)biotic stressors, which all guide to smaller seed yield and difficult natural regeneration. An additional problem is the low percentage of artificial reforestation success.

OBJECTIVES:

The objectives of this research were the isolation of bacteria of the genus *Bacillus* and *Pseudomonas* from the sessile oak rhizosphere, the in vitro investigation of their plant growth-promoting potential, and molecular identification of the most competent plant growth promoters.

METHOD / DESIGN:

The bacteria of the genus *Bacillus* and *Pseudomonas* were isolated by culturing methods from the sessile oak rhizosphere samples from mountain Rudnik where it naturally occurs. The Gram, catalase, and oxidase tests were performed, as did the fluorescent pigment production for potential pseudomonads. In addition, its plant growth-promoting abilities (IAA synthesis, siderophore production, and phosphate solubilization) were investigated *in vitro*. The selected isolates were molecularly identified based on the 16S rRNA gene sequence.

RESULTS:

A total of 179 bacteria were isolated from sessile oak rhizosphere samples, 75 of them being putative *Bacillus* and 48 putative *Pseudomonas* species. Of the total isolates, 155 of them were IAA producers, 81 siderophore producers, and 90 isolates were capable of phosphate solubilization. Fourteen most competent PGPR isolates were moleculary identified based on the 16S rRNA gene sequence as *Lysinibacillus parviboronicapiens, Viridibacillus arvi, Viridibacillus arenosi, Brevibacterium frigoritolerans, Peribacillus simplex, Rahnella variigena, Pseudomonas koreensis, Pseudomonas helmanticensis, Serratia quinivorans, Pseudomonas vancouverensis and Pseudomonas migulae*.

109 Institute of Forestry, Department of Genetics, Plant Breeding, Seed and Nursery Production. Corresponding author: b3028_2019@stud.bio.bg.ac.rs

110 University of Belgrade, Faculty of Biology, Chair of Microbiology

CONCLUSIONS:

The four isolates, out of 179 isolated and investigated, were selected as potential sessile oak plant growth promoters for two of three tested features, being *Viridibacillus arvi, Pseudomonas migulae, Pseudomonas koreensis and Pseudomonas helmanticensis*. Further research is needed to confirm the plant growth-promoting potential of the bacterial isolates *in vivo*.

T1-P-39 Dibutyl phthalate induces migration and angiogenesis of Ea.Hy926 human endothelial cells through Gper/Erk1/2 signaling

Dunja Kokai, Bojana Stanic, Biljana Tesic, Svetlana Fa, Dragana Samardzija Nenadov, Kristina Pogrmic-Majkic, Nebojsa Andric[111]

KEYWORDS: dibutyl phthalate; human endothelial cells; cell migration; angiogenesis; ERK1/2

INTRODUCTION:

Cell adhesion and migration represent two opposing but intricately balanced functions of endothelial cells (ECs) important in maintaining the stability of the endothelium and during wound healing and angiogenesis. Regulation of cell adhesion and migration involves coordinated events including activation of a number of signaling pathways, cytoskeleton rearrangement, and surface integrin redistribution. Dysregulation of angiogenic factors and increased angiogenesis seem to play a key role in the pathophysiology of various diseases in humans such as tumor growth, progression and metastasis, as well as in many other non-malignant diseases but has also been demonstrated in atherosclerotic plaques as an important factor in early plaque development, intraplaque hemorrhage, plaque instability and rupture, and eventually, acute cardiovascular events. Although epidemiological studies suggest a possible association between exposure to dibutyl phthalate (DBP), a man-made chemical widely used in many industrial and consumer products, and cardiovascular diseases (CVDs), the impact that DBP exerts on EC migration and angiogenesis remains unclear.

OBJECTIVES:

Here, we sought to examine cell adhesion to extracellular matrix (ECM), migration, and angiogenesis after acute exposure of human vascular ECs to DBP and investigate the molecular events and signaling pathways involved in these processes.

METHOD / DESIGN:

EA.hy926 cells were exposed to either vehicle (0.05% DMSO – control) or three concentrations of DBP (10^{-6}, 10^{-5}, and 10^{-4} M DBP in 0.05% DMSO) for up to 48 h. Cell viability was monitored using the alamarBlue™ assay. The adhesion assay on gelatin-coated cell culture plates was used to investigate the effect of DBP exposure on cell adhesion to the ECM. Cell migration was assessed using the wound-healing ("scratch") assay. Angiogenesis was assessed by monitoring endothelial tube formation in the growth factor-reduced ECM membrane-loaded cell culture plates. Gelatin zymography was used to detect the latent and activated forms of matrix metalloproteinases (MMPs) in cell culture media. Quantitative real-time PCR was used to determine mRNA expression levels, whereas Western blotting was employed to investigate protein expression. When indicated, pharmacological inhibitors were added 45 min prior to treatments and were present for the entire duration of the treatments. All results were expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) with Dunnett's multiple comparison post-hoc test. A p value of < 0.05 was considered significant.

111 University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, ENDOS Laboratory, Serbia. Corresponding author: dunja.kokai@dbe.uns.ac.rs

RESULTS:

Viability of EA.hy926 cells was not significantly affected after 24 h and 48 h of exposure to all concentrations of DBP. Decreased adhesion to gelatin was observed after 24 h of exposure to 10^{-5} M DBP, followed by diminished expression of five genes encoding the integrin family of proteins: *ITGA2, ITGA5, ITGAV, ITGB1*, and *ITGB5*. Cell migration was increased after 24h of exposure to 10^{-6} M and 10^{-5} M DBP. Observed increase in cell migration was not due to altered expression of *MMP1, MMP2*, and *MMP9*, but rather increased gelatinolytic activity of *MMP-2* in response to a 24 h-long exposure to DBP. After 24h of DBP pre-treatment, angiogenesis was increased after additional 20 h-long exposure of EA.hy926 cells to 10^{-4} M DBP. To investigate signaling pathways involved in DBP-induced migration of EA.hy926 cells, we used several pharmacological inhibitors: ICI 182 780, a specific estrogen receptor (ER) down-regulator, G15, a specific G-protein-coupled ER (GPER) antagonist, wortmannin, a specific inhibitor of the phosphoinositide-3-kinase – protein kinase B/Akt pathway, and U0126, a potent and selective inhibitor of the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway. G15 and U0126 inhibited DBP-induced migration, indicating that DBP acts through the GPER and ERK1/2 pathway. Western blotting analysis confirmed activation of ERK1/2 in DBP-exposed EA.hy926 cells: 10^{-5} M and 10^{-4} M DBP activated ERK1/2 after 15 min, whereas only 10^{-5} M DBP activated ERK1/2 after 30 and 60 min of exposure. ERK1/2 activation was GPER dependent, which was confirmed on Western blots by using G15. DBP-induced increase in angiogenesis was ERK1/2 dependent, as shown by U0126-inhibited tube formation.

CONCLUSIONS:

Obtained results suggest that DBP acts through GPER in EA.hy926 cells to increase ERK1/2 activity, cell migration, and angiogenesis, thereby providing novel information regarding the molecular mechanism of DBP's action in human vascular ECs. Understanding the molecular mechanisms behind different diseases with vascular etiology is critical for formulating effective prevention strategies and identifying novel therapeutic interventions. Additional studies are needed in order to provide a more detailed insight into the exact mechanism of DBP's action in the vasculature.

T1-P-40-ORAL Efficiency of Indian-almond leaf (*Terminalia catappa* linnaeus, 1767) extracts in rearing Siamese fighting fish (*Betta Splendens* Regan, 1910)

Saowalak Malawa[112/113], Karun Thongprajukaew[113/114], Nutt Nuntapong, Naraid Suanyuk[112]

KEYWORDS: *T. catappa; B. splendens*; Growth; Water quality; Hematological parameter

INTRODUCTION:

The lack of adequate management in aquaculture causes stress to aquatic animals, lowering immunity that leads to pathogenic infection and outbreak of diseases. Whereas antibiotic and chemical treatments can cause undesirable side effects and residue problems in the production of aquatic animals and their surrounding environments, herbal treatment based on indigenous knowledge is an alternative approach in aquaculture. Indian-almond (*Terminalia catappa* Linnaeus, 1767) leaf has been widely used in rearing of economically important Siamese fighting fish (*Betta splendens* Regan, 1910). Generally, this plant contains active ingredients include tannins, flavonoids, phenols, alkaloids, triterpenoids, steroids, saponins and quinones. Therefore, some improvements in water quality, and better growth performance of a few aquatic animals have

112 Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90112, Thailand. Corresponding author: 6310630001@psu.ac.th

113 Center of Excellence in Agricultural and Natural Resources Biotechnology Phase 3, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90112, Thailand

114 Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand

been reported after addition of the aqueous *T. catappa* leaves extracts. However, perceptive information is unavailable on the efficiency of *T. catappa* leaves extracts in rearing the growing phase of Siamese fighting fish.

OBJECTIVE:

This study was performed to determine the optimal concentration of Indian-almond leaf extracts for rearing growing phase of male Siamese fighting fish.

METHOD/DESIGN:

Seventy-five solid-red male fish (2-month-old, 0.80-0.95 g initial body weight) were individually distributed into aquaria (8.3 cm diameter × 12.5 cm height, with 8 cm water depth). Aquaria were divided into five groups, containing fifteen fish each. Each group was treated with a different concentration of Indian-almond leaf extracts (0, 0.125, 0.25, 0.5 and 1 g/L of water). The water was 80% exchanged every other day and the qualities were monitored two times weekly. At the end of the 8-week trial, growth performance, feed utilization, skin coloration and hematological parameters of reared fish were investigated.

RESULTS:

No mortality was observed over the eight weeks of trial. Only the fish reared in the highest concentration of leaf extracts exhibited the highest growth (specific growth rate 1.74% body weight/day) relative to control treatment ($P < 0.05$). No significant differences in feed conversion ratio (1.16 g feed/g gain on averages) and protein efficiency ratio (2.28 g gain/g protein on averages) were observed across all groups. The last treatment also improved skin coloration, in terms of lightness and redness, as compared to control group. White blood cells, red blood cells, plasma protein concentration and hematocrit did not differ between the fish reared in the highest concentration of leaf extracts and in control treatment ($P > 0.05$), while minor improvement was observed in fish subjected to 0.125, 0.25 and 0.5 g/L of leaf extracts. Also, rearing the fish in the highest concentration of leaf extracts significantly improved key water quality parameters (pH, ammonia, nitrite and nitrate) as compared to control ($P < 0.05$).

CONCLUSIONS:

These findings suggest that the highest concentration of Indian-almond leaf extracts (1 g/L of water) is suitable in rearing growing phase of male Siamese fighting fish. Application of this herbal extract is environmentally-friendly and can reduce chemical or antibiotic usages in aquaculture, inhibiting residue problems in the production of aquatic animals and the surrounding environments.

T1-P-41 Effects of chronic dietary cadmium on midgut superoxide dismutase (SOD) and catalase (CAT) in larvae from two *Lymantria dispar* populations

Dragana Matić, Milena Vlahović, Larisa Ilijin, Anja Grčić, Aleksandra Filipović, Marija Mrdaković, Vesna Perić-Mataruga[115]

KEYWORDS: superoxide dismutase; catalase; *Lymantria dispar*; cadmium; biomarker

INTRODUCTION:

Cadmium (Cd) levels in the environment have increased during decades of intensive industrial development and urbani-

115 Department of Insect Physiology and Biochemistry, Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, University of Belgrade, Despot Stefan Blv. 142, 11060 Belgrade, Serbia. Corresponding author: dragana.matic@ibiss.bg.ac.rs

zation. *Lymantria dispar* has proved to be a suitable organism indicator to monitor Cd pollution in forest ecosystems. Since insects accumulate heavy metals predominantly in the gut, it is not surprising that several enzymes in the midgut of *L. dispar* larvae, including antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), have been marked as promising biomarkers of Cd presence. Namely, Cd indirectly induces oxidative stress in the cell. However, long-term exposure of the population to pollution often results in increased tolerance and altered sensitivity of biomarkers.

OBJECTIVES:

We aimed to determine specific enzyme activities and isoform patterns of SOD and CAT in the midgut of *Lymantria dispar* larvae after chronic treatment with Cd. To assess these parameters as biomarkers of Cd exposure, we compared the responses of two populations with different histories of exposure to pollution.

METHOD / DESIGN:

Egg-masses of *L. dispar* were collected from two localities in Serbia - the uncontaminated forest in Kosmaj Mountain, which is a protected natural resource, and a polluted site near the busy Ibar highway. Larvae were fed a wheat germ diet containing 0, 50 or 100 µg Cd/g dry food starting from hatching until they were killed on the 3rd day of the 4th instar. Specific activities of SOD and CAT in the midgut homogenates were determined by spectrophotometric assays. Enzyme isoforms were separated by native polyacrylamide gel electrophoresis. Statistical analyses were performed in GraphPad Prism 7 (GraphPad Software, Inc., USA), where enzyme activities were analyzed by one-way ANOVA followed by Tukey's posthoc test. The level of statistical significance was $p<0.05$.

RESULTS:

The specific activity of SOD was higher in control larvae from the polluted locality compared to the control group from the uncontaminated forest. Exposure to both Cd concentrations decreased SOD activity in larvae from the polluted site. Three SOD isoforms were detected in control groups from both populations. While isoform SOD-2 was absent in the population from Kosmaj after the treatment with higher Cd concentration, both SOD-2 and SOD-3 disappeared in all Cd-treated larvae from the site near the highway. In the population from the unpolluted locality, specific activity of CAT was reduced at 100 µg Cd/g dry food, whereas in another population a decrease in enzyme activity was noticed at both Cd concentrations. The same pattern of Cd influence was observed for CAT isoform activity. Only one CAT isoform was present in both control and experimental larvae from both populations.

CONCLUSIONS:

Higher SOD activity in control larvae originating from the site near the highway compared to those from the uncontaminated forest probably indicated the presence of traffic-related pollution that caused oxidative stress. However, neither SOD nor CAT showed activation in response to Cd treatment. A decrease in SOD and CAT activities in both Cd-treated groups from the population inhabiting the polluted site was most likely the result of the trade-off in favor of the alternative defense mechanism(s). Such trade-off might have led to the diminished expression of isoforms SOD-2 and SOD-3. Thus, a decrease in SOD and CAT activities after Cd exposure could be seen as an adaptive strategy in *L. dispar* populations. Specific activities of SOD and CAT with SOD isoform patterns could be used as biomarkers of Cd exposure in contaminated environments.

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T1-P-42 Differences in the root anatomical traits of three *Salix L.* clones in response to increased Cd concentrations

Zorana Hrkić Ilić[116],
Milan Borišev, Lana Zorić, Jadranka Luković[117]

KEYWORDS: *Salix*; cadmium; root; anatomy; phytoremediation

INTRODUCTION:

Among heavy metals, cadmium (Cd) is one of the most widely distributed pollutant, whose concentrations increase in the soil, water and air, globally and over time. Numerous physiological, biochemical and morphological studies have shown that woody species from the genus *Salix L.* (willows) represent suitable tools for phytoremediation of the sites polluted by cadmium. However, only a few studies have followed structural changes of the root tissues exposed to increased concentrations of Cd in soil and water. Previous physiological studies have established high cadmium accumulation in the roots of several willow clones, *Salix alba L.* clone ´B-44´, *Salix viminalis L.* clone ´SV068´, and *Salix matsudana* Koidz. clone ´SM404´.

OBJECTIVES:

The aim of our work was to assess the effects of two singly applied Cd concentrations (10^{-3} and 10^{-6} M Cd) on the roots´ anatomical traits of three willow clones, *Salix alba L.* (clone ´B-44´), *Salix viminalis L.* (clone ´SV068´), and *Salix matsudana* Koidz. (clone ´SM404´), grown in the soil contaminated with cadmium.

METHOD / DESIGN:

Plant materials consisted of one-year old stem cuttings of three willow clones, *Salix alba L.* clone ´B-44´, *Salix viminalis L.* clone ´SV068´, and *Salix matsudana* Koidz. clone ´SM404´, obtained from the Institute for Lowland Forestry and Environment (ILFE) in Novi Sad, Republic of Serbia. The clones were selected according to their properties of good Cd removal from moderately polluted soils, highest Cd content recorded in roots in comparison with aboveground organs, and clone specific response to high Cd concentrations. Willow plants were grown under semi-controled conditions (greenhouse), by soil culture method, in Mitscherlich pots containing 5 kg of soil. Plants were divided into three treatments: control (without Cd), and treatments with 10^{-3} M Cd and 10^{-6} M Cd, each singly added to soil, respectively. Plants were harvested 4 months after the treatments application (May to September). The anatomical response of the selected *Salix* clones to Cd was investigated on the microscopic sections of the part of the roots at a distance of 3 cm from the root neck. Examined roots developed secondary anatomical structure. Measurements of roots´ cross-sections included following traits: root cross-section area, root diametar, thickness of the periderm, secondary phloem (cross-sectional area, percentage and thickness), secondary xylem (cross-sectional area, percentage and diameter), cross-sectional area of parenchyma cells, and cross-sectional area, number and diameter of vessels.

RESULTS:

Results of analyses have shown that on the root level, clones expressed different response to increased concentrations of Cd. Both applied concentrations of Cd led to a significant reduction of measured anatomical root traits of clone ´SV068´ in comparison with the control plants. In root tissues of this clone, concentration of 10^{-3} M Cd had more prominent negative ef-

116 Faculty of Forestry, University of Banja Luka, Bulevar Vojvode Stepe Stepanovića 75A, 78000 Banja Luka, Bosnia and Herzegovina.

Corresponding author: zorana.hrkic-ilic@sf.unibl.org

117 University of Novi Sad Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, Novi Sad 21000, Serbia

fects on most of the anatomical traits than concentration of 10^{-6} M Cd, in comparison with control. On the other hand, clone ´B-44´ has shown even increase in many root traits after treatment with 10^{-3} M Cd concentration. Elevated root cross-sectional area in this clone, under concentration of 10^{-3} M Cd, can be explained by increased thickness of secondary phloem and cross-sectional area of vessels in secondary xylem. In clone ´SM4041´ applied Cd concentrations caused significant and opposite changes in values of measured parameters, specifically in thickness of secondary phloem, cross sectional area of parenchyma cells and number of vessels (N/µm^2 xylem). In this clone values of vessel areas were increased, while their number and percentage decreased. Significant differences in the vessels distribution were observed between the clones, when compared to control plants, with the vessel distribution peaked at a vessel diameter of 20-40 µm. In this class of vessel diameter, values in clones ´B-44´ and ´SM4041´ decreased, while in clone ´SV068´ increased, compared to control, individually.

CONCLUSIONS:

Our research points to the importance of anatomical traits in assessment of the potential use of *Salix sp.* genotypes in phytoremediation. Obtained results indicated genotype-specific response to the presence of excess concentration of Cd in root tissues. The data from this work are in correlation with previous physiological studies of selected willow clones, and indicate further research associated with the possible use of clone ´B-44´ in phytoremediation strategies.

T1-P-43 Positive selection on the mitochondrial nadh dehydrogenase subunit 6 gene in Hares (*Lepus Spp.*)

Maša Janošev, Ines Đurđić, Milomir Stefanović[118]

KEYWORDS: adaptations; hares; mitochondrial DNA; selection

INTRODUCTION:

Mitochondria play a fundamental role in cellular energy production via the oxidative phosphorylation (OXPHOS) pathway. Previous studies on different mitochondrial OXPHOS genes indicated the adaptive effects of amino acid changes in the proteins that constitute the core subunits of the OXPHOS system, possibly as a consequence of adaptation to different environmental pressures. Hares (*Lepus spp.*) are considered a particularly suitable group to study the effects of natural selection on mitochondrial OXPHOS genes because hares represent a highly polymorphic group of closely related species that occur in a range of different habitats with varying environmental conditions.

OBJECTIVES:

The main objective of this study is to test for presence of natural selection shaping the genetic variability of the mitochondrially encoded gene of NADH dehydrogenase subunit 6 (*MT-ND6*) in hare species occurring in different habitats and to assess the functional implications of the observed amino acid changes in the encoded protein variants.

METHOD / DESIGN:

Publicly available nucleotide sequences of the *MT-ND6* gene from nine hare species were retrieved from the GenBank database. The presence of codon-based selection signals was tested by comparing the number of non-synonymous changes per non-synonymous site with the number of synonymous changes per synonymous site using maximum likelihood and Bayesian approaches (PAML, "Datamonkey Adaptive Evolution" web server, TreeSAAP). The potential functional impact of the revealed amino acid substitutions was evaluated by comparing them to known functional protein domains and by assessing the changes in the physicochemical properties of the amino acid's replacements.

118 University of Novi Sad, Faculty of Sciences. Corresponding author: milomir.stefanovic@dbe.uns.ac.rs

RESULTS:

Based on several selection tests, codons 102, 118, and 148 were shown to be under positive selection, as confirmed by at least one of the selection tests applied. Two of these three codons were predicted to be within the transmembrane domains, while one codon was shown to be within the loop region. TreeSAAP analysis showed that the amino acid change at the loop region altered the equilibrium constant (ionization of -COOH) to a moderately radical magnitude. However, no significant effect of the observed amino acid substitutions on the function of the *MT-ND6* proteins was detected. In addition, several codons were found to be subject to negative selection.

CONCLUSIONS:

Positive selection was observed at three codons in the MT-ND6 gene in hares, whereas no pronounced effect of amino acid changes in codons under selection on the structure and function of the encoded proteins was detected. Presence of codons evolving under the positive selection suggest that the evolution of MT-ND6 gene in hares may be shaped by adaptations, with different environmental pressures being the most likely triggers for these adaptations.

T1-P-44 The response and tolerance mechanisms of lettuce (*Lactuca Sativa L.*) exposed to increased zinc concentrations in aquatic cultures

Maja Radojević[119], Jelena Vulinović[119/120], Danijela Arsenov[120], Milan Župunski[120], Nenad Popov[121], Milica Živkov Balaš[121], Milan Borišev[120]

KEYWORDS: Zn; accumulation; antioxidant stress; tolerance

INTRODUCTION:

Zinc (Zn) is an essential microelement of plants. In plants, Zn is required at optimal concentrations, but in combination with heavy metals in the soil it can be a major problem in agricultural production. Under such conditions, Zn accumulates in excess in plant tissues, reaching toxic concentrations for plants and causing physiological changes in plants. Also, zinc has important functions in the cell, because it activates many enzymes, participates in proteosynthesis and metabolism of carbohydrates, lipids and nucleic acids.

OBJECTIVES:

The experiment, which is presented in this paper, is based on the treatment of lettuce *Lactuca sativa L.* with an increased concentration of Zn. The aim of the experiment was to analyze metabolic fingerprint of lettuce *Lactuca sativa L.* exposed to Zn stress in order to look into the tolerance mechanisms and phytoextraction potential of selected species.

METHOD / DESIGN:

Plants were grown by the method of static aqueous cultures with aeration, using Hoagland's 100% nutrient solution (pH = 5.8). The seeds of the plants were grown in hydroponic pots with rock wool as a substrate. After 40 days of plant growth on the nutrient solution, plants were separated in two 14 liter pots. For the next seven days, the control plants were grown on a pure nutrient solution, whereas half of the plants were exposed to twentyfold higher concentrations of zinc comparing to the control solution. After sampling, measurements of photosynthesis parameters, biochemical parameters, water regime,

119 Faculty of Technology, University of East Sarajevo, Zvornik, Republic of Srpska, Bosnia and Hercegovina. Corresponding author: radojevicmaja7@gmail.com

120 Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

121 Scientific Veterinary Institute "Novi Sad", Serbia

activity of antioxidant enzymes and Zn content were measured.

RESULTS:

The application of Zn treatment has led to a significant decrease in the intensity of photosynthesis, stomatal conduction, and water efficiency. Glutathione was slightly elevated, whereas proline was significantly increased. An increased parameter of lipid peroxidation indicates significant stress disturbed the cell membranes. Among the six measured antioxidant enzymes activities, treatment of zinc led to an increase in super-oxide dismutase and ascorbate peroxidase indicating to moderate activation of the antioxidant system. Zn treatment had a moderate effect on the activity of respiratory enzymes, but without statistical significance. The accumulation of Zn in treated plants was significantly higher (49.5 µg/g in leaves of the control and 114.3 µg/g in the treated plants), especially higher in the root than in lettuce leaves (378.8 µg/g in control, and 1175.2 µg/g in the treated plant roots).

CONCLUSIONS:

Based on the obtained results, it is concluded that the application of Zn treatment led to the initiation of tolerance mechanisms in lettuce, with some moderate biochemical distress, which did not result in morphological symptoms of toxicity. Thus in moderate Zn load, lettuce plants proved to be a good potential candidate for phytoextraction applications.

T1-P-45 Overview of bryophyta reserves Zasavica

Mihajlo Stanković[122]

KEY WORDS: bryophyta, Zasavica, terrestrial and aquatic species

INTRODUCTION:

The researched area in phytogeographical terms belongs to the Pannonian province (*Alno-Quercion roboris* Ht. (1937) 1938 and *Quercion pubescentis-petraeae* No.-Bl.1931) within the Central European-Balkan-Illyrian subregion of the Central European region with pronounced influences of the western Moesian province *Quercion frainetto* Ht.1954 and *Quercion petraeae-cerris* (Lakušić, 1976; Lakušić & Jovanović, 1980) of the Central European region, the Pannonian province of the Pontic-South Siberian region and to a lesser extent the Illyrian province of the Central European region (Stevanović et. al., 1999; Gajić, 1984). In the investigated area, mosses inhabit various terrestrial and aquatic habitats (tree bark, stumps, felled trees, land, concrete slabs, coastal parts of the main stream and surrounding ephemeral waters as well as marshes after water withdrawal). Zasavica is a wetland-peat complex which, since 1997, has been placed under protection as a Special Nature Reserve with a total area of over 3400 ha, where 331.64 ha of forest land is located.

OBJECTIVES:

The aim of this paper is to show the total moss diversity of the Zasavica reserve.

METHOD / DESIGN:

The methodology of the work implies the collection of data published so far on the bryophytes of the reserve, to which list the species whose findings have not been published so far have been added. The determination of a species whose findings have not yet been published has been noted by Özenoğlu H, Kirmaci M, Kĭremit FF (2019).

122 Pokret gorana Sremska Mitrovica, zasavica@zasavica.org.rs

RESULTS:

So far, a total of 49 species of mosses have been recorded in the area of SNR Zasavica, of which 46 species are terrestrial and 3 species are aquatic (one floating, submerged and semi-aquatic). The first detailed data on the bryoflora of the reserve are given by Grdović, S., Blaženčić, Ž. (2001) who list 43 terrestrial species, then Stanković,M. (2006) completes the list with two aquatic species of mosses (*Riccia fluitans* and *Ricciocarpus natans*). Fot the needs of the study aiming to elaborate expasnion of the Zasavica reserve, in the list of Perić, R. (2012), a new teerestrial species of moss (*Marchantia polymorpha*) was determined on the site. Dželatović, et al. (2019) conducted a radioactivity analyses expanding the list with two new species (*Anomodon viticulosus* and *Eurhynchium hians*), which are not on the list of mosses that Pantović,et.al. (2020) determined for Serbia. The semi-aquatic species *Riccia scorocarpa* is a new species for the list of bryophytes of Zasavica, found in 2019., in the swamp of the flood zone of the Valjevac pastures. According to Pantović, et. al. (2020), there are data for two of the three aquatic mosses for Northwestern Serbia, which includes the Zasavica reserve and for the species *Riccia fluitans* there are data from before 1990., for the species *Riccia scorocarpa* there are data after 1990., while the species *Ricciocarpus natans* is not mentioned by the same authors for this part of Serbia. Also for the same species in Serbia Sabovljević, M., Natcheva, R. (2006) states that there is data but without a precise location. A total of 49 species of mosses in the reserve is 11.47% of the total diversity of bryophytes in Serbia, because according to Sabovljević, et. al., (2004), 427 species of bryophytes were registered in Serbia. The Red List of Moss and Liverworts of Serbia and Montenegro with endangered status according to the IUCN criteria from 1994., lists 5 species, one (*Callicladium haldanianum*) in the category of endangered (EN), three (*Brachythecium oxycladum, Isopterygiopsis pulchella, Syntrichia papillosa*) in the category dependent on protection (LR), while one (*Hypnum fertile*) due to insufficient data required to assess the exact category of vulnerability does not have the specified category of vulnerability (DD).

CONCLUSIONS:

So far, research on the bryophytes of Zasavica has recorded a total of 49 species of mosses, which is 11.47% of the total diversity of bryophytes in Serbia. Of the endangered species according to the IUCN criteria, there are 5 species, one in the category of endangered (EN) and insufficient data (DD) and three in the category of dependent on protection (LR). The species *Ricciocarpus natans* is a new species for Northwestern Serbia and Mačva, while the species *Riccia scorocarpa* is new species for the Mačva region and this part of Posavina.

T1-P-46 Pinitol as DNA protector against hydroxyl and peroxyl radicals-induced DNA damage

Sanja Matić[123], Snežana Stanić[124]

KEYWORDS: Pinitol; DNA damage; hydroxyl radical; peroxyl radical

INTRODUCTION: Oxidative damage to DNA plays a crucial role in the progression and development of numerous diseases. Preventing DNA from oxidative damage is crucial for any living organism.

OBJECTIVES: The present study was aimed to investigate the *in vitro* DNA protective effect of pinitol, a dietary inositol present in *Ceratonia siliqua L.*, against hydroxyl and peroxyl radicals-induced oxidative DNA damage. Pinitol exhibits numerous

123 University of Kragujevac, Institute for Information Technologies Kragujevac, Department of Science, Jovana Cvijića bb, 34000 Kragujevac, Serbia.

Corresponding author: sanjamatic@kg.ac.rs

124 University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Radoja Domanovića 12, 34000 Kragujevac, Serbia

pharmacological properties such as anticancer, antidiabetic, antioxidant, hepatoprotective, immunosuppressor, antiosteoporosis, antiaging, preventive and ameliorative of Alzheimer's disease etc.

METHOD / DESIGN: The protective activity of pinitol at various concentrations (25, 50, 100, and 200 µg/mL) was assayed *in vitro* using DNA from herring sperm as a model system, $FeSO_4$ and H_2O_2 for the generation of hydroxyl radicals, (2-methylpropionamidine) dihydrochloride (AAPH) for oxidative DNA damage and assays for the detection of hydroxyl and peroxyl radicals-induced DNA damage.

RESULTS: Pinitol showed DNA-protective effect at all tested concentrations against hydroxyl radical in a dose-dependent manner. Concentration-response of pinitol indicated that protection against peroxyl radical induced DNA damage was more significant with the increase in pinitol concentration.

CONCLUSIONS: This investigation showed that pinitol can use as DNA protector against oxidative damage caused by hydroxyl and peroxyl radicals.

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T1-P-47 DNA protective potential of pinitol against ethyl methanesulfonate induced genotoxity in *Drosophila melanogaster*

Sanja Matić[125], Snežana Stanić[126]

KEYWORDS: Pinitol; SLRL assay; *in vivo*; genotoxicity; antigenotoxicity

INTRODUCTION: The search for non-genotoxic bioactive products from natural sources in the interest of prevention and treatment of various diseases is an important research line. Pinitol, a cyclic polyo, isolated from *Ceratonia siliqua L.*, has been suggested to exert a wide range of biological activities, i.e., antioxidant, antitumor, hepatoprotective, antibacterial, insulinomimetic, immunomodulator, and antiaging. Potentially beneficial effects of pinitol have been reported in treatments of osteoporosis and Alzheimer's disease. Although several studies have supported potential health benefits from pinitol, genotoxic and antigenotoxic effects is still unknown.

OBJECTIVES: The aim of the present study was to determine *in vivo* genotoxic effect of pinitol on ethyl methanesulfonate (EMS)-induced DNA damage in germ cells of *Drosophila melanogaster*. In order to identify compounds that might protect DNA from damage, the antigenotoxic effects of pinitol against DNA damage induced with EMS were evaluated in *D. melanogaster* males using the sex-linked recessive lethal (SLRL) test.

METHOD/DESIGN: To assess the genotoxic effect three days old Canton S males were treated with pinitol in concentration

125 University of Kragujevac, Institute for Information Technologies Kragujevac, Department of Science, Jovana Cvijića bb, 34000 Kragujevac, Serbia. Corresponding author: sanjamatic@kg.ac.rs

126 University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Radoja Domanovića 12, 34000 Kragujevac, Serbia

of 100 ppm. In order to detect protective activity against DNA damage, D. melanogaster males were exposed to EMS in concentration of 0.75 ppm, 24 h prior to pinitol in the concentration of 100 ppm. The standard procedure for the detection of sex linked recessive lethal mutations on *D. melanogaster* was applied.

RESULTS: EMC induced a statistically significant sex linked recessive lethal mutations in all three broods at a dose of 0.75 ppm. The treatment with pinitol in concentration of 100 ppm reduced the frequency of sex linked recessive lethal mutations in comparison with the negative control value. Compared with the sucrose, as the negative control, pinitol decreased ($p > 0.05$) the genotoxicity of EMS in postmeiotic germinative cell line – at spermatozoids and spermatids, and in premeiotic line – spermatocytes. The frequency of germinative mutations induced by EMS decreased with high significance ($p < 0.001$***) after post-treatments with pinitol.

CONCLUSIONS: The results indicated that pinitol exhibited a DNA protective potential against EMS and also it did not induce the genotoxic effect alone in tested concentration in *D. melanogaster* males using the sex-linked recessive lethal test.

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T1-P-48 Improvement of kombucha fermentation using Box Behnken experimental design

Anja Saveljić, Dragoljub Cvetković, Aleksandra Ranitović, Olja Šovljanski, Ana Tomić, Siniša Markov[127]

KEYWORDS: kombucha; Box Behnken; fermentation; experimental design;

INTRODUCTION:

High beverage consumption worldwide has opened the opportunity for the improvement of different traditional drinks as part of the functional food concept. In recent years, the development of kombucha fermentation is a part of the scientific and industrial focus in the field of functional drinks. Kombucha optimization has become very important because of the large importance of the definition of chemical composition from health-improving, economic, and industrial points of view..

OBJECTIVES:

In this study, the influence of the specific surface area of the vessel, inoculum size, initial tea concentration, and fermentation time on the efficiency of kombucha fermentation was examined. The focus of this study is the optimization and standardization of kombucha fermentation conditions using Box- Behnken experimental design.

METHOD / DESIGN:

Fermentation was performed using the local tea fungus culture with at least five yeast strains (*Saccharomycodes ludwigii, S. cerevisiae, S. bisporus, Torulopsis sp., and Zygosaccharomyces sp.*) and two bacterial strains of *Acetobacter genera*. Three operating parameters of kombucha fermentation (specific surface area of the vessel (0.06, 0.15, and 0.3 cm^{-1}), inoculum (2.5, 5, or 10% (*v/v*)), and initial tea concentration (0.15, 0.3, or 0.45% (*w/v*)) were independent factors in the selected Box-Behnken experimental design, while the output variables were the pH values and the titratable acidity of kombucha.

127 University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, Novi Sad, Serbia. Corresponding author: anjasaveljicc@gmail.com

RESULTS:

Examination of kombucha fermentation was performed to determine the influence of the selected operating parameters on the efficiency of kombucha fermentation. The very important parameter was also the final fermentation time, which was observed through archived values of experiment outputs. Observing fermentation processes through Box-Behnken experimental design, a higher concentration of total acids was recorded in the cultivation medium with a larger inoculum size, and the time required to obtain the beverage of optimal acidity was shorter. It can be observed that minimal initial tea concentration (0.15%) provided sufficient nitrogen compounds and mineral elements that are necessary for kombucha fermentation under stationary fermentation conditions. Based on unattainable adequate values of titratable acidity and pH value that despite the high C and N source contents, the fermentation process with high tea concentration can be slower and therefore economically less acceptable.

CONCLUSIONS:

Investigation of Kombucha fermentation optimization was performed with the aim to determine the possibility of optimizing the kombucha fermentation based on the pH value and titratable acidity using three input variables: specific surface area of the vessel, inoculum size, initial concentration of tea, as well as fermentation time as output which values defined achieving optimal acidity in the system. In summary, Box Behnken experimental design was applied to establish the optimum kombucha fermentation process which is very important in a view that the scale-up process from a laboratory scale to a commercial product is very challenging and may influence the quality of fermentation.

T1-P-49 Do the shape and size of the Forcipular Apparatus significantly differ between sexes in centipede *Lithobius Melanops* Newport, 1845 (Chilopoda: Lithobiomorpha: Lithobiidae)?

Vukica Vujić, Sofija Pavković-Lučić, Bojan Ilić, Zvezdana Jovanović, Jelena Milovanović, Boris Dudić, Dalibor Stojanović[128]

KEYWORDS: Myriapoda; centipedes; centroid size; landmarks; sexual dimorphism

INTRODUCTION: Sexual dimorphism, as one of the most investigated phenomena in evolutionary biology, has been widely analysed in arthropods. The investigations of size and shape sexual dimorphism in morphological structures which have significant impact on adaptive value are especially important. Bearing that in mind, the forcipular apparatus, as one of the apomorphic and diagnostic features in the class Chilopoda (centipedes), with a crucial role in survival (i.e. feeding and defence), is suitable as the morphological trait in studying sexual dimorphism. However, in centipedes, size and shape sexual dimorphism in morphological structures has been poorly investigated using geometric morphometrics.

OBJECTIVES: The aim of this study was to research size and shape sexual dimorphism in the parts of the forcipular segment (forcipular coxosternite + forcipules) in widespread European centipede *Lithobius melanops* Newport, 1845, by using aforementioned approach.

METHOD/DESIGN: Analysed sample, which contains adults of each sex (both maturus junior stage and maturus stage), was collected from the locality Dobanovci, near Belgrade, Serbia. In statistical data processing, several different programs were used: TpsDig (to digitize 30 landmarks on each morphological structure), CoordGen (to calculate centroid size - CS), MorphoJ (to perform Principal component analyses and Canonical variate analyses for both symmetric and asymmetric components),

128 University of Belgrade, Faculty of Biology, Studentski Trg 16, 11000 Belgrade, Serbia. Corresponding author: vukica.vujic@bio.bg.ac.rs

and R program (to test the presence of size sexual dimorphism in the forcipular apparatus).

RESULTS: Results of this study indicate that neither size nor shape sexual dimorphism of forcipular apparatus is present in the studied population of *L. melanops*. Namely, ANOVA indicated no size sexual dimorphism ($F_{1,44} = 2.05$; $p = 0.16$), while Canonical variate analyses on both symmetric and asymmetric components showed no shape sexual dimorphism in this morphological structure in analysed species.

CONCLUSIONS: The fact that significant differences in size and shape of the forcipular apparatus between sexes were not observed indicates that this morphological structure might be conserved during the evolution of these myriapods. Conservation of traits, which are crucial for feeding and defense, provides these arthropods higher adaptive value, i.e. increases their chance to survive in changeable environmental conditions.

T1-P-50 Growth trade-off of fast-growing species grown in Cd perturbed environment

Milan Župunski, Danijela Arsenov*, Milan Borišev, Nataša Nikolić, Slobodanka Pajević[129]

KEYWORDS: biomass-partitioning; meta-analysis; willow; poplar; black-locust

INTRODUCTION:
Rhizosphere nutrient composition is essential for plant growth and survival. It is not uncommon that the rhizosphere contains higher amounts of non-essential elements, which might impair plants' physiology and lead to severe structural and functional alterations.

OBJECTIVES:
Even though a plethora of research has been done, we still do not truly understand which strategies of biomass allocation do fast-growing species acquire to overcome the disturbances in the rhizosphere.

METHOD / DESIGN:
We explored the biomass allocation patterns of 18 fast-growing genotypes under both favorable and Cd-perturbed soil conditions (fluvisol type). Even though these genotypes are distinguished as fast-growing, the intra and interspecific differences between tested plants were evident and showed that under unfavorable conditions they do choose different response strategies.

RESULTS:
We showed that fast-growers overall tend to strengthen their roots towards the Cd-triggered perturbances in the rhizosphere and allocate more biomass to that particular plant organ. Intraspecies analyses pointed to differences in resource use efficiency and acquisition strategy based on specific leaf area, targeting genotypes PE19/66 and PD3 (both *Populus deltoides*), and *Salix alba* B44 as strong fast-growing oriented genotypes. Others exhibited more or less the conservative resource use and acquisition strategy under-explored cadmium pollution.

CONCLUSIONS:
To summarize, our study highlights the intra and interspecies specificity of fast-growing species to Cd occurrence in the rhizosphere. Association of growth traits and Cd-related traits tested with structural equation model highlighted the shoots bioconcentration index as a proxy-trait which directly interplay with the functional traits performance and modify the biomass shift.

*129 University of Novi Sad, Faculty of Sciences, Trg Dositeja Obradovića 3, Novi Sad, Serbia. Corresponding author: milan.zupunski@dbe.uns.ac.rs with *Danijela Arsenov*

T2-IL-1 *In silico* safety assessment of probiotics for human use using genomics and bioinformatics analysis approach

Komwit Surachat[1]

KEYWORDS: *in silico* safety assessment; probiotics; genomics; bioinformatics; bacteria

INTRODUCTION: Probiotics are live microorganisms that provide health benefits when consumed by the body. They are used widely in commercial products including fermented foods, yogurts, and dietary supplements. The safety assessment of probiotics for use in commercial products is very important for food industries and public health issues since they can pass transferable antibiotic resistance genes and other mobile genetic elements directly to humans.

OBJECTIVES: Therefore, *in silico* safety assessment of probiotics for human use should be performed to identify related genetic traits that might affect host health. Also, it can provide deep genetic information insight into a bacterial strain to increase the value of the product.

METHOD / DESIGN: Whole-genome sequencing and *in silico* analysis of genomic data using next-generation sequencing technology and comprehensive bioinformatics tools are popular approaches nowadays. They can be performed with many bacterial strains simultaneously and identified species, virulence factors, transferable antibiotic resistance elements, and antimicrobial encoding genes information with online tools and databases.

RESULTS: All related genomic information can be extracted from the analysis including general genome characteristics, all encoding genes, and functional annotation, pathogenic information.

CONCLUSIONS: The obtained information from *in silico* analysis can be used as a preliminary screening and guideline for the safety assessment of probiotics using the genomics and bioinformatics analysis approach.

T2-IL-2 Triticale in beer production

Jelena Pejin[2], Milana Pribić[2], Saša Despotović[3], Sunčica Kocić-Tanasković[2]

KEYWORDS: beer, brewing, triticale; adjuncts; wort; brewing

INTRODUCTION:

Beer is one of the oldest known beverage in the world and is still a staple low-alcohol product. Malted barley is the favored cereal grain used in traditional brewing process. Barley is modified during the malting process to ensure biochemical changes occur within the grain required in the wort production. Malting is an energy intensive process and brewing with a proportion of unmalted adjuncts has become an attratcive option for cost and carbon footprint reduction. Adjuncts like corn, wheat, barley, and triticale are utilized by brewers to increase extract yield, to modify beer quality (flovour, foam, colloidal

1 *Molecular Evolution and Computational Biology Research Unit, Faculty of Science, Prince of Songkla University. Corresponding author: komwit.s@psu.ac.th*
Division of Computational Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand

2 *University of Novi Sad, Faculty of Technology, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia*

3 *University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia*

stability) and to enable production of the innovative products. The inclusion of even a little unmalted raw material in the grist can alter the sensory properties of beer. Thus, it is possible to obtain a product of new flavour and aroma without having to change the production process. Barley and corn are the most commonly used adjuncts in the decrease in amylolytic, cylolytic, and proteolytic enzymatic activity in the grist, as these enzyme systems are synthesized during the malting process. But, the first man-made cereal - triticale is an exception. Triticale is a hybrid between wheat and rye and shows a number of advantages for the grower. It shows promising brewing properties because of the high levels of amylolytic and proteolytic enzymes activity even in the unmalted form. In combination with the low temperature regimes similar to those used for barley malt. The usage of triticale in brewing could give viscosous mash, because of the solubilisation of triticale arabinoxylans, which could lead to a slower beer filtration.

OBJECTIVES:

The objective of this study was to evaluate the possibility of triticale application as a partial substitute for barley malt in beer production. Triticale variety NS Paun was used in a different proportions in wort production (10, 30, and 50%) with or without addition of commercial enzyme for wort viscosity reduction - Shearzyme.

RESULTS:

With an increase in triticale content in the grist, viscosity increased, which was corrected with the addition of commercial enzyme Shearzyme. The highest value viscosity was obtained in wort produced with 50% of triticale content in the grist without enzyme addition (1.640 mPa·s) which was reduces to 1.446 mPa·s when enzyme was added during mashing process. The content of the soluble nitrogen in obtained beers was lower in relation to the content of this parameter in the boiled worts, which indicates the fact that the yeast metaolized it. The lowest content of soluble nitrogen was determined in the beer produced with 50% of triticale in the grist without enzyme addition (462 mg/L). The highest ethanol content was obtained in the beer with the 50% of triticale content in the grist (2.95%), without enzyme addition. Produced beers with enzyme addition, showed reduced color (3.5 EBC units) in comparison with beers produced without Shearzyme addition (5.0 EBC units).

CONCLUSIONS:

Extract contents of obtained worts and boiled worts decreased with an increase in triticale content in the grist. Enzyme addition decreased wort viscosity, especially when higher triticale ratios in mash were used. Replacement of the barley malt with native triticale did not have a negative impact on beer fermentation, even at the highest triticale content in the grist (50%). 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.

T2-IL-3 From butterfly diversity to peptide drug discovery

Patamarerk Engsontia[4]

KEYWORDS: caterpillar toxin; neurotoxins; antimicrobial peptides; next-generation sequencing; novel peptide drugs

INTRODUCTION:

Biodiversity is a valuable resource for discovering new drugs that can save millions of lives from diseases, such as cancers, stroke, diabetes, and cardiovascular disease. Bioactive peptides in animal venoms, particularly from 5s animals (snake, scor-

4 *Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla Thailand, 90110.*

Corresponding author: patamarerk.e@psu.ac.th

pion spider, cone snail, and sea anemone), have long been a subject of interest. Some toxins have been intensively investigated and developed into approved drugs. Toxins from caterpillars have been overlooked until recently. At least 70 species of caterpillars from 15 family taxa are venomous, which cause varying effects from mild iteration to death due to hemorrhagic syndrome. In 2021, three new papers used next-generation sequencing and proteomic approach to identify toxin peptide components from three caterpillar species (*Parasa lepida, Doratifera vulnerans*, and *Premolis semirufa*). The growing knowledge can help elucidate the hidden diversity of peptides with pharmacologic properties from the caterpillar venoms.

OBJECTIVES:

This study aims to identify toxin peptides from the stinging nettle caterpillar, *Parasa consocia*, and compare its toxin component with other caterpillar species from the same family taxa (*Limacodidae*) to identify their common characteristics.

METHOD / DESIGN:

The RNA-seq data from stinging hairs of *Parasa consocia* was downloaded from the NCBI database and assembled into a transcriptome using tools in the UseGalaxy cloud platform. Its toxin genes were identified following a previously published annotation pipeline. Orthologous relationships of toxin genes from different caterpillar species were analyzed using OrthoVenn2. Phylogenetic relationships of genes were analyzed using the maximum likelihood method.

RESULTS:

A total of 142 candidate toxin genes from *P. consocia* were identified. This includes proteolytic enzymes (serine protease; peptidase; metalloproteinase), peptidase inhibitors (serpin, kazal-type inhibitor, trypsin inhibitor-like), and allergens (carboxylesterase, CAP superfamily, acid phosphatase, antimicrobial peptide, and phospholipase A2). Comparing these results with *P. lepida* and *Doratifera vulnerans* (Family Limacodidae) and *Premolis semirufa* (Family Erebidae) suggest that the common components in Limacodids venoms are antimicrobial peptides (cecropin-like), knottin-like peptides (predicted structure similar to spider neurotoxins), and allergens commonly found in bee venoms (carboxylesterase-6 and venom acid phosphatase).

CONCLUSIONS:

Caterpillar venoms contain diverse peptides with potential pharmaceutical properties. Some interesting candidate drugs from Limacodids include cecropin-like antimicrobial peptides and knottin-like neurotoxins, which could inhibit pain receptors. Future functional analyses are essential to validate their antimicrobial properties or their use for treating chronic pain.

T2-P-1 Effect of climatic variables and sowing date on winter rapeseed (*Brassica Napus L.*) development and yield

Ana Marjanović-Jeromela[5], Sreten Terzić[5], Mirjana Jankulovska[6], Miroslav Zorić[5], Ankica Kondić-Špika[5], Nevena Nagl[5]

KEYWORDS: climate change; cultivar x environment interaction; growth stage; winter rapeseed; seed yield.

INTRODUCTION:

Climate change differentially affects crops, since the effects are caused by combination of changes in growing conditions and the timing of phenological phases. Winter rapeseed is vulnerable to local climatic conditions because of its lengthy growth period and overwintering ability. The information on the cultivar x environment interaction provides valuable data to plant breeders and agronomists for the identification of superior cultivars in specific environments, and defines site-specific best management practices. A further step in the cultivar (C) and year (Y) interaction analysis (C x Y) for rapeseed, would be to investigate the effect of specific climatic variables throughout developmental stages. Such data could be used to dissect the year effect and determine which variables are the most significant for an optimal plant development at each growth stage. The effect of climatic variables on the winter rapeseed developmental stages and yield in Southeast Europe has not yet been analysed simultaneously, although their interaction is important to breeders and growers.

OBJECTIVES:

The aim of the study was to understand year-related interactions and the effect of climatic variables in different growth stages on seed yield and oil content.

METHOD / DESIGN:

Sources of variability for the seed yield and oil content of four rapeseed cultivars were evaluated, during the four growing seasons, under the influence of three sowing dates. Six climatic factors: the temperature (minimum on 5 cm above ground; minimum; maximum; and mean), total precipitation, and relative air humidity, were observed during the germination, over-wintering, budding, flowering and ripening.

RESULTS:

A Wald F test showed a highly significant effect of C × Y for both, the seed yield and oil content. The treatment × year (T × Y) interaction was significant for the oil content. A set of individual factorial regression models was developed in order to test the hypothesis about the effect of climatic variables on C x Y and T x Y interactions. Out of thirty available climatic variables, nineteen had a highly significant effect on the C x Y interaction for the oil content and six variables had a significant effect. The largest proportion of the explained interaction variance was obtained for precipitation at the budding stage (60.3%), the maximum temperature at overwintering (60.2%), and the relative air humidity at flowering (59.0%). As a consequence of the decreased level of significance of the T x Y interaction for the oil content, only three climatic variables were found to be important. A highly significant effect was observed only for precipitation at overwintering (81.4%), whereas the effect of the relative air humidity at the budding stage (76.4%) and precipitation at the germination stage (61.1%) accounted for a significant proportion of the T x Y interaction.

CONCLUSIONS:

The study successfully dissected the effect of year-related climatic variables on the agronomical traits in winter rapeseed.

5 *Institute of Field and Vegetable Crops, Novi Sad, Serbia. Corresponding author: ana.jeromelal@ifvcns.ns.ac.rs*

6 *Department of Genetics and Plant Breeding, Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University, Skopje, North Macedonia*

T2-P-2 Utilization of organicsoil obtained from food waste recycling system for growing salad vegetable in pot plant system

Aunkamol Kumngen[7], Usmana Meehae[7], Pattamavadee Kunwandee[8], Siwapong Leunram[9] and Jutarut Iewkittayakorn[7*]

KEYWORDS: food waste; green oak lettuce; organic soil; compost; pot plant system

INTRODUCTION:

Thailand generated average amount of 27.93 million tons of municipal waste per year, which is 18 million tons of food waste. Landfill is the method to manage this waste. However, this method not only uses a large area but also has affected on environment problems. The easy way to get rid of this waste is to make compost. BIOAXEL Co., Ltd., Surat Thani, Thailand has developed a food waste recycling system to change the food waste to compost or BA organic soil (BA) in commercial name. After checking the BA organic soil quality, it was found to be within acceptable limits stipulated by the Thai Agriculture Standard for compost (TAS 9503 2005). However, utilization of BA organic soil has been lacking of information. Thus, this work aimed to study the utilization of BA organic soil for growing salad vegetable in pot plant system.

OBJECTIVES:

The aim of this study was to utilize BA organic soil for growing salad vegetable in pot plant system. The growth parameters were investigated including root length, plant length, number of leaves, thickness, width of bush, fresh plant weight, dry plant weight plant.

METHOD / DESIGN:

BA organic soils were obtained from BIOAXEL Co., Ltd., Koh Sa-mui, Surat Thani, Thailand. For ensuring confidentiality, this compost was identified quality according to Thai Agriculture Standards for compost (TAS 9503, 2005) before further studying. Thai Agriculture Standards for compost included of temperature, pH, electrical conductivity, organic matter, total nitrogen, total phosphorus, total potassium, and carbon nitrogen (C:N) ratio. Planting materials including soil, coconut coil and BA compost were applied to samples of the planting soil. BA organic soils were mixed with soil and coconut dust in different ratios (0% - 70% by volume) in order to find suitable plant soil formula that gave the highest plant growth parameters. The green oak lettuce plants were grown under field conditions at the Faculty of Natural Resources, Prince of Songkla University, Songkhla, Thailand from January to April 2020. The lettuce plants were planted and watered regularly for 45 days before harvesting to measure plant growth parameters including root length, plant length, number of leaves, thickness, width of bush, fresh plant weight, dry plant weight.

RESULTS:

The results showed that BA organic soil quality was found to be within acceptable limits stipulated by the Thai Agriculture Standard for compost (TAS 9503 2005). After adding BA more than 30%, the plants did not grow well. The best ratio of planting materials was found to be 10% v/v BA adding, giving the highest root length (7.00±1.00 cm), plant length (30±1.00 cm), number of leaves (14±2.00 number of leaves per plant), thickness (11.66±0.67 mm), width of bush (28±2.00 cm), fresh plant weight (66.03±2.56 g), and dry plant weight (3.19±0.17 g). The growth parameters of planting material without BA adding

7 *Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.*

** Corresponding author: jutarut.p@psu.ac.th*

8 *Agricultural Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand*

9 *BIOAXEL Co., Ltd. 6/9 Moo 3 Bophut Chaweng Beach Road, Koh Sa-mui, Surat Thani 84320, Thailand*

gave 5±1.00 cm of root length, 6.00±1.32 cm of plant length, 7.67±1.53 number of leaves per plant, 3.36±0.61 mm of thickness, 7.00±1.00 cm of width of bush, 2.01±0.68 g of fresh plant weight and 0.32±0.05 g dry plant weight.

CONCLUSIONS:

Physicochemical characteristics and the nutrient contents of BA organic soils obtained from BIOAXEL Co., Ltd., Koh Sa-mui, Surat Thani, were found to be within acceptable limits stipulated by the Thai Agriculture standards for compost (TAS 9503 2005). BA organic soil utilization were observed and measured in the experimental planting of green oak lettuce using this BA organic soils. The use of this BA organic soils with 10%v/v adding in soil and coconut gave the best outcome. Thus, BA organic oil could be used as compost for planting.

T2-P-3 Comparison of physico-chemical characteristics and content of lipid oxidation products in cheese analogues based on palm and coconut oil

Mirela Iličić, Jovana Degenek, Ranko Romanić, Katarina Kanurić, Vladimir Vukić, Dajana Vukić[10]

KEYWORDS: cheese analogues, fat content, lipid oxidation, palm oil, coconut oil

INTRODUCTION:

Increased consumption of saturated fats, most prevalent in milk, has been shown to be associated with an increased risk of obesity, atherosclerosis, coronary heart disease, elevated blood pressure and tissue injury diseases. One of the possibilities related to the improvement of fatty acid composition in dairy products such as cheese is certainly the partial or complete replacement of milk fat with vegetable oils and fats rich in ω-6 and ω-3 fatty acids. In order to prevent mentioned health problems, but also to provide novelties on the market of increasingly popular functional food, production of reduced-fat and low-fat cheeses is in constant growth. Vegetable oils are a source of other important bioactive substances for the human body, such as liposoluble vitamins, tocopherols, phytosterols, lecithin, pigments and other unsaponifiable matter in small quantities. Although a wide range of vegetable oils has been used worldwide in this purpose, the most common cheese analogues present at the market in our country are those which contain palm and coconut oil as a fatty phase.

OBJECTIVES:

Comparative analysis of cheese analogues based on different oils is not much represented in the literature. Therefore, this study aims to determine the differences in physico-chemical characteristics (pH value, a_w value, fat content, total proteins content, ash content and lactose content) and in the content of conjugated dienes and trienes, as products obtained from primary and secondary lipid oxidation processes, in cheese analogues based on palm and coconut oil.

METHOD / DESIGN:

Cheese analogues based on palm and coconut oil were purchased from the market. Chemical analysis was performed by employing standard methods: fat content according to Van Gulik (ISO 3343:2008 [IDF 222:2008]), total proteins content (ISO 8968-1:2014 [IDF 20-1:2014]), dry matter content after drying at 105° C (ISO 5534:2004 [IDF 4:2004]), and ash content after mineralization at 550 °C (ISO 5545:2008 [IDF 90:2008]). The lactose content was calculated by subtracing the sum of the total protein, fat and ash contents from the dry matter content. Value of pH was determined by use of a pH meter (pH Spear,

10 *University of Novi Sad, Faculty of Technology Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia. Corresponding author: dajanavukic@uns.ac.rs*

Eutech Instruments, Oakton, England), and a_w value was measured by use of LabSwift aw device (Novasina AG, Switzerland). Content of conjugated dienes and trienes was determined spectrofotometrically by measuring absorbances of 1% sample solutions in n-hexane at 232 nm and 270 nm, according to Dimić i Turkulov, 2000.

RESULTS:

The obtained results showed that cheese analogues based on palm and coconut oil differ from each other in pH values (6.03 and 3.74, respectively). In contrast, no significant differences between a_w values of these samples were observed. Cheese analogue with coconut oil in its fatty phase had higher fat content in comparison with palm oil-based cheese analogue. As for the total proteins content, their presence in coconut oil-based cheese analogue was not confirmed, while the palm oil-based cheese analogue contained 1.53%. The dry matter, ash and lactose contents determined in the palm-oil cheese analogue were higher than in the cheese analogue with coconut oil in fatty phase. When it comes to the content of conjugated dienes determined in the cheese analogues samples, there is an insignificant difference between them. However, the palm oil-based cheese analogue showed a significantly higher content of conjugated trienes (0.51) compared to the coconut oil-based analogue (0.05).

CONCLUSIONS:

By comparing the chemical characteristics of cheese analogues based on palm and coconut oil, it can be concluded that there are differences between them in terms of pH value, fat, total protein, dry matter and lactose contents, which is closely related to the differences in their formulations and the oils used as a fatty phase. The increased content of conjugated dienes and trienes in the palm oil-based cheese analogue indicates a higher concentration of primary and secondary lipid oxidation products, as a consequence of higher content of unsaturated fatty acids.

T2-P-4 Proteolysis and *In vitro* digestion of kombuha fresh cheese

Mirela Iličić, Dajana Vukić, Katarina Kanurić, Vladimir Vukić, Ljiljana Popović, Maja Bjekić, Jovana Degenek[11]

KEYWORDS: kombucha; fresh cheese; proteolysis, digestion

INTRODUCTION:

Fresh (acid coagulated) cheeses belong to a group of soft unriped cheeses. Produced from the enzymatic coagulation of milk with rennet and starter cultures, these cheeses exhibit a soft texture, a slightly acidic flavour, a high moisture and low price. Recent studies have intensively investigated the possibility of kombucha application as non-conventional starter culture in dairy technology. Milk proteins are the important source of bioactive peptides, which can be released by hydrolysis (e.g. pepsin, trypsin and chymotrypsin) during gastrointestinal digestion or by enzymatic activity of applied starter culture.

OBJECTIVES: Up to now, there is no literature data about proteolysis of fresh cheese obtained by kombucha inoculum. Therefore, the main objective of this research was investigation of effect of non-conventional starter culture (kombucha inoculum) and traditional starter culture (XPL-1) on proteolysis and in vitro gastrointestinal digestion (GI) of produced fresh cheeses.

METHOD / DESIGN:

Two types of fresh cheese with different starter culture were produced in laboratory conditions: 1. XFC - cheese with XPL-1 culture (a concentration of 0.02 g/L) and 2. KFC - cheese with kombucha inoculum (a concentration of 100 mL/L). Traditional starter culture and kombucha inoculum were added to milk heated to 35°C, a coagulation enzyme was added after 30 min-

11 *University of Novi Sad, Faculty of Technology Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia. Dr Dajana Vukic: dajanavukic@uns.ac.rs*

utes. Coagulation at 35°C lasted until a pH of ~ 4.5-4.6 was reached, the coagulum was cut, pasteurized by gently stirring at 60°C for 5 min, quickly cooled to 25°C and drained. Determination of protein concentration was performed according the modified spectrophotometric methods by Lowry et al. (1951). The bovine serum albumin as the standard protein (0.5 mg / mL) was used for calibration. The degree of hydrolyses (DH) was calculated as the ratio of 0.22 mol/dm3 trichloroacetic acid soluble proteins to total proteins in the hydrolysate and expressed in percentage. *In vitro* gastrointestinal digestion was performed in a laboratory glass beaker using a combination of two enzymes: pepsin and pancreatin. All analysis were measured in three phases: 1. before the GI digestion (first phase); 2. after the digestion by pepsin (second phase) and 3. after the digestion by pancreatin (third phase).

RESULTS:

The concentration of soluble proteins increased during *in vitro* GI digestion for both fresh cheese samples. In both samples, low content of soluble proteins was obtained before addition of enzymes (the first phase) and it was 9.91 mg/g for kombuha and 12 mg/g for traditional fresh cheese. In the second phase, after GI digestion with pepsin, content of soluble proteins ranged from 39.5 (XFC) to 105.25 mg/g (KFC). The highest increase of soluble proteins was observed after the third phase (digestion by pancreatin) in both samples. The content of soluble proteins after duodenal GI digestion (after pepsin and pancreatin addition) was significantly higher in the kombucha fresh cheese than in the cheese obtained by traditional starter culture. After the third phase, the content of soluble proteins in kombucha fresh cheese, was 100% higher in relation to the solubility of proteins in the XFC sample. The initial value of the degree of proteolysis in both cheese samples was less than 5% and was slightly higher in the XFC sample. Deegre of hydrolysis after pepsin digestion was higer in KFC sample (30 %). Maximum proteolysis was achieved in the third phase of GI digestion and it was 53.60% in KFC sample.

CONCLUSIONS:

With respect to the obtained results the use of kombucha inoculum as a starter culture for cheese production resulted with high deegre of hydrolisis and the content of soluble proteins compared to the sample with traditional starter culture in all phases of GI digestion. The hydrolysates of prepared kombucha fresh cheeses showed high digestibility and therefore, these products could be a rich sources of bioactive peptides.

T2-P-5 Medicinal and aromatic herbs as functional ingredients for specialty beverages

Saša Despotović[12], Sofija Djordjević[13], Dragana Paunović[12], Sonja Veljović[14], Jovana Marković[12], Aleksandra Martinović[15], Viktor Nedović[12]

KEYWORDS: medicinal herbs; aromatic herbs; ingredients; beverages.

INTRODUCTION:

The quest for foods that have a health-promoting impact began many years ago as a functional food. Nowadays, the varieties of food products and food ingredients are more about how they impact the health and well-being of consumers. Throughout history, herbs have been used to add taste and/or preservation to foods. The creative use of herbs can make food much

12 *Department of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Serbia. Corresponding author: sdespot@agrif.bg.ac.rs*

13 *Institute for Medicinal Plants Research "Dr. Josif Pančić", Belgrade, Serbia*

14 *Institute of General and Physical Chemistry, University of Belgrade, Serbia*

15 *Faculty of Food Technology, University of Donja Gorica, Montenegro*

more enjoyable, and not less healthy. Various herbal infusions can be added to beverage compositions to increase nutritional qualities and health benefits while maintaining a sensory and pleasant balance throughout the fortification process.

OBJECTIVES:

The primary objective of the study was to develop and manufacture specialty drinks made from fruit juices and extracts of medicinal and aromatic plants that had a high concentration of biologically active chemicals and a high antioxidant activity. Three types of soft drinks have been developed: those with potentially targeted physiologically beneficial effects on metabolism, cardiovascular system, and body resistance, as well as those with medicinal and aromatic herbs whose positive healing effects have already been documented in previous research.

METHOD / DESIGN:

Fruit juices were made by mechanically processing mature fruits, that have not been fermented and have been preserved only via physical methods. The plant material was dried in ambient conditions and ground shortly before extraction. A single percolation method was used to create liquid plant extracts. Extracts of medicinal and aromatic herbs were mixed in combinations with specific functional characteristics sensory acceptable and compatible with fruit blends. Total flavonoid content, polyphenols, and antioxidant capacity were determined.

RESULTS:

Plant extracts and fruit juices were first classified in terms of total phenols, and their antioxidant activity was assessed using the FRAP and DPPH tests. The total antioxidant activity determined by the FRAP assay and the antioxidant activity determined by the DPPH test were correlated with the total phenol content. The number of phenolic compounds in tested herbal extracts and fruit juices differs significantly at the level of statistical significance of p 0.05. Given that antioxidant activity is directly proportional to phenolic component concentration, the FRAP and DPPH test both demonstrated statistically significant antioxidant activity.

CONCLUSIONS:

For the production of specialty beverages with targeted effects on accelerating metabolism, protection of the cardiovascular system, and strengthening the body's resistance. While it is evident that a wide range of medicinal and aromatic plants may be utilized to improve the functional and sensory characteristics of beverages, the results are often not favorable. Blending various medicinal and aromatic herbs can provide a remedy, specialty beverage. The most essential issue is to select the best plant composition so that maximum functional characteristics may be balanced with pleasant sensory features.

T2-P-6-ORAL Suppression of ring artifacts in reconstructed holographic images using graph signal processing

Dimitrije Stefanović, Marko Panić, Vladimir Crnojević[16],
Nikša Jakovljević[17]

KEYWORDS: ring artifacts; digital holography; graph signal processing; Taubin filter; optimal graph filter

INTRODUCTION:

Monitoring of pollen concentration is an important task that can help people suffering from pollen allergies. Currently, the most reliable methods for determining concentration and classification of pollen particles require a lot of time and labor. In recent years new devices based on reconstructed holographic images of pollen particles for pollen classification and automatic concentration monitoring have been introduced. Although it is possible to obtain high quality particle images, the acquisition process also introduces various artifacts such as ring artifacts which leads to wrong estimation of the final pollen concentration.

OBJECTIVES:

The aim of this research was to find a method to remove ring artifacts from pollen holographic image reconstructions while preserving important features of particles such as shape and size.

METHOD / DESIGN:

For removing ring artifacts, two methods based on graph signal processing[18] framework were proposed: Taubin smoothing filter (TSF) and optimal graph filter (OGF). Both proposed methods represent translation of basic filter design concepts from classical digital signal processing into a graph domain. The reconstructed holographic images of pollen are modeled as a graph with a 2D lattice structure, where each vertex represents a pixel, and edges only exist between adjacent pixels. While TSF can be treated as a finite impulse response (FIR) filter which is iteratively applied to obtain better denoising results, OGF tends to achieve the same results through only one iteration and it can be considered as an infinite impulse response (IIR) filter.

RESULTS:

We evaluated the proposed methods by using visual inspection of filtered images and regions of interest (ROI) containing only pollen particles. As there are no reference images of pollen particles without artifacts, for objective evaluation it is necessary to use non-reference metric for image quality such as total variation (TV) metric. This metric is often used as a loss-function in the optimization process for image denoising. There are several other ring artifacts removal methods[19] in literature addressing similar problems, but artifacts that are of interest in these cases manifest mainly as ideal circles, while ring artifacts in our data depend on the shape of pollen particles. As a reference method we used Chambolle-Pock (CP) algo-

16 *BioSense Institute, University of Novi Sad. Corresponding author: dimitrije.stefanovic@biosense.rs*

17 *Faculty of Technical Sciences, University of Novi Sad*

18 *L. Stanković; D.P. Mandic; M. Daković; M. Brajović; B. Scalzo; S. Li; and A. G. Constantinides, Data analytics on graphs Part I: Graphs and spectra on graphs now, 2020*

19 *P. Paleo, "Iterative methods in regularized tomographic reconstruction," PhD Thesis, Univ. Grenoble Alpes, 2017, https://tel.archives-ouvertes.fr/tel-01731514*

rithm[20] and results, obtained by calculating TV on ROIs, show that only OGF manages to outperform CP in terms of TV minimization, while results obtained with TSF are slightly worse then with CP. In terms of visual inspection, each method successfully eliminated ring artifacts, while preserving details within pollen particles and their shape. Results are shown in *Table 1*.

CONCLUSIONS:

Two methods, TSF and OGF, were presented and compared in terms of TV with CP algorithm. Efficiency of both methods was tested on different varieties and shapes of pollen particles and results show that this type of image processing has a potential as a standard preprocessing of holographic image reconstructions.

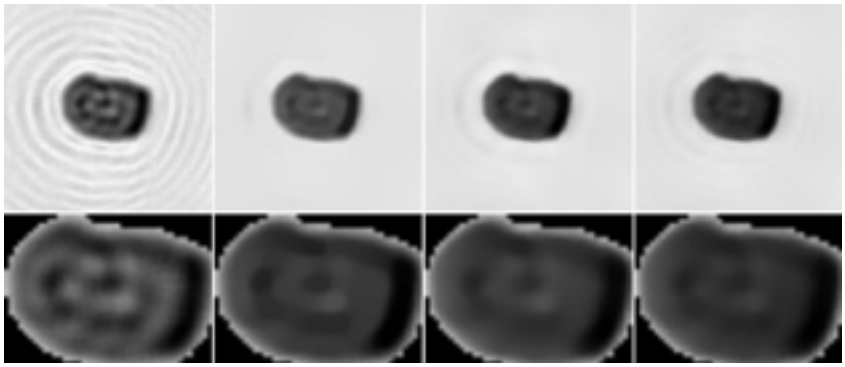

Figure 1. 100x100 regions around particles (top) and ROI (bottom) in: original (a) and filtered hologram reconstructions using the following algorithms: CP (b),TSF (c) and OGF (d)

TOTAL VARIATION	Average	Standard Deviation	Decrease Factor	
Original Images	61278	20052	Average	Standard Deviation
Chambolle-Pock	50706	14489	1.21	1.38
Taubin Smoothing Filter	51655	14738	1.19	1.36
Optimal Graph Filter	48910	13479	1.25	1.49

Table 1. Total variation statistics for 19 original and filtered ROI images

T2-P-7 Quality characteristics of pasta enriched with wild garlic powder

Branislava Đermanović, Jovana Kojić, Jelena Krulj, Olivera Šimurina, Bojana Šarić, Dubravka Škrobot, Bojana Filipčev[21]

KEYWORDS: pasta products, cooking quality, wild garlic powder, stickiness

INTRODUCTION:

Wild garlic (*Allium ursinum L.*) is a wild edible, spicy plant, very similar to garlic, whose exceptional healing properties have been known since ancient times. Due to the multitude of bioactive compounds that are extremely important for human

20 *Antonin Chambolle, Thomas Pock. A first-order primal-dual algorithm for convex problems with applications to imaging. 2010. ffhal-00490826f*

21 *Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia.*

Corresponding author: branislava.djermanovic@fins.uns.ac.rs

nutrition, such as tannins, flavonoids, phenolic acids, phytosterols, carotenoids, vitamins and minerals, it can be considered as a potential carrier of food functionality. Wild garlic is ideal for removing toxic substances that are stored in the body, and for antiseptic and antimicrobial action. Wild garlic stimulates the immune system, lowers cholesterol, lowers blood pressure and, is suitable for heart diseases. The modern eating habits of the population lead to an increase in the interest of the food industry in the placement of food products that have a beneficial effect on human health.

OBJECTIVES:

Therefore the main aim was to produce the functional paste enriched with wild garlic in a way which could potentially positive influence on human health.

METHOD / DESIGN:

Pasta from durum wheat flour supplemented by wild garlic powder (WGP) at the ratio of 5%, 7%, and 9% was produced on electric pasta machine "Pasta Fresca". Control sample was prepared from durum wheat flour. Technological quality properties of cooked pasta were measured by determining optimal cooking time (min), cooking loss (%) and swelling index (%). Additionally, the stickiness of pasta was determined using a TA-XT2 Texture Analyzer using P / 35 cylindrical probe and a 5kg load cell.

RESULTS:

Based on the obtained results, the optimal cooking time of pasta enriched with WGP decreased in relation to control sample in the percentage from 23.1% to 61.5%, depending on the applied level of WGP. The optimal cooking time for pasta with the addition of 5% and 7% WGP is less than 10 minutes, which is in line with today's lifestyle. The percentage of cooking loss indicates the loss of solids that occur during cooking pasta, more precisely the ability of the protein-starch matrix to retain physical properties. Cooking loss in all samples was in the optimal range from 6.10% to 8.20%. The cooking loss measured for pasta with 7% WGP was 6.15%, which is very similar to the control sample (6.10%), while losses in pasta with 5% and 9% WGP were slightly higher, 7.42% and 8.10%, respectively. On the other hand, swelling index indicates the possibility to form matrix in order to prevent the penetration of water into the structure of the pasta. The largest measured value of the swelling index was in the control sample (3.47%). Swelling index with 7% WGP (2.29%) is lower than the control, while swelling index for paste with 5% and 9% WGP were 2.43% and 2.86%, respectively. Stickiness of cooked pasta in the control sample was 41.9 g·sec, while the lowest value was in the sample with 7% WGP (64.54 g·sec). Higher values of the stickiness were determined in the sample with 5% and 9% WGP (72.28% and 76.40%, respectively).

TECHNOLOGICAL QUALITY OF COOKED PASTA				
	COOKED PASTA			
	Optimal cooking time (min)	Cooking loss (%)	Swelling index	Sticknes (g*sec)
Control	13 ± 1.0^{c}	6.10 ± 1.14^{a}	3.47 ± 0.30^{c}	41.90 ± 3.58^{a}
5% WGP	5 ± 1.0^{a}	7.42 ± 0.01^{b}	2.43 ± 0.03^{a}	72.28 ± 1.95^{b}
7% WGP	7 ± 1.0^{a}	6.15 ± 0.02^{a}	2.29 ± 0.02^{a}	64.54 ± 0.73^{b}
9% WGP	10 ± 1.0^{b}	8.20 ± 0.18^{c}	2.86 ± 0.07^{b}	76.40 ± 9.04^{b}

Table 1. Quality parameters of cooked pasta enriched with WGP

CONCLUSIONS:

In conclusion, taking into account all the above parameters of cooked pasta quality, the sample with 7% WGP is considered the optimal functional pasta with the addition of WGP.

ACKNOWLEDGMENTS: This work is financed by The Ministry of Education, Science and Technological Development 451-03-9/2021-14/ 200222 of the Republic of Serbia.

T2-P-8 Kinetic study of molassigenic metal ions biosorption on sugar beet pulp

Lidija Peić Tukuljac[22], Jelena Krulj[22], Lato Pezo[23], Nikola Maravić[24], Zita Šereš[24], Jovana Kojić[22], Branislava Đermanović[22]

KEYWORDS: biosorption; sugar beet pulp; molassigenic metal ions; kinetics

INTRODUCTION: Sugar beet pulp as a cheap and abundant sugar industry solid waste from the environmental and economical point of view is appropriate for valorization as biosorbent for metal ions removal. The sugar industry are facing problems with high amount of molassigenic metal ions' remained after purification step in sugar juice. To overcome challenges occurred with purification stage, utilization of biomass as cation-exchange material imposes as a potential solution for more successful sugar juice purification. Modeling of biosorption kinetics is one of the main requirements for characterization of novel biomass used as a biosorbent and describing efficient sequestering of metal ions, process mechanism and dynamics.

OBJECTIVES: In the present study, the biosorption of Na(I), K(I) and Ca(II) from alkalized sugar juice on sugar beet pulp has been investigated. The main objective of current work was to study kinetic aspects of the metal ions biosorption on sugar beet pulp including non-linear kinetic and diffusion models.

METHOD / DESIGN: Batch biosorption experiments were performed at temperature (70oC) and pH (10.5) of alkalized juice similar to industrial conditions. The molassigenic metal ions content in sugar juice was determined by atomic absorption spectrometer (AAS). Removal effect (%) was calculated from the difference in molassigenic metal ions amount in alkalized sugar juice before and after the applied process. Nature of the biosorption process was evaluated by using kinetic models (pseudo-first, pseudo-second and Elovich) and diffusion model (Weber -Morris).

RESULTS: The obtained results show maximum amount of retained metal ions after 90 min of contact time for all investigated metals. The highest removal effect was noticed with divalent calcium ions (30.19%), while sodium and potassium ions were removed with 10.94 and 9.09%-efficiency, respectively. According to appropriate statistical features (such as the sum of squared errors, the coefficient of determination and the average absolute relative deviation) the Elovich kinetic model was observed to provide the best correlation of the experimental data among the kinetic models studied. The applicability of this kinetic model indicated that biosorption process involved chemical adsorption and ion- exchange mechanism. The rate-controlling step in biosorption process was interaction between functional groups from the sugar beet pulp and mola sigenic metals. By applying Weber-Morris model, it could be concluded that intraparticle diffusion was not the only rate-limiting step of the process. The liquid layer around the particle had also an effect on the adsorption rate. Proposed kinetic models for calcium removal from the alkalized sugar juice had excellent model agreement (R2=0.968) which was in an accordance with literature since these models have been originally developed for the divalent cation removal mechanisms.

CONCLUSIONS: The results of this study indicate that the sugar beet pulp is suitable biosorbent for the biosorption of Na(I), K(I) and Ca(II) from the alkalized sugar juice. Sugar beet pulp can be valorized in effective and environmental-friendly way.
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22 Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia. Corresponding author: lidija.peictukuljac@fins.uns.ac.rs

23 Institute of General and Physical Chemistry, University of Belgrade, Studentski Trg 12-16, 11000 Belgrade, Serbia

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

T2-P-9 Edible oil enrichment with carotenoids from carrot waste

Aleksandra Gavarić, Senka Vidović, Vanja Šeregelj, Gordana Ćetković[25]

KEYWORDS: carrot waste; carotenoids; ultrasonic extraction

INTRODUCTION:

Edible oils include a large variety of products, representing an important source of essential fatty acids and liposoluble vitamins. However, polyunsaturated oils like sunflower oil, pumpkin seed oil or linseed oil are susceptible to oxidation, so their protection by enrichment with antioxidants represents a promising way to extend their shelf-life. Synthetic compounds such as butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), and tert-butyl-hydroquinone (TBHQ) are widely used antioxidants for this purposes due to their low cost, high stability and efficacy. However, possible toxicological effects on human health have been observed and some countries banned their utilization. Recent research has focused on the utilization of fruit and vegetable by-products as potential sources of natural antioxidants. Carrot (*Daucus carota L.*) waste has attracted considerable attention due to the potential health benefits of its lipophilic bioactive compounds, mainly carotenoids. Carrot processing generates wastes in the form of peels and pomaces, which can create serious nutritional, economic and environmental issues. Consumption of carotenoids has been associated with various health benefits, including a reduced risk of age-related macular degeneration and cataract, some cancers and coronary heart disease.

OBJECTIVES:

The main aim of this research was to investigate the possibility of edible oil enrichment using carrot waste as a source of carotenoids.

METHOD / DESIGN:

In all experimental runs, 10 g of lyophilized carrot waste was mixed with 100 mL of selected edible oils (sunflower, linseed and pumpkin seed oil) and exposed to ultrasonic probe (Hielscher UP400St) at different amplitude levels (20, 40 and 60%) until temperature of mixture reached 60°C. Fresh carrot waste was obtained from the "Nectar" beverage industry (Bačka Palanka, Serbia), immediately packed, freeze-dried and stored at −20 °C until further use.

RESULTS:

The total carotenoid content in the carrot waste was 600.93 mg/kg; the β-carotene was predominant (455.96 mg/kg), followed by α-carotene (144.97 mg/kg). The carotenoids were completely absent in sunflower oil, whereas linseed and pumpkin seed oils contained low concentrations of xanthophylls (lutein and zeaxanthin).

CONCLUSIONS:

Carrot waste contains high amounts of residual bioactives with currently low commercial value. Enrichment of linseed and pumpkin seed oils with carotenoids from carrot waste significantly increased its β-carotene and α-carotene contents which contributed to stability towards oxidation reactions and provided highquality value-added edible oil.

25 *University of Novi Sad Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad. Corresponding author: cvejina@uns.ac.rs*

T2-P-10 Extraction of phenols from peppermint by ultrasonic probe

Zorana Mutavski[26], Jelena Rodić[26/27], Aleksandra Gavarić[26], Senka Vidović[26], Nataša Nastić[26]

KEYWORDS: peppermint leaves; ultrasonic extraction; polyphenols

INTRODUCTION:

Peppermint (*Mentha piperita L.*) is a widely distributed aromatic and perennial medicinal plant from the Lamiaceae family. Due to the outstanding antioxidant activity of leaf, pedicle and flower extracts, rich in phenolic compounds, they are widely used for pharmaceutical, food and cosmetics purposes.

OBJECTIVES:

The main aim of this research was to compare conventional solid/liquid (S/L) extraction with ultrasound assisted extraction of peppermint leaves in respect to yield of polyphenols. Another target was to determine the optimal extraction time and amplitude of ultrasonic extraction at which the highest total phenols and flavonoids contents were provided.

METHOD / DESIGN:

In all experimental runs, 10 g of peppermint leaves was mixed with 200 mL of solvent (30, 50 and 70% ethanol). The highest total phenols (TP) and total flavonoids (TF) contents were obtained spectrophotometrically using 50% ethanol as solvent, which was further used as solvent in ultrasonic extractions. The ultrasonic probe (Hielscher UP400St) at different amplitude levels (20, 60 and 100%) and extraction time (2, 4, 6, 8 and 10 min) was engaged for providing 15 different extracts.

RESULTS:

The maximal TP (413,4698 mg GAE/g DW) value was obtained after 2 min of ultrasonic extraction at 20% amplitude, whereas minimal TP (237,9772 mg GAE/g DW) value was obtained after 6 min of extraction at 100% amplitude. The TF varied from 216,1222 mg CE/g DW to 368,7847 mg CE/g DW. The maximal TF was obtained at the same extraction parameters as maximal TP.

CONCLUSIONS:

Ultrasonic extraction of peppermint leaves with extraction time 2 min and amplitude 20% provided significantly higher TP (413,4698 mg GAE/g DW) and TF (368,7847 mg CE/g DW) values in comparison to S/L extraction which delivered TP (236,8546 mg GAE/g DW) and TF (232,8792 mg CE/g DW) values. Furthermore, ultrasonic extraction with probe lasted only 2 min in comparison with S/L extraction which proceeded for 24 h.

26 *University of Novi Sad Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad. Corresponding author: senka.vidovic@uns.ac.rs*

27 *Euro Prima doo, Zorana Radmilovića bb, 21203 Veternik*

T2-P-11 Comparison of process alternatives for bioethanol production from milling industry by-product

Jovana Gucunski, Bojana Bajić, Vesna Vučurović, Damjan Vučurović, Đurđina Belić, Siniša Dodić[28]

KEYWORDS: bioethanol, milling industry by-product, SHF, SSF, SLSaF

INTRODUCTION: By-products of the milling industry (shrunken and damaged wheat grains) with a significant amount of starch can be considered as a substrate for bioethanol production. There are several process alternatives for this, and each has specific advantages and disadvantages. Separated hydrolysis and fermentation (SHF) can ensure that enzymatic hydrolysis of starch (liquefaction and saccharification) and bioethanol fermentation are performed separately, under optimal conditions for each step. However, the overall process time is longer and the substrate inhibition of the production microorganism may occur. Simultaneous saccharification and fermentation (SSF) takes less time and utilizes less energy compared to SHF. Still, it is challenging to optimize SSF, considering that optimal conditions need to be achieved for the saccharification enzyme and fermenting yeast. In simultaneous liquefaction, and saccharification and separated fermentation (SLSaF) the main goal is to decompose starch molecules in one-step hydrolysis process. The disadvantage of this process can be the substrate inhibition due to the high concentration of glucose.

OBJECTIVES: This study aimed to compare three different process alternatives, SHF, SSF and, SLSaF for bioethanol production, from milling industry by-product, using commercial enzymes and yeast *Saccharomyces cerevisiae*. Additionally, the influence of different liquefaction temperatures (65°C, 75°C, 85°C) on bioethanol production were investigated.

METHOD / DESIGN: The grinded wheat milling by-products were suspended into the tap water (to obtain ratio at 1:3), and the pH value was adjusted at 6. Enzymatic hydrolysis was performed with enzymes α-amylases (Termoamyl 120 L, Novozyme) and glucoamylases (SAN Extra L, Novozyme). The production microorganism was *S. cerevisiae,* and fermentation was performed at 30°C for 42h with constant stirring (150 rpm), under anaerobic conditions.

RESULTS: Bioethanol yield (ml/100g of raw material) was calculated and the results are shown in *Figure 1*. Additionally, the highest raw material utilization was obtained for SLSaF (50.97 g/100g) at liquefaction temperature of 65°C, for SHF (51.15 g/100g) at 75°C, and the SSF (51.95 g/100g) at 75°C. The starch utilization for each production alternative with the highest bioethanol yield, considering the initial concentration, were 93.94% for SLSaF, 94.26% for SHF and 95.73% for SSF.
CONCLUSIONS: Based on the obtained results, the SSF process alternative was considered superior compared to SLSaF and SHF, due to the higher bioethanol yield and raw material utilization. Further research will focus on the optimization of simultaneous saccharification and fermentation bioethanol production process from the milling industry by-product.

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28 University of Novi Sad, Faculty of Technology Novi Sad, Department of Biotechnology and Pharmaceutical Engineering, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia. Corresponding author: jgucunski@uns.ac.rs

T2-P-12 The effect of ph and chitosan concentration on flocculation efficiency of *Bacillus Sp.* biomass

Selena Dmitrović, Ivana Pajčin, Vanja Vlajkov, Jovana Grahovac, Aleksandar Jokić, Nataša Lukić[29]

KEYWORDS: flocculation; chitosan; antimicrobial activity; *Bacillus sp.*; *Aspergillus spp.*

INTRODUCTION:

Chitosan is a biopolymer widely distributed in the nature, derived from chitin, with good physical and chemical properties for separation of solid particles from liquid phase in a process known as flocculation. In recent years, chitosan has been investigated as a suitable alternative to synthetic polymeric flocculants. Chitosan and its derivatives show antimicrobial activity against fungi, gram-positive and gram-negative bacteria, which is an additional benefit when it comes to suppression of microbial plant pathogens. Furthermore, chitosan could be applied as flocculation agent for separation of microbial biomass used in plant pathogens biological control. The flocculation efficiency is influenced by many factors, including pH value, chitosan molecular mass (Mw), temperature, chitosan concentration, biomass concentration and cell growth phase.

OBJECTIVES:

In this study two types of chitosan were applied to flocculate cultivation broth of *Bacillus sp.* aimed to be used as biocontrol agent for suppression of mycotoxigenic *Aspergillus* phytopathogens, isolated from diseased corn. The aim of this study was to select appropriate flocculant and to relate its dose and pH value to the flocculation efficiency and antimicrobial activity of the harvested *Bacillus sp.* biomass against the tested phytopathogens.

METHOD / DESIGN:

The jar test was applied to determine the optimum pH value (3.0, 5.0, 7.0) and the optimum flocculant dose (5,15, 25, 35, 45, 55, 65, 80, 100, 120 mg/L) using two different types of chitosan (A-obtained from shrimp shells, ≥75% deacetylated, SIGMA-ALDRICH, USA; B-low molecular mass chitosan 310,000-375,000 Da, SIGMA-ALDRICH, USA). Flocculation experiments were performed using 100 mL of *Bacillus sp.* cultivation broth produced in the laboratory scale bioreactor. Experiments were performed in a 250 mL beaker, in which 100 mL of sample was weighed, then the appropriate amount of flocculant was added (pH value was adjusted using 0.1M HCl/0.1M NaOH).The sample beaker was placed on a magnetic stirrer, first at 350 rpm for 5 minutes to disperse the flocculant and then the speed was reduced to 100 rpm for 30 minutes to enhance particle collisions. After the flocculation tests, antimicrobial activity of the precipitate/supernatant against phytopathogenic *Aspergillus spp.* (2 strains) was examined using the well diffusion method. Data obtained by measuring inhibition zone diameters were processed by several statistical methods (ANOVA - analysis of variance and Duncan's multiple range test) using Statistica 13.5 software (Tibco Software Inc., USA). All statistical analyses were performed at the significance level of 95%.

RESULTS:

According to the obtained ANOVA results, the type of flocculant and dose, as well as pH value, had a significant impact on a flocculation efficiency and antimicrobial activity. The highest degree of flocculation efficiency for each examined pH value was achieved in the chitosan concentration range from 45 mg/L to 65 mg/L. It was found that the flocculation efficiency has been the highest at pH value 3.0, while it has been the lowest at pH value 7.0. It was also confirmed that lower pH values didn't have significant effect to reduction of viable cell number, due to *Bacillus sp.* sporulation ability. Low molecular mass chitosan B had slightly lower flocculation efficiency than chitosan A, obtained from shrimp shells. Based on the results, the

29 *University of Novi Sad, Faculty of Technology Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia. Selena Dmitrović: selena.dmitrovic@uns.ac.rs*

precipitate sample obtained at pH value 5.0 and with flocculant dose of 55 mg/L for both chitosan A and B showed the best antimicrobial activity against *Aspergillus spp.* strains.

CONCLUSIONS:

It can be concluded that different types of chitosan could be successfully applied as the flocculating agents for separation of *Bacillus sp.* biomass aimed to be applied in biological control of plant pathogens. The highest degree of flocculation efficiency (99,77%) was achieved when pH value was 3.0 and chitosan concentration was 45 mg/L. It is necessary to perform further optimization of the flocculation parameters in order to achieve maximal flocculation efficiency, as well as high antimicrobial activity of the final product, which will be the subject of future research. Furthermore, formulation of biocontrol agents based on flocculated microbial biomass, as well as their testing against wider spectra of plant pathogens, will be further investigated.

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T2-P-13 Protease production potential of *Bacillus Spp.* isolated from vegetables' rhizosphere

Ivana Pajčin, Vanja Vlajkov, Selena Dmitrović, Aleksandar Jokić, Jovana Grahovac[30]

KEYWORDS: waste valorization, proteolytic activity, biocatalyst, circular economy, biotechnological production

INTRODUCTION:

Industrial waste management is one the major problems when it comes to environmental protection and complying to legislation regarding waste treatment and disposal. On the other hand, a circular economy approach including waste valorization through microbial conversion in value-added products is one of the promising solutions. However, there is a necessity to find suitable microorganisms as the main biocatalysts in biotechnological processes of waste conversion.

OBJECTIVES:

The aim of this study was to isolate and select *Bacillus spp.* strains capable of producing proteases, as potential biocatalysts for valorization of dairy and meat industry waste.

METHOD/DESIGN:

The strains tested in this study were isolated from the rhizosphere of onion, beans, green beans, pea, tomato, potato, cabbage, pepper, carrot, cucumber, parsnip, and beet. The soil sampling was performed at the locality Tovariševo, Serbia. The strain isolation procedure was performed as follows: 1 g of soil sample was suspended in 9 mL of saline and subjected to heat treatment (7 min at 100 °C). Thermally treated sample dilutions (0.5 mL of 10, 100, and 1000 fold dilutions) were mixed with nutrient agar medium and transferred to Petri dishes for incubation at 28 °C for 48 h. Colonies that macromorphologically corresponded to the *Bacillus* genus according to Bergey's Manual of Systematic Bacteriology were picked and transferred to fresh nutrient agar, followed by incubation under the similar conditions as in the previous phase. This procedure was repeated until visually pure cultures were obtained. Afterwards, a selective medium for protease production screening was

30 *University of Novi Sad, Faculty of Technology Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia. Corresponding author: ivana.pajcin@uns.ac.rs*

prepared, containing skim milk (28 g/L), tryptone (5 g/L), yeast extract (2.5 g/L), glucose (1 g/L), and agar (15 g/L). The isolated strains were inoculated on the modified skim milk agar and incubated at 28 °C for 48 h. Detection of halo zones around colonies, resulting from proteolytic degradation of skim milk, was considered as positive result regarding ability of protease production, while measurement of halo zones' diameter was used as a semi-quantitative indicator of protease production.

RESULTS:

Protease production capability screening included totally 76 strains isolated from the rhizosphere of different vegetables. The results describe the proteolytic potential of the isolated *Bacillus spp.* strains (*Table 1*), taking into account the strain origin. The presented results have shown that each *Bacillus spp.* strain isolated from the rhizosphere of tomato, carrot, and parsnip has shown the ability to produce proteases, while the absence of proteolytic activity was observed in strains isolated from the rhizosphere of pea and cucumber. The best results in terms of halo zones indicating proteolytic potential were achieved in strains isolated from the rhizosphere of parsnip (13.17 mm), onion (12.83 mm), green beans (12.67 mm), tomato (12.50 mm), cabbage (12.33 mm), and potato (12.00 mm).

PLANT	Percent of isolated strains exhibiting proteolytic activity (%)	Plant	Percent of isolated strains exhibiting proteolytic activity (%)
Onion	83.33	Cabbage	20.00
Beans	50.00	Pepper	42.86
Green beans	55.56	Carrot	100.00
Pea	0.00	Cucumber	0.00
Tomato	100.00	Parsnip	100.00
Potato	71.43	Beet	20.00

Table 1. Proteolytic activity of Bacillus spp. isolated from the rhizosphere of different vegetables

CONCLUSIONS:

The obtained results indicated high potential of *Bacillus spp.* strains isolated from the rhizosphere of onion, green beans, tomato, potato, cabbage, and parsnip to produce proteases. The selected strains could be potentially applied as biocatalysts for degradation of protein-rich dairy and meat industry waste. Utilization of industrial waste loaded with organic substances in biotechnological production significantly reduces waste treatment and disposal costs, with simultaneous contribution to circular economy development. Hence this research topic will be further investigated in terms of molecular identification of selected biocatalysts, as well as optimization of biotechnological processes for waste valorization, with a special emphasis on production of microbial biocontrol agents for suppression of microbially-induced plant diseases as value-added products.

ACKNOWLEDGMENT: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia in the framework of the program 451-03-68/2021-14/200134.

T2-P-14 Production of 3rd generation bioethanol from micro and macroalgal biomass: a review

Aleksandra Katanski[31], Vesna Vučurović[32], Zorana Trivunović[33]

ABSTRACT

The constant energy demand due to the continuous population increase clearly emphasizes the need to find additional energy sources. Fossil fuels are the main source of energy worldwide at the moment. However, their combustion leads to the emission gases release that disrupt environmental stability. Recent researches have pointed out the significance of biofuels as a complementary energy source. Bioethanol has emerged as a perspective and renewable alternative to fossil fuels. Different substrates have been used for the production of bioethanol, such as different edible crops, lignocelluloses biomass and agricultural waste. Nevertheless, algae have proven to be one of the most efficient and promising feedstock for bioethanol production. Therefore, a lot of researches are directed towards finding the most productive algae strains and optimal conditions that would enable bioethanol mass production and at the same time commercialization. In this study, the current processes for the conversion of the algae into bioethanol are analyzed and described. The possibility of using macro and microalgae as raw materials, as well as a review of indispensable pretreatments such as algae cultivation, hydrolysis, and harvesting, highlighted the great potential of algal biomass as a suitable substrate for bioethanol production.

KEYWORDS: Bioethanol; microalgae; macroalgae; biofuel; raw material

T2-P-15 Phenotypic variation of spike index of winter wheat (*Triticum aestivum L.*) grown under limited soil conditions

Nataša Ljubičić[34], Vera Popović[35], Bojana Ivošević[34], Vuk Radojević[35], Dragana Popović[36], Miloš Pajić[34], Marko Kostić[37]

KEYWORDS: wheat, spike index, interaction, soil

INTRODUCTION:

Soil and climatic conditions are one of the most important factors affecting grain yield of wheat. The ability of a wheat cultivar to produce high and stable yield over a wide range of environments plays a major role in food security. However, since different morpho-physiological traits have been proposed as key traits associated with grain yield potential of wheat, its assessment could greatly contribute to improve grain yield.

31 University of Novi Sad, Faculty of Technology Novi Sad, Department of Biotechnology and Pharmaceutical Engineering, Novi Sad, Serbia. Corresponding author: aleksandra.katanski@gmail.com

32 University of Novi Sad, Faculty of Technology Novi Sad, Department of Biotechnology and Pharmaceutical Engineering, Novi Sad, Serbia

33 University of Novi Sad, Faculty of Technology Novi Sad, Department of Biotechnology and Pharmaceutical Engineering, Novi Sad, Serbia

34 BioSense Institute-Research and Development Institute for Information Technologies in Biosystems, University of Novi Sad, Dr. Zorana Đinđića 1, 21000 Novi Sad, Serbia. Corresponding author: ljubicic.natasa@gmail.com; natasa.ljubicic@biosense.rs; drvvpopovic@gmail.com

35 Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21 000 Novi Sad, Serbia

36 Faculty of Economics in Subotica, University of Novi Sad, 21000 Novi Sad, Serbia

37 Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21 000 Novi Sad, Serbia

OBJECTIVES:

The objective of this research was to estimate the responses of eleven winter wheat genotypes (Mina, Sofija, Tiha, Anastazija, Nevesinjka, Evropa 90, Novosadska rana-5, Dragana, Ljiljana, Simonida, Zugoly) under specific growing conditions of halomorphic soil, solonetz type. During the two vegetation seasons, phenotypic variability and genotype by environment interaction (GEI) for spike index of wheat genotypes was studied.

METHOD / DESIGN:

The experiment was set up on the solonetz soil and consisted of control (non-ameliorated solonetz soil) and treatments with two levels of soil amelioration using phosphor gypsum, in amounts of 25 and 50 tha^{-1}. The additive main effects and multiplicative interaction (AMMI) models were used to quantify the genotype by environment interaction (GEI).

RESULTS:

The combined ANOVA showed that the phenotypic expression of spike index was significantly influenced by environmental variations, because the significant variance explained 48.8 % of the total variation, while genotype contributed with 7.8 % of the total variation of the experiment. Genotype by environment interaction expressed no significant mean square, while additional analysis of GEI using the IPCA (Interaction Principal Components) analysis showed a statistical significance of the first main component IPCA1. First source of variation IPCA1 explained 55.6 % of the GEI variation for the spike index of wheat.

CONCLUSIONS:

The results of this study showed that wheat genotypes responded differently to different levels of soil amelioration and significant wheat spike index variation was noticed due to different environment conditions. Spike index variation also depended on tested genotype, as well as vegetation season. Genetics analysis of different wheat genotypes grown in different agroecological conditions contributes to their better utilization, as well as selection for crosses in wheat breeding programs.

T2-P-16 Correlation analysis of yield components in winter wheat grown on less productive soil

Nataša Ljubičić[38], Vera Popović[39], Bojana Ivošević1, Dragana Popović[40], Maša Buđen1, Miloš Pajić1, Marko Kostić[41]

KEYWORDS: wheat, yield components, soil, correlations

INTRODUCTION:

The less productive soils present one of the major problems in wheat production over the world. Considering the importance of wheat production, it is necessary to better utilize the less productive soils and to select wheat varieties that can be successfully grown on such soils. Since that the grain yield of wheat is complex and variable trait that depends on numerous yield components and environmental factors, individual characteristics of the plant, such as the number of grains per spike, grain weight per spike, plant height and harvest index, are important in the formation of grain yield, especially in the stressful conditions of wheat cultivation. The investigation of variability and assessment the interrelationship of yield components

38 *BioSense Institute - Research and Development Institute for Information Technologies in Biosystems, University of Novi Sad, Dr. Zorana Đinđića 1, 21000 Novi Sad, Serbia. Corresponding author: natasa.ljubicic@biosense.rs; ljubicic.natasa@gmail.com*

39 *Institute for Field and Vegetable Crops, Maksima Gorkog 30, 21 000 Novi Sad, Serbia*

40 *Faculty of Economics in Subotica, University of Novi Sad, 21000 Novi Sad, Serbia*

41 *Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21 000 Novi Sad, Serbia*

could improve cultivar creation, selection and ability of a wheat cultivar to produce high and stable yield over a wide range of environments.

OBJECTIVES:

The objective of this study was to estimate the mean values the yield components (the number of grains per spike, grain weight per spike, plant height and harvest index) of ten winter wheat cultivars growing under stressful conditions of halomorphic soil, solonetz type, as well as, the correlations between them.

METHOD / DESIGN:

The field trial was carried out at solonetz soil type and consisted of control (solonetz without amelioration) and treatments with two levels of soil amelioration using phosphor gypsum in amounts of 25 and 50 tha^{-1} during two vegetation seasons. The experimental material in the study was comprised of ten Serbian winter wheat varieties (*Triticum aestivum L.*), chosen on the basis of their differences in yield and performance of several morpho-physiological traits. The relationship between grain yield components was determined by calculating the Pearson's correlation coefficient (r) measuring strength of association between traits.

RESULTS:

Unfavorable environmental conditions in this study, type of soil and weather conditions, during wheat development decreased the mean values of the investigated components of wheat yield. Statistically significant and strong positive correlation was established between the grain weight per spike and the number of grains per spike in both seasons within each treatment, which indicates that the number of grains per spike is a trait through positive selection, can increase the grain weight per spike.

CONCLUSIONS:

A different reaction of wheat cultivars to soil repair levels was observed, in relation to each treatment and vegetation season. The lowest variability of the yield components of certain cultivars indicates good adaptation of these cultivars to the conditions of abiotic stress on holomorphic soil. Significant or highly significant correlation obtained between the examined yield components on all examined treatments indicated the possibility that improvement of one component affects the improvement of another, which could consequently lead to improvement the final yield.

T2-P-17 Isolation of bioactive compounds from hemp (*Cannabis Sativa L.*) by conventional and novel extraction techniques

Sanja Milošević[42], Nemanja Brkljača[43], Saša Đurović[44], Branimir Pavlić[42]

KEYWORDS: *Cannabis sativa L.*; supercritical fluid extraction; microwave extraction; essential oil; polyphenols

INTRODUCTION: Industrial hemp (*Cannabis sativa L.*) is one of the oldest medicinal plants that is a source of valuable bioactive compounds (fiber, protein, oil, cannabinoids, polyphenols etc.). Due to the uniqueness of its composition, hemp is used as a highly valuable product usable in the food, pharmaceutical, and cosmetic industries. The nutritious and bioactive com-

42 *Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia. Corresponding author: sanjamilosevic9898@gmail.com*

43 *Educons University, Vojvode Putnika 87, Sremska Kamenica 21208, Serbia*

44 *Institute of General and Physical Chemistry, Studentski trg 12/V, 11158 Belgrade, Serbia*

position of hemp contributes to the prevention and treatment of some diseases, so it can be used in medicine as: analgesic, antiepileptic, anticonvulsant, anti-neurodegenerative, antibacterial, and anticancer agent.

OBJECTIVES: The main goal of this paper was to investigate the most convenient extraction techniques for isolation of bioactive compounds (cannabinoids, phenols and fatty acids) from dry inflorescence of three *Cannabis sativa L.* varieties. In this research, modern extraction techniques (microwave-assisted hydrodistillation, microwave-assisted extraction, and supercritical fluid extraction) were applied and compared with conventional techniques in order to obtain the maximum yield. Since the chemical composition of the extracts depends on the extraction techniques and the applied conditions, this will enable a comparison of the examined techniques and the definition of the best extraction technique for the target group of compounds.

METHOD/DESIGN: Essential oils and lipid extracts were obtained by hydrodistillation, microwave-assisted hydrodistillation, and supercritical fluid extraction. Extracts from the primary raw material of hemp (*Cannabis sativa L.*) and SFE raffinate were processed by microwave-assisted extraction in order to isolate polyphenolic fraction.

RESULTS: The obtained results show that supercritical carbon dioxide extraction provides the highest yield of hemp oil in relation to hydrodistillation (HD) and microwave hydrodistillation (MWHD). The highest yield of essential oil in relation to the extraction time was obtained in extraction of variety Fedora 13 (14.01%), while the lowest was of variety Marina (5.33%). Total phenols and flavonoids obtained from the primary raw material, but also from SFE raffinates by microwave extraction could be used as important molecules that are potential natural antioxidants.

CONCLUSIONS: Supercritical extraction has proven to be the best technique for obtaining essential oil from *Cannabis sativa L.* The high content of phenols and flavonoids obtained from SFE raffinates indicates that the by-products of supercritical extraction are a good material for isolation of bioactive compounds. Thanks to the extraction of bioactive molecules, hemp could be an important plant material in the pharmaceutical and food industries for creating potential new products.

T2-P-18 Antioxidant and neuroprotective activity of *Helichrysum italicum* extracts obtained by modern and conventional extraction techniques

Vladislava Nebrigić[45], Milena Terzić[45], Göhkan Zengin[46], Marija Radojković[45]

KEYWORDS: *Helichrysum italicum*, extraction techniques, biological activity

INTRODUCTION:

Helichrysum italicum (Roth) G. Don is a medicinal plant with promising pharmacological activities. In traditional medicine of Mediterranean countries this plant was used in the treatment of health disorders such as allergies, colds, cough, skin, liver and gallbladder disorders, inflammation and infections. In recent years, it has been showed that bioactive compounds from plants and plant extracts can improve cognitive functions in neurodegenerative conditions. One of the good therapeutic targets for the treatment and/or management of Alzheimer and Parkinson diseases could be inhibition of cholinesterases.

45 *Faculty of Technology, University of Novi Sad, Novi Sad, Serbia. Corresponding author: ramarija@uns.ac.rs*

46 *Selçuk Üniversitesi, Fen Fakültesi Biyoloji Bölümü, Kampüs/Konya, Turkey*

Furthermore, oxidative stress which is induced by the formation of free radicals may impair endogenous antioxidant defence and take part in the progression of many neurodegenerative diseases. Thus, screening of plant extracts for their property as antioxidants and to inhibit enzymes is considered as a promising tool in the discovery of lead molecules against targeted diseases.

OBJECTIVES:

The aim of this study was to determine is there possibility for the use of *Helichrysum* extracts as potential supplementary therapy in management of neurodegenerative diseases.

METHOD / DESIGN:

After collecting, removing impurities, and shade drying, plants were used to make extracts. Extract was obtained using traditional (maceration) and modern extraction techniques (microwave-assisted extraction-MAE), where 50% ethanol was used as solvent. Antioxidant potential was analyzed using three different in vitro antioxidant assays which included antiradical ($DPPH^{\cdot}$ and $ABTS^{\cdot+}$) and reducing power (FRAP) assays. Enzyme inhibitory effects were tested against acetylcholinesterase (AChE), butrylcholinesterase (BChE).

RESULTS:

The results of total phenolic and flavonoid content of the extracts showed that extract obtained by MAE had higher phenolic (106.65±1.79 mg GAE/g) and flavonoid (10.86±0.29 mg RE/g) content than extract obtained by maceration (total phenol 100.85±1.44 mg GAE/g, and total flavonoid (8.14±0.45 mg RE/g). In antioxidant assays MAE extract had higher antiradical activity against both the $DPPH^{\cdot}$ (114.18±12.16mgTE/g) and $ABTS^{\cdot+}$ (143.70±6.94 mg TE/g) radicals and stronger capacity to reduce Fe^{3+} ion (FRAP 273.45±0.67 mgTE/g) than extracts obtained by maceration. However, the AChE inhibitory activity of the extracts revealed that the extract obtained by maceration had higher inhibitory activity (3.01±0.17 mg GALAE/g), than MAE extract (2.71±0.11 mg GALAE/g). Similarly, the macerate (1.38±0.32 mg GALAE/g) also had higher inhibitory effect on BChE activity than the MAE extract (0.68±0.01 mg GALAE/g).

CONCLUSIONS:

According to the obtained results it was shown that *Helichrysum italicum* could be a rich natural source of bioactive agents. Further studies are needed on isolation and identification of individual compounds from the extracts and to investigate in depth their modes of action.

T2-P-19-ORAL Characterization and purification of a novel halothermotolerant L-asparaginase from *Bacillus licheniformis* PPD37 and its anti-proliferative activity against cancer cell lines

Payal Patel, Haren Gosai, Bharti Dave[47]

KEYWORDS: L-asparaginase; enzyme purification; enzyme characterization; halotolerant, thermotolerant, anti-proliferative activity.

INTRODUCTION:

L-asparaginase is an amidohydrolase that catalyzes deamidation of L-asparagine and cleaves it into aspartate and ammo-

47 *Department of Bioscience, School of Science, Indrashil University, Rajpur Dist- Kadi, Gujarat, India. Corresponding author: payalpatel00025@gmail.com*

nia. This property of L-asparaginase (LA) is critical for the treatment of different types of cancer. Here, a novel source of LA production – a halotolerant *Bacillus licheniformis* PPD37 strain was derived from solar salterns of Gujarat, India. LA produced by this halotolerant strain underwent purification, characterization and its anti-proliferative activity against cancer cell lines was determined.

OBJECTIVES:

The objectives of the current study were:

- Production and purification of LA produced by *B. licheniformis* PPD37 strain using column chromatography
- Characterization of the purified LA to determine its optimum activity conditions and its tolerance against different pH, temperature, salt concentration, heavy metals, and effectors.
- Determination of molecular weight of the enzyme and its kinetic parameters.
- Testing its anti-proliferative activity against cancer cell-lines.

METHOD / DESIGN:

1. Purification of LA was achieved by applying methods of centrifugation, ultrafiltration, ethanol precipitation, and ion-exchange chromatography.
2. Optimal activity and tolerance to various factors was determined by checking the enzyme activity at different conditions. Kinetic parameters of Km and Vmax were determined based on its enzyme activity against a range of substrate concentration.
3. SDS-PAGE method was used to determine the molecular weight of the enzyme.
4. MTT assay was performed to determine the IC_{50} value of the purified enzyme against different cancer cell lines.

RESULTS:

After purification the specific activity of LA was found to be 7.707 U/µg. The purified LA proved to be halothermotolerant and demonstrated 92.74 % and 92.15% enzyme activity at 70°C and 10% NaCl after 1 hour of incubation, respectively. The enzyme also retained its activity over a wide range of pH 3-10. Purified LA was observed to be a heterodimer showing two bands of 65 kDA and 63 kDA on SDS-PAGE. The purified LA had low Km value 1.518 µM and Vmax value 6.94 µM/min/mL. More than 70% LA activity was maintained against heavy metals while activity inhibition was observed in the presence of Triton X-100 and Tween 80. The enzyme exhibited significant anti-cancer activity against cancer cell lines – HeLa, SH-SY-5Y, A549 and SiHa, with IC_{50} values being less than 0.5 U/mL.

CONCLUSIONS:

A novel halothermotolerant LA was extracted from *B. licheniformis* PPD37 strain. The purified enzyme has the ability to withstand a wide range of pH, temperature, and salt concentration. It also has high specific activity and demonstrated its anti-proliferative activity even at low concentrations of less than 0.5 U/mL. This shows that the novel LA derived from this bacterial strain could be highly beneficial as a therapeutic agent.

T2-P-20 Biological potential of elderberry wine

Milena Terzić[48], Milica Stožinić[48], Tatjana Majkić[49], Ivana Beara[49], Uroš Miljić[48], Saša Đurović[50], Marija Radojković[48]

KEYWORDS: elderberry; fruits wine; phenolic compounds, biological activity;

INTRODUCTION: *Sambucus nigra L.*, commonly known as the European elder, belongs to the family Adoxaceae. This plant species has a long tradition in folk medicine throughout Europe. Mature elder berries are used in the form of herbal tea, syrup, or juice against colds, as a laxative, diaphoretic, diuretic, and analgesic. Elderberry is present in various food products as a dietary supplement. Market and consumer interest in elderberry-based products are growing, so numerous research is based on creating new products that are not available on the market yet.

OBJECTIVES:
The aim of this study was to obtain elderberry wines as new potential food products with added value, and analyzed their biological potential.

METHOD / DESIGN:
Elderberry wines were prepared according to the standard procedure of red wine production in laboratory conditions. Plant species *S. nigra* is characterized by the content of cyanogenic glycosides which are potentially toxic compounds, due to which the obtained wines were treated with different temperature treatments (60°C during 10 minutes, and 70°C during 5 minutes), in order to degrade these molecules. Elderberry wines were analyzed spectrophotometrically with the idea to determine the total content of phenolics, flavonoids, anthocyanins, and tannins. Antioxidant activity was examined using several in vitro assays (DPPH, ABTS, FRAP, CUPRAC, and Metal chelating), also, elderberry wines were examined for neuroprotective, antityrosinase, and antidiabetic potential.

RESULTS: Based on the obtained results in terms of the content of total phenolic molecules, wine with a temperature profile of 70°C, 5 minutes was a richer source of total phenols (5.12 mg GAE/mL wine), flavonoids (0.42 mg RE/mL wine), anthocyanins (0.76 mg CyG/mL wine) and tannins (3.84 mg CA/mL wine), compared to wine exposed to temperature 60°C for 10 minutes (total phenols 4.46 mg GAE/mL wine, total flavonoids 0.24 mg RE/mL wine, total anthocyanins 0.63 CyG/mL wine and total tannins 3.18 mg CA/mL wine). When it comes to biological activity, wine exposed to temperature 70°C for 5 minutes proved to be a very potent antioxidant agent, because in all applied antioxidant assays it showed better activity, compared to wine with a temperature profile of 60°C, 10 minutes. On the other hand, neuroprotective activity was more pronounced in wines with a temperature profile of 60°C, 10 minutes (0.34 mg GALAE/mL wine, for AChE and 0.18 mg GALAE/mL wine for BChE), while antityrosinase and antidiabetic potential was stronger in wines exposed to 70°C, 5 minutes.

CONCLUSIONS:
Based on the conducted research, the obtained results indicate an exceptional biological potential of elderberry wine. Temperature treatment of 70°C, 5 minutes was more suitable for the production of elderberry wine because temperature 70°C

48 *University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia. Corresponding author: milicas09@gmail.com*

49 *University of Novi Sad, Faculty of Sciences, Trg Dostiteja Obradovića 3, 21000 Novi Sad, Serbia*

50 *Institute of General and Physical Chemistry, Studentski trg 12/V, 11158 Belgrade, Serbia*

facilitates the isolation of secondary metabolites from fruits, which explains the better biological activity of this wine, and a time period of 5 minutes is sufficient to affect the degradation of cyanogenic glycosides but does not cause disturbance of the chemical structure of phenolic compounds.

T2-P-21 The influence of carbon source on the antagonistic activity of *Bacillus sp.* against the aflatoxigenic *Aspergillus flavus*

Vanja Vlajkov[51], Ivana Pajčin[51], Mila Grahovac[52], Dragana Budakov[52], Marta Loc[52], Jovana Grahovac[51]

KEYWORDS: *Bacillus*, *Aspergillus flavus*, carbon source, cellulose, waste

INTRODUCTION: Besides yield losses, considered a minor problem, mycotoxigenic fungi belonging to the *Aspergillus flavus* are recognized as a severe threat to food safety. Economic losses caused by crop contamination and harmful effects on human health due to aflatoxins entering the food chain indicate the high priority for developing the strategy to suppress those fungal pathogens. Global climate changes enhance conditions suitable for the development of mycotoxigenic fungi, even in the geographical regions characterized by a low incidence of their appearance. This kind of scenario makes the task mentioned above even more urgent and attracts the attention of scientific society, resulting in much research conducted to find a promising solution to address the existing problem. The growing trend of biopesticides in the world market proves the changes occurring in modern farming regarding plant disease management. The constantly rising popularity of biocontrol agents confirms it is just a matter of time when their domination over synthetic products will occur. Bacterial antagonists, with the emphasis on the *Bacillus genus* members, are considered leaders in the category of microbial biopesticides, highly effective in the suppression of phytopathogenic species. Even though biocontrol agents are defined as a versatile weapon in plant diseases control, there is a significant gap between the potential of microbial-based biopesticides and the number of products registered and utilized in agricultural practice. The explanation for this scenario could be found in high production costs and overall cost-effectiveness of the industrial level production. Cultivation medium preparation is one of the most critical elements in the cost structure of microbial biopesticides production (30–40% of total production costs). Carbon sources play a crucial role in the production of biocontrol agents and influence the growth of bacterial cells as well as the synthesis of secondary metabolites. Reducing the cultivation media preparation costs using alternative nutrient sources was recognized as a solution that could significantly decrease overall production costs. This approach would contribute to the industrialization of biocontrol agents' biotechnological production and result in increased chances of microbial pesticides' adoption and positioning in the world market.

OBJECTIVES: The present study includes the selection of optimal carbon source for cultivation of novel isolate of *Bacillus genus*, originating from the rhizosphere sample of *Phaseolus vulgaris*. The antagonistic effect of the producing strain was evaluated against aflatoxigenic *Aspergillus flavus* isolated from the infected maize grown in the Republic of Serbia.

METHOD / DESIGN: Seven different carbon sources, including glucose, fructose, glycerol, maltose, lactose, cellulose, and starch, were used for cultivation media preparation. The selected carbon sources were chosen to represent the most common components of the industrial waste streams, which could serve as an alternative source of nutrients. The cultivation was carried out in an Erlenmeyer flask for 96 h, at the temperature of 28°C, with an agitation rate of 180 rpm. The percentage of mycelial growth inhibition was estimated based on the antimicrobial activity testing of cultivation broth by the diffusion

51 *Faculty of Technology Novi Sad, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia. Corresponding author: vanja.vlajkov@uns.ac.rs*

52 *Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia*

method. One-way ANOVA was performed to determine the carbon sources' effect statistical significance on inhibition zone diameter. Duncan's multiple range test was conducted to define homogenous groups of medium with different carbon sources with the same level of statistical significance regarding their effect on antimicrobial activity of the tested cultivation broth against tested aflatoxigenic strain.

RESULTS: The results from the present study pointed out a significant influence of cultivation medium composition on the antifungal activity of the selected producing microorganism. Considering the overall experimental data, it was estimated that the best result was achieved by using a medium consisting of cellulose as the carbon source. A possible solution, which could contribute to the decrease of the cultivation medium preparation costs, is the utilization of lignocellulose waste material as an alternative source of cellulose.

CONCLUSIONS: Cellulose being chosen as the most promising carbon source opens a new chapter of possibilities regarding agricultural waste utilization as an alternative source of a key nutrient. The conversion of lignocellulosic waste as a raw material in biotechnological production represents a promising approach for creating bio-based products in a cost-efficient way. Additionally, it is also important from the aspect of production sustainability and contributing to agricultural waste recycling. As the present study results revealed high antagonistic activity of the producing strain using a cellulose-based medium, further research will include the utilization of various agricultural waste in the media preparation. The following investigation would be a key step in developing viable and eco-friendly bioprocess solutions for the production of biocontrol agents, with the possibility of scaling up to the industrial level and potential commercialization.

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T2-P-22 Dilute acid hydrolysis of spent coffee grounds at mild conditions: A response surface methodology approach

Panagiota Tsafrakidou, Athanasia Goula, Ioannis Mourtzinos, Irene Pieria, Charilaos Voulgaridis, George Menexes, Costas G. Biliaderis, Alexandra-Maria Michaelidou[53]

KEYWORDS: dilute acid; hydrolysis; optimization; response surface methodology; spent coffee grounds

INTRODUCTION:

More than 6 million tons of spent coffee grounds (SCG) are produced annually during coffee beverage preparation. Up to date, they are being discarded in landfills due to the lack of efficient waste management methods. As a lignocellulosic waste stream, SCG could serve as an excellent sustainable raw material in biotechnological processes for the production of added-value chemicals/commodities. In this context, the present study examines the potential for sugars recovery from SCG, as a stepping stone to explore the use of this biomass in biotechnological processes.

OBJECTIVES:

Response surface methodology was used to develop a prediction model, as well as to identify the optimum conditions for the recovery of fermentable sugars from SCG, by employing dilute acid hydrolysis under mild conditions (temperature below 100°C, ambient pressure) in order to prevent the acid mediated thermal decomposition of monomeric sugars (intermolecu-

53 *Faculty of Agriculture, Aristotle University, Thessaloniki, Greece. Corresponding author: amichail@agro.auth.gr*

lar dehydration and polymerization processes).

METHOD / DESIGN:

A central composite design was employed to evaluate the four selected variables, i.e., reaction time, liquid to solid ratio, temperature, and H_2SO_4 concentration) for the determination of the optimum dilute acid hydrolysis conditions, in order to maximize the total sugars yield. The experiments were designed using the statistical software package Design Expert (Release 6.0.8). Sugar concentration was determined as glucose equivalent spectrophotometrically using the phenol-sulfuric method and the total sugar yield (Y_{TS}) was calculated according to the equation:

$$Y_{TS} = C_{TS} V_H / m_{dSCG} \times 100$$

Where C_{TS} is the total sugar concentration obtained from the calibration curve, VH is the hydrolysate volume and m_{dSCG} is the mass of the initial dried SCG before hydrolysis.

RESULTS:

Regression analysis was used to identify the influence of the independent variables on the sugars yield. In the final model, the non-significant factors ($p > 0.05$) were excluded from the model, which thus contains the linear effect of time (X_1), temperature (X_3), and H_2SO_4 concentration (X_4), as well as the quadratic effect of liquid to solid ratio (X_2). The following regression equation was obtained:

$$Y_{TS} = -51.27+0.05X_1+3.37X_2+0.43X_3+2.12X4-0.16X_2^2$$

The determination coefficient (R^2) of the proposed model was 0.816 ($p < 0.001$).

CONCLUSIONS:

Mild conditions for SCG acid hydrolysis pretreatment proved to be an efficient method for the extraction of hemicellulose type carbohydrates (soluble sugars). Response Surface Methodology (RSM) was successfully implemented to optimize the conditions of the process (i.e., reaction time, liquid to solid ratio, temperature, and H_2SO_4 concentration) and allowed the rapid screening for a large experimental domain. According to the analysis, maximization of the sugar yield can be obtained at 149.73 (~150) min, an L/S ratio of 10.25, 90°C, and 3% H_2SO_4. Under these conditions, hydrolysis efficiency reached 73,88% expressed in glucose equivalents, corresponding to an average of 0.1876 $g_{gluc.eq}/g_{scg}$ yield. The optimized process points to new possibilities for SCG's biotechnological valorization through the production of several chemical compounds by chemical and/or fermentation processes.

T2-P-23 The effect of *Trichoderma Spp.* on physiological parameters of two tomato cultivars grown under greenhouse conditions

Mirjana Bojović[54], Igor Vukelić[54], Zorana Srećkov[54], Zorica Mrkonjić[54], Danka Radić[55], Gordana Racić[54], Dejana Panković[54]

KEYWORDS: tomato; *Trichoderma*; greenhouse; non-destructive measurements

INTRODUCTION:

54 *Educons University, Faculty of Ecological Agriculture, Vojvode Putnika 87, 21208 Sremska Kamenica.*

Corresponding author: dejana.pankovic@educons.edu.rs

55 *University of Belgrade, Institute of General and Physical Chemistry Studentski trg 12/V, 11000 Belgrade*

Fungi that belong to the genus *Trichoderma* colonize plant rhizosphere and are considered as good candidates for the use in environmentally friendly agriculture as plant growth promoters and biocontrol agents. In recent years, investigations are focused on the use of non-destructive characterization of plant responses to different abiotic and biotic stresses.

OBJECTIVES:

Dualex sensor was used in order to pre-screen the reaction of tomato plants treated with different *Trichoderma* isolates, based on the previous investigations that imply that the positive effects of this fungi depend on the plant genotype.

METHOD / DESIGN:

The experiment was conducted in a randomized block design under greenhouse conditions with two replicates. In total 30 plants of two tomato cultivars were transplanted in soil, per treatment: NC-control Narvik, GZC- control Gruzanski zlatni, NT1 - *T. harzianum*; NT2 - *T. brevicompactum*; NT3 - *T. harzianum* + *T. brevicompactum*; GZT1 - *T. harzianum*; GZT2 - *T. brevicompactum*; GZT3 - *T. harzianum* + *T. brevicompactum*). The suspensions of *Trichoderma* isolates were applied in the root zone of tomato plants, in the phase of three established leaves per plant. Measurements of chlorophyll (Chl), flavonols (Flav) and anthocyanins (Ant) content were done in vivo on fully developed leaves of the tomato plants, using Dualex optical sensor (Force-A, Orsay, France), once per week during 50 days of plant growth. NBI (Nitrogen Balance Index) was calculated as Chl/Flav ratio.

RESULTS:

Results obtained in this experiment showed that in both cultivars, the content of chlorophyll did not change significantly after *Trichoderma* application. However, positive trend for Flav content was observed for the cultivar GZ in the treatment with T2. In contrast, at the beginning of the experiment, NBI index decreased in GZ as influenced with T1 and T2 treatments, which could indicate a shift from primary to secondary metabolism in mentioned cultivar. Moreover, it could be noticed that during plant growth content of anthocyanin decreased in both control and *Trichoderma* treatment conditions.

CONCLUSIONS:

In conclusion, we can suggest that use of non-destructive measurements with Dualex sensor could serve as starting point to better understanding of plant responses to *Trichoderma* presence. Moreover, measurements with Dualex can serve as a pre-screening method for testing the effect of larger number of *Trichoderma* isolates and their effect on more different tomato genotypes.

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T2-P-24 Xanthan production on winery wastewaters: optimization of process parameters important for biopolymer quality

Zorana Trivunović, Ida Zahović, Siniša Dodić, Jovana Grahovac, Jelena Dodić[56]

KEYWORDS: Biotechnological production; xanthan; winery wastewater; biopolymer quality; bioprocess optimization;

56 University of Novi Sad, Faculty of Technology Novi Sad, Department of Biotechnology and Pharmaceutical Engineering, Novi Sad, Serbia.

Corresponding author: ida.zahovic@uns.ac.rs

INTRODUCTION: Intensive growth of wine industry during the last few decades is responsible for various ecological problems due to disposal of huge volumes of untreated wastewaters. Winery wastewaters are difficult to treat by conventional methods because of its seasonal nature, unequal composition and substantial flow variations. Over the past years, biotechnological processes for obtaining market-valuable products have received a considerable attention for reuse of various industrial effluents and utilization of inorganic and organic pollutants. In previous research, the biotechnological production of xanthan, commercially the most important microbial biopolymer widely used in different branches of industry, is proven as sustainable solution for winery wastewaters utilization. Considering that xanthan application directly depends on its quality, it is important to ensure that cultivation of selected xanthan-producing microorganism, on winery wastewaters always results in accumulation of high-quality biopolymer. Temperature, aeration intensity and agitation speed are among the most important bioprocess parameters that affects the xanthan quality, and therefore its possible application.

OBJECTIVES: The objective of this study was to optimize the values of bioprocess parameters important for quality of xanthan produced on winery wastewaters.

METHOD/DESIGN: The reference strain *Xanthomonas campestris* ATCC 13951 was used as xanthan-producing microorganism. Medium for xanthan biosynthesis was prepared by mixing the wastewaters generated in different stages of white wine production (crusher washing, press washing and flotation tank washing) to achieve initial sugar content of 30 g/L. Xanthan production process was carried out in 7 L laboratory stirred tank bioreactor under aerobic conditions for 96 h at different values of temperature (25-35°C), air flow rate (1.0-2.5 vvm) and agitation speed (200-800 rpm) that were varied according to Box-Behnken design (3^3). The average molecular weight of xanthan and apparent viscosity of its 1% (w/v) aqueous solution were determined as indicators of produced biopolymer quality. Response surface methodology was used to define mathematical models, which describe the individual and interactive effects of examined parameters on the xanthan quality, while desirability function approach was applied to optimize their values.

RESULTS: According to the predictions of developed optimization model with the highest value of the overall desirability function (0.964), the xanthan of maximum possible quality is produced by cultivation of reference strain on winery wastewaters at temperature of 28.43°C, air flow rate of 1.91 vvm and agitation speed of 410.53 rpm. The predicted average molecular weight of synthesized biopolymer and apparent viscosity of its aqueous solution are $7.65 \cdot 10^5$ g/moL and 60.96 mPa·s, respectively.

CONCLUSIONS: The results obtained in this study represent valuable information about quality of xanthan produced under different bioprocess conditions that can be used in further investigation related to development of biotechnological production of xanthan on winery wastewaters.

ACKNOWLEDGMENT: This research is part of the project (451-03-9/2021-14/200134) funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

T2-P-25 Xanthan production on crude glycerol-based medium: optimization of time for inoculum preparation

Ida Zahović, Jelena Dodić, Zorana Trivunović[57]

KEYWORDS: Xanthan; *Xanthomonas campestris*; incubation time; crude glycerol; inoculum preparation;

INTRODUCTION: Xanthan is a non-toxic, biocompatible and biodegradable polysaccharide of microbiological origin. This biopolymer possesses exceptional rheological characteristics that make it widely used in the food, pharmaceutical, petrochemical, chemical and textile industry. Xanthan is produced industrially by submerged aerobic cultivation of reference strain *Xanthomonas campestris* ATCC 13951 on the medium of appropriate composition and under optimal conditions. Although glucose and sucrose are the predominantly used carbon sources in cultivation media for xanthan production, the rise in prices and the increasing demands for these sugars indicate the need for exploration of alternative substrates with lower market value. Among them, crude glycerol proved to be one of the most promising for the production of various high-value products including xanthan. The development of biotechnological process for the production of xanthan on crude glycerol-based media is still in initial stages due to variation in the tolerance of different *Xanthomonas* strains on the impurities present in this effluent. In order to eliminate the mentioned difficulties, it is necessary to optimize all phases of the xanthan production process for each selected *Xanthomonas* strain. The first step towards that goal involves optimization of the inoculum preparation procedure, which includes definition of the time for producing strain incubation.

OBJECTIVES: The objective of this work was to optimize inoculum preparation for xanthan production on crude glycerol based-medium in terms of incubation time of applied producing microorganism.

METHOD/DESIGN: Inoculum preparation was divided into two stages: inoculum I and inoculum II preparation. Experiments were performed according to a 3-level factorial design to evaluate the effects of two independent variables, i.e. incubation time of inoculum I and incubation time of inoculum II on xanthan quantity in crude glycerol-based production medium at the end of bioprocess. The commercial medium (YMB®) was used for inoculum I preparation and crude glycerol-based growth medium was used for inoculum II preparation. Both inoculums were prepared in aerobic conditions at 25°C and 150 rpm. For optimization of incubation time, the inoculated flasks of growth media were incubated at different times (24 h, 36 h and 48 h). The xanthan production was performed by cultivation of *Xanthomonas* PL4 strain, isolated from pepper leaves, in 300 mL Erlenmeyer flasks with 100 mL of the crude glycerol-based medium. The biosynthesis was performed under aerobic conditions at 30°C and 150 rpm for 168 h. Bioprocess efficacy was estimated based on the xanthan concentration in medium at the end of biosynthesis.

RESULTS: The most significant results of this research are shown graphically in *Figure 1*. The illustrated results show the predicted effect of incubation times of the inoculum I and inoculum II on the xanthan concentration in crude glycerol-based production medium at the end of bioprocess. According to the presented response surface plot, the applied *Xanthomonas* strain is the most productive if the incubation time of inoculum I is between 32 h and 40 h, and the incubation time of inoculum II in the range from 40 h to 48 h.

57 University of Novi Sad, Faculty of Technology Novi Sad, Department of Biotechnology and Pharmaceutical Engineering, Novi Sad, Serbia.

Corresponding author: ida.zahovic@uns.ac.rs

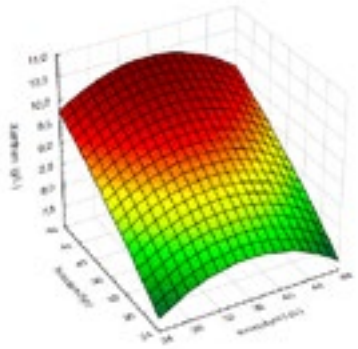

Figure 1. The effect of incubation time of inoculum I and inoculum II on xanthan concentration in crude glycerol-based production medium

CONCLUSIONS: The change in the duration of the second phase of inoculum preparation significantly affects the xanthan production on crude glycerol-based medium in applied experimental conditions. The developed model predicts that the maximum xanthan concentration of about 10.5 g /L can be achieved if the incubation times of inoculum I and inoculum II are 36 h and 48 h, respectively.

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T3-IL-1 Stability and color evolution of anthocyanins from *Cornelian cherry* extracts in different food systems

Ioannis Mourtzinos[1], Anastasia Loukri[1], Stamatia Christaki[1], Stella Chatzigavrill[2]

KEYWORDS: Anthocyanins; Colorants; Co-pigmentation; Cyclodextrin; *Cornelian cherry*

INTRODUCTION:

Food colorants are of great interest both in industrial and scientific basis. Nowadays, environmental concerns as well as the consumers' growing interest for natural and clean-label products contribute to the replacement of synthetic colorants with natural ones. To this respect, natural ingredients are being employed as food colorants. *Cornelian cherry* is a medicinal plant of the Mediterranean basin and it is a source of bioactive compounds, such as phenolics and anthocyanins. Due to its properties, it can be used for the production of innovative products either by using the whole fruit or by isolating the anthocyanins.

OBJECTIVES:

The aim of the present study was the evaluation of 'green' extracts from *Cornelian cherry* as food colorants. The extracts were evaluated as red colorants in real food systems.

METHOD / DESIGN:

Aqueous solutions of b-cyclodextrin were used as extraction media as well as means of anthocyanins' stabilization. FTIR analysis was used for the identification of possible interactions of cyclodextrin and phenolic compounds. The interactions of anthocyanins in the presence of metal ions i.e. Fe^{3+} and Ca^{2+} have also been evaluated. Spectrum analysis was used for the investigation of co-pigmentation effect. Colorimetric values and total anthocyanin content were evaluated in respect to cyclodextrin and metals presence. Model foods, i.e. acidic non-carbonated beverage and meat balls, were used for the evaluation of *Cornelian cherry* extracts as red colorants for the food industry.

RESULTS:

FTIR spectrum changes appear as a result of possible interactions of the *Cornelian cherry* compounds with cyclodextrin. The metal ions contribute also to spectrum changes as a result of the co-pigmentation effect. The final color of the extract in the presence of iron becomes darker and a blue color appears in pH=5. Cyclodextrin contributes to the stability enhancement of anthocyanins during storage under different conditions. The non-carbonated beverage containing the cyclodextrin extract had the lowest ΔE values during storage and highest content of total anthocyanins. On the other hand, in the meat product, the redness was slightly affected by the addition of the red extract.

CONCLUSIONS:

In acid conditions, the produced extracts have a clear red color and the anthocyanins show a good stability. In this study, blue derivatives were created in almost neutral conditions as a result of the co-pigmentation effect of Fe^{2+}. Cyclodextrin, despite the fading effect, contributed to the stability of anthocyanins in all examined conditions. For the first time, Cornelian cherry was used for the formulation of a red acidic non-carbonated beverage with attractive and stable color. Hence, the developed extraction conditions with cyclodextrin can induce the use of the extract in the food and beverage industry.

1 *Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.*

Corresponding author: mourtzinos@agro.auth.gr

2 *ORIZON Diatrofiki, 62100 Serres, Greece*

T3-IL-2 Rethinking plants as excelent sources of health and wellness-promoting compounds: inspired by nature and science

Sílvia M. Rocha[3]

KEYWORDS: plants; autochthonous species; bioactive compounds; sustainability; novel foods

INTRODUCTION:

Since the beginning of life on earth, plants have been used as a source of nutrients and compounds with therapeutic effects and/or promoters of well-being. Over the centuries, eating habits have changed, as well as the strategies for the treatment and prevention of diseases. Today, society and several public and private institutions have expressed their concerns regarding many of these changes and the negative impact they have on our quality of life.

According to World Health Organization (WHO), despite we are living on a planet with more than 50000 edible plants, 60% of our intake comes from just three types of plants: rice, maize and wheat, and the global demand for these three crops is projected to increase 33% by 2050. The lack of dietary diversity has huge impact on health and environment and is fueling the "double burden", i.e. it is not uncommon to find undernutrition and obesity co-existing within the same country, and even within the same community. On the other side, it is known that we are over-producing some foods, like red meat and whole grains, and under-producing others, such as vegetables, fruits and nuts.

Thus, taking advantage of the great developments that have been observed in terms of analytical technologies that have allowed the prospecting of numerous bioactive compounds in plants, as well as the evaluation of their physical-chemical, sensory and biological properties, we are in time to rethink their role in the diet of today's society. The diversity of the diet can be achieved even by the rational use of natural resources, in which non-conventional parts of plants can be safely used, as well as by-products or residues from agricultural and industrial activities. Local species can also play a very important role, especially those that are forgotten and have never entered the large food distribution networks, whose marketing can have a very significant impact on the economy of local communities. The strategies to be used to rethink the use of plants as a raw material of excellence for obtaining bioactive compounds and their role in the relationship between food and health will be discussed in this presentation, based on recent knowledge in the area. Finally, the TOP Covid-19, a 100% vegetable training kit for olfactory deviations recovery will also be presented.

ACKNOWLEDGEMENTS: Thanks are due to FCT/MEC for the financial support of QOPNA (UID/QUI/00062/2019) and LAQV-REQUIMTE (UIDB/50006/2020) Research Units and the following projects: ***SambucusValor*** (Integrated valorization of *Sambucus nigra L.* plant based-materials according to healthy consumption patterns: from the plant to the creation of new value-added food products - PDR2020-101-031117, Parceria no 146/Iniciativa no 341, funded by PDR2020), **ReStoragePear** (Development of strategies to prevent superficial scald and internal browning in Rocha pear and quality assurance in long-term storage - POCI-01-0247-FEDER-017777, supported by COMPETE 2020), and **AgroForWealth** (Biorefining of agricultural and forest products, by-products and wastes: integrated strategic valorisation of resources towards society wealth and sustainability - CENTRO-01-0145-FEDER-000001, funded by Centro2020), through national funds and co-funded by FEDER, within the PT2020 Partnership Agreement.

3 *LAQV-REQUIMTE & Chemistry Department, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal. Corresponding author: smrocha@ua.pt*

T3-IL-3 Algal bioactive metabolites for biomedical applications

Vassilios Roussis, Efstathia Ioannou, Stefanos Kikionis[4]

KEYWORDS: Marine biopolymers; Polysaccharides; Electrospinning; Biomedical applications

INTRODUCTION:

Natural polymers, due to their inherent biocompatibility and biodegradability, are highly appreciated as valuable ingredients for biomaterials and therefore are widely exploited in the biomedical field. Polysaccharides, incorporating various functionalities in their structures and exhibiting interesting physicochemical properties and significant biological activities, are considered attractive materials for the development of novel systems for biomedical applications, such as drug delivery and tissue engineering.

OBJECTIVES:

The aim of the study was the exploitation of marine polysaccharides as biopolymers for the preparation of nanofibrous matrices for drug release and scaffolds for cell cultures and tissue engineering.

METHOD / DESIGN:

Polysaccharides were extracted from green and brown algae and their molecular size was determined by size exclusion chromatography. The nanofibrous matrices were prepared by the electrospinning technique involving a variety of other copolymers and bioactive agents in the spinning solutions. The morphological characterization was performed by Scanning Electron Microscopy and the physicochemical characterization by Infrared Spectroscopy and Thermogravimetric and Differential Scanning Analyses.

RESULTS:

Nano- microfibrous matrices, based on marine polysaccharides and incorporating bioactive reagents were prepared by the electrospinning technique and tested on hairless mice or humans for their wound healing properties, exhibiting very high activity.

CONCLUSIONS:

The current results, in combination with the inherent cytocompatibility and the wide spectrum of biological properties of marine biopolymers, highlight the potential uses of the prepared polysaccharide-based biomaterials as novel nanofibrous matrices for biomedical applications.

4 *Section of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, Athens 15771, Greece. Corresponding author: roussis@pharm.uoa.gr*

T3-IL-4 Proteomic analysis of antimicrobial effects of lupinifolin in vancomycin-resistant enterococci

Wipawadee Sianglum[5], Chutimon Promthong[5], Sittirak Roytrakul[6], Nantiya Joycharat[7]

KEYWORDS: antimicrobial; lupinifolin; mechanism of action; multidrug resistance; vancomycin-resistant ***Enterococcus*** (VRE)

INTRODUCTION:

Enterococci are recognized as serious nosocomial pathogens that can produce biofilms, which contribute to their virulence and antimicrobial resistance. The organisms have confirmed the worldwide emergence of multiple-drug resistant (MDR) enterococci, particularly to vancomycin. Our previous studies have reported that lupinifolin, a prenylated flavonoid isolated from *Albizia myriophylla* Benth., showed great antibacterial properties against biofilm formers, MDR- and vancomycin- resistant enterococci (VRE). However, the detailed mechanisms of lupinifolin remained poorly understood.

OBJECTIVES:

The aim of this study was to assess the lupinifolin effects against VRE growth, biofilm formation, and proteome profile and gene expression changes.

METHOD / DESIGN:

The proteomic analysis using liquid chromatography-tandem mass spectrometry (LC–MS/MS) was performed on untreated control and VRE exposed to lupinifolin. Gene expression alterations after treatment were investigated by quantitative real- time PCR. Effect of various concentrations of the compound on the bacterial growth was assessed by time-kill analysis. Biofilm quantification after treatment were conducted using a crystal violet assay.

RESULTS:

The proteome profile changes involved in cell division and cell wall biosynthesis, cell membrane, stress response, cell surface antigen and virulence factor, and various metabolic pathways. Moreover, biofilm formation and the survival rate of the bacteria were reduced after exposure to lupinifolin in a dose-dependent manner.

CONCLUSIONS:

The data obtained in this study provide further evidence and knowledge of the anti-bacterial and anti-virulence properties, and the mechanism of action of lupinifolin. The findings may lead to the development of an effective and safe antimicrobial agents for treatment of VRE and MDR bacterial infections.

5 Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand. Corresponding author: wipawadee.s@psu.ac.th

6 Proteomics Research Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), The National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand

7 Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand

T3-IL-5 Impact of climate change in the adaptation and virulence of marine luminous bacterium *Vibrio campbellii*

Pimonsri Mittraparp-arthorn[8/9], Jiranan Pattano[9], Komwit Surachat[8/10]

KEYWORDS: bioluminescence; climate change; quorum sensing; virulence; Vibrio campbellii

Vibrio campbellii is a lumionous marine bacterium that occur in marine environments worldwide and is capable to survive and persist under unsuitable conditions such as seasonal change and salinity fluctuation. Some strains are highly pathogenic causing massive loss in shrimp during hatchery and on-growing phases. Climate change can result in outcomes that favor bacterial survival and virulence. The metabolism, fitness, and virulence of many marine pathogens has been previously reported to be correlated with the elevated ocean temperatures driven by climate change. Since this bacterium is globally distributed, this may allow it to infect a broader range of marine organisms.
In our study, *V. campbellii* HY01 isolated from shrimp that died with luminous vibriosis in Thailand was selected as a representative strain for the study of its metabolism, transcriptome profile, and virulence under changing environmental conditions (temperature, salinity, and pH). The differences in adaptation were also compared with a strain isolated from difference climate and geographical origins.
The results showed that temperatures, salinity, and pH are important for the light production, growth, hemolytic activity, biofilm formation, and virulence of V. campbellii. Therefore, this will be useful for environmental control of this pathogen in shrimp aquaculture and given the ongoing concerns about impact of climate change on disease in marine ecosystems.

T3-IL-6 The current status of *Cannabis sativa L.* therapeutic potential

Nebojša Kladar, Ljilja Torović, Biljana Božin[11]

KEYWORDS: *Cannabis L.*; cannabinoids; biological activity; pharmacological potential

Cannabis sativa L. Cannabaceae is, according to currently relevant taxonomy, the only species of genus *Cannabis L.* The history of its exploitation dates 5000 years BC, during which the co-existence of two types of *Cannabis sp.* could be observed. Glandular trichomes are the most important structures of *C. sativa* regarding the production of secondary metabolites of interest. Plants predominantly containing glandular trichomes are producing more resin and are used, or better to say abused, for their psychoactive effects. On the other hand, the plants predominantly containing non-glandular trichomes are being cultivated for production of fiber, as well as for food production. Although containing some common secondary metabolites, such as phenolics, flavonoids and alkaloids, *C. sativa* is of particular interest because of its resin, which is highly abundant in terpenoids and cannabinoids. Cannabinoids, commonly termed as phytocannabinoids, are terpenophenolic compounds derived from cannabigerolic acid. More than 100 cannabinoids have been isolated from *C. sativa*, but only ten of them have been studied in detail, of which tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) are attracting the

8 *Molecular Evolution and Computational Biology Research Unit, Faculty of Science, Prince of Songkla University, Hat Yai 90110, Songkhla, Thailand. Corresponding author: pimonsri.m@psu.ac.th*

9 *Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai 90110, Songkla, Thailand*

10 *Division of Computational Science, Faculty of Science, Prince of Songkla University, Hat Yai 90110, Songkhla, Thailand*

11 *University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, Novi Sad, Serbia*

most of research interest. It is important to highlight that THC and CBD do not exist in fresh plant material, but are being derived after harvest (or heat treatment) by decarboxylation of corresponding acidic forms – tetrahydrocannabinolic (THCA) and cannabidiolic (CBDA) acids. Furthermore, CBN is an oxidation product of THC and is primarly of interest in the area of forensics. The crucial difference between THC and CBD lays in their psychoactive potential. Cannabis plants containing resin rich in THC are consumed for their psychoactive effect, while CBD-rich plants (containing low amounts of THC) are not psychoactive, and are termed as hemp (or industrial hemp). The importance of phytocannabinoids for human physiology has been better understood after discovery of endocannabinoid system and corresponding cannabinoid receptors (CB1 and CB2). Namely, it was concluded that endocannabinoids regulate a number of processes in immunological and central nervous system. Therefore, phytocannabinoids as CB1 and CB2 agonists have the potential to interfere with these interactions (activation of potassium- and inhibition of calcium-dependent channels, regulation of GABA and glutamate release, activation of adenyl cyclase and extracellular kinases) which could be used in treatment of various pathological conditions. The previously stated was scientifically confirmed since anti-inflammatory, analgesic, anticonvulsive, neuroprotective, antipsychotic and immunosuppressive effects were reported for THC, CBD, cannabichromene (CBC), cannabigerolic acid (CBGA), etc. The successfully conducted clinical studies have shown benefits of CBD application in specific forms of child epilepsy – Lennox - Gastaut and Dravet syndrome. Furthermore, THC has been proved as effective supportive therapy in cancer and HIV-patients, as well as in patients with multiple sclerosis (MS). Although, a large number of studies reported cytotoxic and antiproliferative effects of THC, it must be stated that these *in vitro* studies were found as clinically irrelevant. However, the clinical significance of THC as partial agonist of CB1 and CB2 is reflected through exhibiting antiemetic effect and increasing the pain threshold, acting as anticonvulsive and increasing the patients' appetite, which is of particular importance in pathological conditions resulting with cachexia. The adverse effects of phytocannabinoids are mostly limited to THC-related psychoactive effects, while mode of interactions relate to increasing the P-glycoprotein expression and additive effects with drugs exhibiting similar pharmacological effect.

T3-P-1-ORAL Medial prefrontal cortex local field potential oscillations and attenuated craving behaviors in methamphetamine-induced addictive-like behaviors mice in response to *Mitragyna speciosa* (Korth.) Havil. leaves extract treatment

Jakkrit Nukitram[12/14], Dania Cheaha[13/14], Ekkasit Kumarnsit[12/14]

KEYWORDS: Conditioned place preference; Craving behaviors; Kratom; Local field potential; Medial prefrontal cortex; *Mitragyna speciosa*

INTRODUCTION:

Mitragyna speciosa (Korth.) Havil. (MS), also known as kratom plant, has been used to alleviate the craving for methamphetamine (METH) in communities of southeastern Asian countries. However, it remained to be confirmed with scientific proofs particularly in animal models.

OBJECTIVES:

This experiment examined the effects of MS extract on behaviors and local field potential (LFP) oscillatory rhythms in the medial prefrontal cortex (mPFC) of mice using METH conditioned place preference (CPP) model.

METHOD / DESIGN:

Mice were implanted with electrodes in the mPFC. They were randomly divided into control group treated with vehicle (saline+CMC) and 3 METH CPP groups treated with vehicle (METH+CMC), 40 (METH+MS40) and 80 (METH+MS80) mg/kg MS, respectively. To induce METH CPP, mice were given intraperitoneal injection of 1 mg/kg METH and 0.9% w/v NaCl solution in alternate days for 10 sessions and confined to METH and saline compartments, respectively, for 30 min in each session. Parallel manners were done in control mice, but 0.9% w/v NaCl solution was given on all days for both compartments. LFP signals and animal behaviors were recorded simultaneously during pre-conditioning and post-conditioning phases for 15 min. LFP signals were analyzed and expressed as percent relative powers of 6 discrete frequency bands (delta, theta, alpha, beta, gamma I and gamma II). CPP scores were analyzed for METH compartment preference. During post-conditioning phase, mice were treated with kratom extracts 60 min prior to CPP test.

RESULTS:

The results revealed significantly induced CPP scores in METH+CMC group. However, the elevated CPP score was significantly reduced by 80 mg/kg MS extract. Moreover, delta power was significantly increased and as well as alleviations of theta and alpha power were detected in METH CPP mice. Successful reversion of all parameters was accompanied with 80 mg/kg MS treatment.

CONCLUSIONS:

Collectively, this investigation provided the possible properties of MS for combating METH seeking. Taken together, MS leaves extract may be developed to be an alternative drug to ameliorate craving and other withdrawal symptoms from METH dependence.

12 Physiology program, Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Hatyai Campus, Hatyai, Songkhla 90112, Thailand. Corresponding author: 6110230003@psu.ac.th

13 Biology program, Division of Biological Science, Faculty of Science, Prince of Songkla University, Hatyai Campus, Hatyai, Songkhla 90112, Thailand

14 Biosignal Research Center for Health, Faculty of Science, Prince of Songkla University, Hatyai Campus, Hatyai, Songkhla 90112, Thailand

T3-P-2 Cytotoxic prenylated phenols of false indigo-bush (*Amorpha fruticosa L.*)

Stevan Samardžić[15], Marija Đorđić[16], Miloš Petković[17], Tatjana Stanojković[16], Zoran Maksimović[15]

KEYWORDS: Amorpha fruticosa; 5,7-dihydroxy-8-geranylflavanone; 2-carboxy-3,5-dihydroxy-4-geranylbibenzyl; amorfrutin A; cytotoxic activity

INTRODUCTION:

False indigo-bush (*Amorpha fruticosa L.*, Fabaceae) is an invasive shrub native to central and eastern North America. In Serbia, it can be found along roads, railways, and riverbeds, where it spreads easily. Previous studies have shown that *A. fruticosa* contains several classes of secondary metabolites (e.g. rotenoids, isoflavonoids, prenylflavanones, chalcones, and stilbenoids) and exhibits various biological activities (e.g. antioxidant, anti-inflammatory, antidiabetic, insecticidal).

OBJECTIVES:

This study aimed to examine the *in vitro* cytotoxic activity of prenylated phenolic constituents of false indigo-bush.

METHOD / DESIGN:

An aliquot (0.82 g) of ethanol-soluble part of n-hexane fruit extract (1:10, m/V) was loaded onto silica gel column (3 × 43 cm) and eluted with mixtures of n-hexane and ethyl acetate (100:0 _ 0:100, V/V). 96 Fractions were collected, analyzed by silica gel TLC and combined based on chemical composition similarity. Compound 1 (6.9 mg) precipitated as the white powder during solvent removal from the united fractions 59–69. The pooled fractions 89–92 (80.6 mg) and 95–96 (59.6 mg) gave compounds 2 (29.8 mg, Rf = 0.5) and 3 (5.8 mg, Rf = 0.53), respectively, after separation by preparative silica gel TLC in a solvent system consisting of n-hexane, ethyl acetate and formic acid (80:20:1, V/V/V). The structure determination of the isolated prenylated phenols were based on their UV, MS, and ^{1}H and ^{13}C NMR spectra. MTT assay was employed to assess their cytotoxicity against human tumor cell lines (HeLa, HT^{-29}, HCT^{-116}, LS174) derived from the cervix adenocarcinoma and colorectal carcinomas, as well as human fetal lung fibroblasts (MRC-5). cis-Diamminedichloroplatinum (CDDP) was used as a control substance. The activity is expressed as the IC_{50} value, i.e. the concentration of the tested substance that led to 50% inhibition of survival, compared to the vehicle-treated control group. The statistical analysis was performed using the Student's *t*-test.

RESULTS:

Based on a comparison of recorded spectral data and literature data, compound 1 was identified as 5,7-dihydroxy-8-geranylflavanone, compound 2 as 2-carboxy-3,5-dihydroxy-4-geranylbibenzyl, and compound 3 as 2-carboxy-3-hydroxy-4-prenyl-5-metoxybibenzyl (amorfrutin A). All tested constituents showed cytotoxicity with the IC_{50} values in the range 10.55–166.11 μg/mL. The non-selectivity of compounds 2 and 3 could be observed. On the other hand, compound 1 was selective and exhibited pronounced activity against the HeLa cell line (IC_{50} = 10.55 μg/mL). HT-29 (IC_{50} = 122.28 μg/mL) and HCT-116 (IC_{50} = 147.09 μg/mL) cancer cell lines, as well as normal cell line MRC-5 (IC50 = 166.11 μg/mL), were less susceptible to the action of compound 1, whereas the survival of LS174 cell line was not affected at all by compound 1 in the tested concentration range. The effectiveness of the control substance CDDP in reducing the survival of the HeLa cancer cell line was the

15 *Department of Pharmacognosy, University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, 11221 Belgrade, Serbia.*

Corresponding author: stevans@pharmacy.bg.ac.rs

16 *Institute of Oncology and Radiology of Serbia, Pasterova 14, 11000 Belgrade, Serbia*

17 *Department of Organic Chemistry, University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, 11221 Belgrade, Serbia*

highest (IC_{50} = 5.20 µg/mL). It should be emphasized that CDDP, among tested substances, was also the most toxic against the MRC-5 cell line (IC_{50} = 8.28 µg/mL). IC_{50} values ranges of compounds 2 and 3 were 31.97–56.29 µg/mL and 35.67–53.21 µg/mL, respectively.

CONCLUSIONS:
5,7-Dihydroxy-8-geranylflavanone, the constituent of false indigo-bush fruit, exhibited strong and selective activity against the human cervix adenocarcinoma cell line in the MTT assay, therefore, its cytotoxic potential can be considered significant. Further studies are needed to fully assess the demonstrated effect.

T3-P-3 Excipients with potential to cause adverse drug reactions in approved hormonal medicines for systemic use

Dejan Kusonić, Nemanja Todorović, Jelena Čanji, Nebojša Pavlović, Dragana Zaklan[18],
Dejana Bajić[19],
Mladena Lalić-Popović[18/20]

KEY WORDS: excipients with known effect; side effects; pharmacovigilance

INTRODUCTION: In addition to the active pharmaceutical ingredient (API), the composition of the medicines also includes excipients which are only ideally completely pharmacologically inactive. It has been shown that excipients can cause effects opposite to the pharmacological effect of the medicine. Therefore, it is very important to talk about excipients with known effect (EKE), which are excipients that have been proven to cause adverse drug reactions (ADRs) when they are in the composition of medicines.

OBJECTIVES: The aim of the study was to identify potentially harmful excipients in hormonal medicines for systemic use approved in the Republic of Serbia.

METHOD: The study was conducted during August 2021 and included the analysis of medicines that received a marketing authorization from the Medicines and Medical Devices Agency of Serbia (ALIMS). Qualitative compositions of hormonal medicines for systemic use (ATC group H) available in SmPC documents on the ALIMS's official website were observed. Excipients considered potentially harmful if they are recognized as excipients with known effect (EKE) in European regulation, Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use', available from European Medicines Agency's official website.

RESULTS: We analyzed 92 hormonal medicines for systemic use that are approved in Serbia. In their composition we found 70 different excipients. By comparing detected excipients with appropriate European regulation we identified 21 excipients from examined preparations that represent potential causes of ADRs: phosphate buffers (8 different buffers), mannitol, benzyl alcohol, benzalkonium-chloride, lactose-monohydrate, sodium, ethanol, glycerol, propylene-glycol, glucose, sodium-laurilsulfate, butylated hydroxytoluene, methyl p-hydroxybenzoate and propyl p-hydroxybenzoate.

18 *University of Novi Sad, Faculty of Medicine Novi Sad, Department of Pharmacy, Novi Sad, Serbia. Corresponding author: dejan.kusonic@mf.uns.ac.rs*

19 *University of Novi Sad, Faculty of Medicine Novi Sad, Department of Biochemistry, Novi Sad, Serbia*

20 *University of Novi Sad, Center for Medical and Pharmaceutical Investigations and Quality Control, Novi Sad, Serbia*

CONCLUSIONS: It has been shown that most detected EKE can cause mild ADRs after oral and parenteral administration such as irritation, headache, abdominal pain, mild diarrhea and allergic reactions (most often due to intolerance), while some excipients such as benzalkonium-chloride, sodium-laurilsulfate, phosphate buffers and butylated hydroxytoluene by the given method of administration do not cause any side effects. In addition, it has been shown that mannitol (34/92), lactose-monohydrate (27/92) and sodium compounds (21/92) are the most common excipients in composition of hormonal medicines for systemic use.

T3-P-4 Distinct nucleus accumben local field potential (LFP) fingerprints between two different types of addictive substances, morphine, and methamphetamine

Dania Cheaha[21], Chayaporn Reakkamnuan[22]

KEYWORDS: Nucleus accumben; Addiction; Morphine; Methamphetamine; local field potential

INTRODUCTION:

Nucleus accumbens (NAc), which is part of the ventral striatum, plays a major role in reward processing, reinforcement learning, and impulsivity. Reward-related stimuli including addictive substances activate dopamine release in the NAc and produce euphoria. This brain area has been used as a target site for the study of addictive drug effects. Power in local field potential oscillations (LFPs) in NAc reflect changes in dopamine neurotransmission and reward-related behaviours. While many addictive drugs involve various neurotransmission systems and produce a distinct behavioural effect, NAc LFP oscillation of different addictive drug classes might reveal some unique pattern.

OBJECTIVES:

This study was conducted to investigate the NAc LFP oscillation induced by acute administration of two different addictive drugs, morphine and methamphetamine.

METHOD / DESIGN:

Two-month-old male Swiss albino ICR mice with an intracranial implanted electrode in the NAc were treated either with saline, 15 mg/kg morphine, or 3 mg/kg methamphetamine subcutaneously. The LFP signal was recorded 30-minute prior to the treatment as the baseline, while the post-treatment was 180 minutes. The NAc LFP signal was analyzed as a spectral power within 1 – 500 Hz frequency range using the Fast Fourier transform (FFT). Normalized spectral power (%baseline) were averaged with a specific frequency range including delta (1 – 4.5 Hz), theta (4.75 – 6.75), alpha (7 – 12.5), beta (12.75 - 35), and gamma (35.5 – 500 Hz) and represented as a color code bar chart called LFP fingerprints.

RESULTS:

The results indicated that either morphine or methamphetamine administration produced a specific NAc LFP fingerprint. Morphine administration enhanced the low gamma (35.5 – 45 Hz) spectral power while methamphetamine predominantly increased spectral power within the high-frequency oscillation (145 – 500 Hz).

21 Division of Biological Science (Biology), Faculty of Science, Prince of Songkla University, Hatyai, Songkhla, 90110, Thailand.

Corresponding author: dania.c@psu.ac.th

22 Division of Health and Applied Sciences (Physiology), Faculty of Science, Prince of Songkla University, Hatyai, Songkhla, 90110, Thailand

CONCLUSIONS:

These findings suggest that various psychoactive substances are differentially produced a specific pattern of LFP power spectrum depending on its pharmacological action. Different addictive substances, morphine, and methamphetamine that modulated specific neurotransmitter systems in the NAc-related reward circuit revealed a unique LFP profile which is a fingerprint of its action.

T3-P-5 Orange carbon dots change the total phenolic content in maize

Ivana Milenković[23], Yiqun Zhou[24], Sladjana Z. Spasić[23/25], Roger Leblanc[24], Milan Borišev[26], Ksenija Radotić[23]

KEYWORDS: carbon dots; maize; nanoparticles; total phenolic content.

INTRODUCTION:

Carbon dots (CDs) are considered a green alternative to metal nanoparticles because they can be used where metal nanoparticles cannot be applied. Orange carbon dots (oCDs), synthesized from citric acid and *o*-phenylenediamine as precursors, are organic spherical nanoparticles with a lot of applications in various biomedical purposes such as drug delivery, bioimaging, and sensing. Ease of preparation, high photoluminescence, solubility in water, and biocompatibility are their main advantages.

OBJECTIVES:

The main aim of this research was to investigate the effect of oCDs on total phenolic content (TPC) in maize as an agricultural species. TPC reflects the contribution of phenolics as a group of secondary metabolites participating in the regulation of plant growth and in the defense responses. Also, it is one of the main indicators of oxidative stress which can cause a metabolic disorder in plants.

METHOD / DESIGN:

Three different concentrations (1 mg L^{-1}, 5 mg L^{-1} and 10 mg L^{-1}) of oCDs nanoparticles were used for the treatment of maize plants via KNOP/2 hydroponic solution during 2 week-growth under 16 h/8 h photoperiod. TPC was analyzed from extracts obtained from the roots and leaves of plants after foliar and solution treatments. Folin-Ciocalteu's spectrophotometric procedure was used for the determination of TPC in the samples.

RESULTS:

The results showed that foliar applications with all concentrations of oCDs induced decreases of TPC in maize leaves but did not affect these parameters in the roots. In solution treatment, the concentration of 1 mg/L of oCDs increased TPC in the leaves, but decreased in roots.

CONCLUSIONS:

The higher efficiency achieved with the lowest oCD concentration (1 mg/L) in foliar treatment makes this way of application advantageous compared with the solution counterpart.

23 University of Belgrade, Institute for Multidisciplinary Research, Department of Life Sciences, Kneza Višeslava 1, Belgrade, Serbia.

Corresponding author: ivana.milenkovic@imsi.rs

24 University of Miami, Department of Chemistry, Coral Gables, FL 33124, Florida, USA

25 Singidunum University, Danijelova 32, 11010 Belgrade, Serbia

26 Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia

T3-P-6 Sustained release of lignin model compound dehydrogenate polymer (DHP) from alginate beads

Dragica Spasojević, Mira Stanković, Miloš Prokopijević, Olivera Prodanović, Jasmina Stojkovska[27], Bojana Obradović[28], Ksenija Radotić[27]

KEYWORDS: dehydrogenate polymer; alginate; drug release

INTRODUCTION:

Alginate dressings are widely used in the treatment of exuding wounds[29]. The enzymatically synthesized lignin model compound dehydrogenate polymer (DHP) from coniferyl alcohol by the enzyme peroxidase, is the best lignin substitute used in various experiments[30]. In our previous work, we have shown that synthesized DHP has antibacterial and antibiofilm properties, and in combination with alginate has good potential for wound treatment[31].

OBJECTIVES:

The objective of this paper was to study the sustained release of DHP from low and medium viscosity alginate beads.

METHOD / DESIGN:

Synthesized DHP powder (0.8 mg) was added to 2 ml 2% (w/v) sodium alginate low (4-12 cP, 1% at 25 °C) and medium (≥2000 cP, 2% at 25°C) viscosity solution. The solution was transferred dropwise to calcium chloride in water for making the gel beads. In vitro release of DHP was monitored in 10 mL of distilled water. Aliquots were taken at predetermined time intervals (1, 2, 3, 4 and 24 h) and concentration of DHP was determined spectrophotometrically at 272 nm. The dissolution tests were performed in duplicate.

RESULTS:

Figure 1 shows release profiles of DHP from low and medium viscosity alginate beads. Low viscosity alginate showed slightly faster DHP release (~5%) compared to medium viscosity beads. After an initial burst release in the first hours, microbeads allowed slow and continuous release of DHP. After 24 hours 35% (for low) and 40% (for medium viscosity alginate) of entrapped DHP was released, suggesting that the time of monitoring should be prolonged. Fitting experimental data into different mathematical models for drug release kinetics, the highest value of correlation coefficient (R2) was obtained for the Korsmeyer-Peppas model. The n value was smaller than 0.5, indicating that the DHP release can be characterized as quasi-Fickian diffusion.

27 *Institute for Multidisciplinary Research, University of Belgrade, Kneza Višeslava 1, 11000 Belgrade, Serbia. Corresponding author: dragica@imsi.rs*

28 *Faculty of Technology and Metallurgy, Karnegijeva 4, University of Belgrade, Serbia*

29 *M. Ip, in Advanced Wound Repair Therapies, ed. David Farrar, Woodhead Publishing Series in Biomaterials, Woodhead Publishing, 2011, 416-449.*

30 *Radotic K, Micic M, Jeremic M. New insights into the structural organization of the plant polymer lignin. Ann N Y Acad Sci, 2005, 1048, 215–229*

31 *Spasojević D, Zmejkoski D, Glamočlija J, Nikolić M, Soković M, Milošević V, Jarić I, Stojanović M, Marinković E, Barisan-Asenbauer T, Prodanović R, Jovanović M, Radotić K. Lignin model compound in alginate hydrogel: a strong antimicrobial agent with high potential in wound treatment. Int. J. of Antimicrobial Agents, 2016, 48: 732–735.*

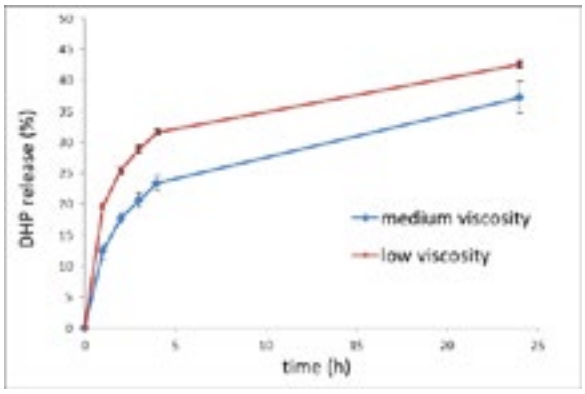


Figure 1. Release profiles of DHP from alginate beads (low and medium viscosity)

CONCLUSIONS:

The obtained results showed that alginate beads could be suitable for sustained DHP release. Prolonged release of antibacterial compound is beneficial for maintaining antimicrobial capacity, so it can be concluded from this study that DHP- alginate beads have good potential as chronic wounds healing agent.

T3-P-7 Effect of spermidine supplementation on autophagy genes expression in honey bee

Srđana Đorđievski[32], Tatjana Čelić[32], Elvira Vukašinović[32], Ivan Pihler[33], Danijela Kojić[32], Jelena Purać[32]

KEYWORDS: polyamines; longevity; health; pollinators.

INTRODUCTION:

Honey bee (*Apis mellifera L.*) is one of the most important pollinators in the world, however the number of colonies has been decreasing in the last few decades. Scientist revealed that major reasons of this problem are poor quality of winter feeding, pathogens, climate changes and excessive use of pesticides. Spermidine is a naturally occurring polyamine that participate in multiple biological processes. Its mechanisms of action are just beginning to be understood. Exogenous supply of spermidine prolongs the life span of several model organisms, significantly reduces oxidative damages and induces autophagy. Autophagy is a cytoprotective cell mechanism by which cell recycles damaged molecules and regulates metabolism. It has been proven that loss of autophagy gene function significantly influences health and shortens life span.

OBJECTIVES:

The aim of this experiment was to examine the effect of spermidine supplemented food on the expression of autophagy genes, Atg3, Atg5, Atg9 and Atg13, in honey bee.

METHOD / DESIGN:

The honey bees (*Apis mellifera L.*) were collected from bee hives and sorted in plastic boxes, each representing one experimental group. Three experimental groups were formed: the control group (C) in which bees were fed with 50% sucrose solution, and two treatment groups S1 and S2, where the sucrose solution contained 1 mM and 0.1 mM spermidine, respectively.

32 *University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia. Corresponding author: srdjana@dbe.uns.ac.rs*

33 *University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Novi Sad, Serbia*

The experiment was conducted for 20 days, after which the bees were instantly frozen. RNA was isolated from abdomen using RNA extracol. Further, cDNA was synthesized in reverse transcription reaction and qPCR was performed using the specific primers for autophagy genes Atg3, Atg5, Atg9 and Atg13.

RESULTS:

The results showed that the expression of selected autophagy genes, Atg3, Atg5, Atg9 and Atg13 were significantly increased in both S1 and S2 experimental groups, compared to control C.

CONCLUSIONS:

These results indicates that spermidine supplementation of honey bee diet induces autophagy and by that strongly influences their health and longevity.

T3-P-8 Effect of spermidine suplementation on vitellogenin gene expression in honey bees

Srđana Đorđievski[34], Tatjana Čelić[34], Elvira Vukašinović[34], Ivan Pihler[35], Danijela Kojić[34], Jelena Purać[34]

KEYWORDS: polyamines; longevity; health; pollinators.

INTRODUCTION:

Honey bee (*Apis mellifera L.*), one of the most important pollinators on the planet, provides a vital service to the ecosystem that is essential for maintaining natural biodiversity. However, the number of colonies has been decreasing for the last few decades, and scientists revealed that the main cause of this problem might be the reduced level of quality food during the wintertime, because of the disappearance of flowering plants. As a result of the mass colony disappearance, the health and survival of the honey bees have been attracting significant attention. There are many experimental tests of nutrition supplementation in many organisms, focusing on the improvements in health. Many studies have shown that spermidine, the naturally occurring ubiquitous polyamine, in different species has a positive effect on health and longevity, exhibits anti-oxidative activity, and is involved in normal vitellogenin synthesis. The vitellogenin is a fosfolipoglycoprotein that has diverse functions, including the regulation of a life span, and is believed to be important to honey bee social organization.

OBJECTIVES:

The aim of this study was to monitor the effect of dietary spermidine supplementation on the vitellogenin gene expression in honey bees.

METHOD / DESIGN:

Honey bees were collected from a bee hive and placed into plastic boxes with average number of 30 honey bees per box. Three experimental groups were formed, control C and treatments S1 and S2, each represented by three biological replicates. The control group (C) was fed by 50% (w/v) sucrose solution, while the feeding solution of the experimental groups S1 and S2 was supplemented with spermidine in concentration of 1 mM and 0.1 mM, respectively. The experiment was conducted for 20 days, after which the bees were instantly frozen. RNA was isolated from abdomens using RNA extracol. Further, cDNA was synthesized in reverse transcription reaction and qPCR was performed using the specific primer for vitellogenin gene.

34 *University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology. Corresponding author: srdjana@dbe.uns.ac.rs*

35 *University of Novi Sad, Faculty of Agriculture, Department of animal science*

RESULTS:

The results of the experiment showed that the expression of vitellogenin gene was significantly upregulated in experimental group S2 compared to control C.

CONCLUSIONS:

These results confirm the specific role vitellogenin might have in regulating honey bee longevity and stress resistance. These are the first results and further research is needed to confirm them and to investigate the mechanisms of spermidine action and exact role of vitellogenin in honey bees.

T3-P-9-ORAL Genomic analysis of carbapenem-resistant *Acinetobacter baumannii* clinical isolates using whole-genome sequencing data

Arnon Chukamnerd[36], Chanida Sakunrang[37], Kongpop Jeenkeawpiam[37], Rattanaruji Pomwised[38], Kamonnut Singkhamanan[36], Virasakdi Chongsuvivatwong[39], Prasit Palittapongarnpim[40], Mingkwan Yingkajorn[41], Sarunyou Chusri[42]*, and Komwit Surachat[37/43]*

KEYWORDS: carbapenem-resistant *Acinetobacter baumannii* (CRAB); whole-genome sequencing (WGS); antimicrobial resistance (AMR) genes; plasmid replicon types; virulence genes

INTRODUCTION:

Infection caused by carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a global healthcare challenge associating with a high mortality rate. CRAB commonly resists many antimicrobial classes due to it carries various antimicrobial resistance (AMR) genes and certain AMR genes are located on plasmids.

OBJECTIVES:

We aimed to assess the presence of AMR genes as well as plasmid replicon types and virulence genes using whole-genome sequencing (WGS) data.

METHOD / DESIGN:

The genomic DNA of 221 CRAB isolates was first extracted. The extracted DNA was then sequenced using sequencing of the BGIseq-500 system. Afterwards, the WGS results were analyzed using bioinformatics tools. The genome assembly and annotation were initially performed. The staramr software was used to identify AMR genes, while plasmid replicon types were detected using blastn with the database of *Acinetobacter* plasmids. In addition, virulence genes were investigated using blastn with virulence factor database (VFDB) of *Acinetobacter spp.*

36 Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand.

*Corresponding author: *sarunyouchusri@hotmail.com (S.C.) and komwit.s@psu.ac.th (K.S.)*

37 Molecular Evolution and Computational Biology Research Unit, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

38 Division of Biological Science, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

39 Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

40 Pornchai Matangkasombut Center for Microbial Genomics, Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand

41 Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

42 Infectious Disease Unit, Division of Internal Medicine, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

43 Division of Computational Science, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

RESULTS:

We found that 91.86% of CRAB isolates harbored bla_{OXA-23} gene, while only 8.14% of CRAB isolates carried bla_{NDM-1} or both bla_{OXA-23} and bla_{NDM-1} genes. These genes are responsible for carbapenem resistance in CRAB isolates. Other genes conferring resistance to aminoglycosides, fluoroquinolones, tetracyclines, sulfonamides, and macrolides were also detected in these CRAB isolates. For plasmid typing, the finding demonstrated that repAci1 was predominantly found in the CRAB isolates, following by repAci7, repM-Aci9, and repApAB49. Besides, the virulence genes associating with adherence, biofilm formation, phospholipase, immune evasion, iron uptake, regulation, and serum resistance were detected in most CRAB isolates.

CONCLUSIONS:

The WGS provides important information in the bacterial genome. In the present study, CRAB isolates contained various AMR genes and plasmid replicon types, which can transfer to other bacteria causing the widespread of AMR. Thus, if we can rapidly understand the molecular features of CRAB isolates, it might be easy to control the spread of these pathogens in the future.

T3-P-10 Lipophilicity as key factor for oral bioavailability of 1-aryl-3-methyl succinimide derivates

Dunja Marjanović, Nataša Milošević, Jelena Čanji, Nebojša Pavlović, Mladena Lalić Popović[44], Nebojša Banjac[45]

KEYWORDS: lipophilicity; permeability; bioavailability; in silico; drug design

INTRODUCTION:

Prediction of bioavailability of a drug candidate in the early stage of drug design and development followed by the selection of compounds with favorable structural properties are the key for later clinical efficiency of the potential therapeutic molecule. Lipophilicity is the pivotal physico-chemical property affecting permeability through biological barriers, potency, distribution, and elimination of a drug in the body. Since lipophilicity influence many biological properties, it is the most frequently applied parameter in drug discovery SAR studies.

OBJECTIVES:

To assess the influence of lipophilicity on the oral absorption of newly synthesized 1-aryl-3-methyl succinimide derivates which is determined in silico as Caco-2 permeability, absorption constant and percent of absorbed molecules.

METHOD / DESIGN:

Reversed chromatography was applied to determine experimentally the anisotropic lipophilicity of thirteen newly synthesized 1-aryl-3-methyl succinimide derivates. The mobile phase was mixture of water and methanol with a varying fraction of the organic solvent while as the stationary phase precoated RP-18W/UV 254 plates (Macherey-Nagel GMBH and Co., Düren, Germany) were applied. After development, the spots were detected at 254 nm with UV lamp. Software package i-lab 2.0 (https://ilab.acdlabs.com/iLab2/) was used for determining absorption constant, ka, while Caco-2 permeability and percent of the absorbed molecules (%absorbed) were predicted with pkCSM software (http://biosig.unimelb.edu.au/pkcsm/) for all compounds observed based on their structure.

44 *University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia. Corresponding author: m.dunja@hotmail.com*

45 *University of Belgrade, Faculty of Agriculture, Belgrade, Serbia*

RESULTS:

Anisotropic lipophilicity was quantified with retention constants, R_M^0 obtained by applying thin-layer reversed-phase chromatography for 13 newly synthesized 1-aryl-3-methyl succinimide derivates. All observed compounds are expected to have favorable Caco-2 permeability except compound 5 with carboxylic group which ionizes in physiological fluids. For all analyzed succinimide derivates short absorption times are expected as well as high absorption rate. Statistically significant parabolic correlation (r^2=0.447, p=0.021) was determined between Caco-2 permeability (calculated with pkCSM software) and anisotropic lipophilicity. Moreover, statistically significant parabolic association (r^2=0.710, p<0.001) was obtained between absorption constant, ka (i-lab 2.0 software) and experimentally determined anisotropic lipophilicity, RM0 for the observed compounds. Finally, the percent of the absorbed molecules, %absorbed (pkCSM software) was influenced by anisotropic lipophilicity with statistical significance (r^2=0.622, p=0.003) and described with parabolic function.

CONCLUSIONS:

Lipophilicity is the key characteristic in transport processes, including intestinal absorption and membrane permeability of 1-aryl-3-methyl succinimide derivates. The increment of lipophilicity of the 1-aryl-3-methyl succinimide core results in enhanced permeability, elevated absorption constants and enlarged bioavailability. However, the augmentation of the permeability through membranes and intestinal absorption as a result of increased lipophilicity is limited probably due to consequent solubility decrement of the studied compounds.

T3-P-11 Subchronic acrylamide treatment induces superoxide dismutase 1 expression in rat liver

Jelena Marković Filipović, Ivana Ivelja, Jelena Karan, Marko Miler, Verica Milošević[46], Milica Matavulj[47]

KEYWORDS: acrylamide; liver; superoxide dismutase 1

INTRODUCTION:

Acrylamide (AA) is a widely used chemical and an important monomer in various industrial and laboratory purposes. In addition, AA is formed in many types of fried and oven-baked foods during cooking. Considering proven neurotoxic, carcinogenic and mutagenic effects on living organisms, AA became a main topic of interest for many research groups.

OBJECTIVES:

The objective of our study was to determine whether acrylamide treatment affects superoxide dismutase 1 (SOD1) expression in rat liver.

METHOD / DESIGN:

Adult male Wistar rats were subchronicly (three weeks) treated with 25 mg/kg or 50 mg/kg body weight (b.w.) of AA. Formalin-fixed paraffin-embedded liver tissue was cut into 5 μm thin sections and immunostained with anti-SOD1 antibody. The amount of SOD1 in immunostained sections was determined using Windows based ImageJ program (ImageJ, Version 1.50f). The optical density (OD) and stained percentage color area of immunolabeled SOD1 were measured.

46 *Department of Cytology, Institute for Biological Research "Siniša Stanković", Serbia. Corresponding author: jelena.markovic@dbe.uns.ac.rs*

47 *University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia. Corresponding author: jelena.markovic@dbe.uns.ac.rs*

RESULTS:

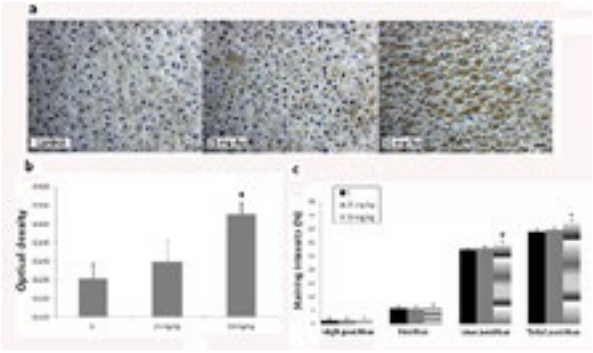


Figure 1. *Representative micrographs of superoxide dismutase 1 (SOD1) immunohistochemical staining in liver of control rats, rats treated with acrylamide (AA) in dose of 25 mg/kg b.w., and rats treated with acrylamide in dose of 50 mg/kg b.w. (a). Optical density of SOD1 immunopositive cells in control and AA-treated rats in doses of 25 mg/kg b.w. and 50 mg/kg b.w. (b). Percentage contribution of high positive, positive, low positive and total positive immunohistochemical staining of SOD1 in control and AA-treated rats in doses of 25 mg/kg b.w. and 50 mg/kg b.w. (c). Values in charts are means ± SEM; n = 10, *p < 0.05. In statistical analysis AA-treated animals were compared with the control group.*

CONCLUSIONS:

Immunostaining of SOD1 in liver of control rats showed weak cytoplasmic immunoreactivity in hepatocytes (*Fig. 1a*). AA application induced dose-dependent increase of immunostaining intensity (*Fig. 1a*). Significant increase of OD and percentage contribution of low positive and total positive cells of immunostained SOD1 was detected in group treated with AA in a dose of 50 mg/kg (*Fig.1b, c*).

T3-P-12 Correlation between anisotropic lipophilicity and *in silico* predicted human distribution of 1-aryl-3-methyl succinimide derivates

Dunja Marjanović, Nataša Milošević, Maja Milanović, Nataša Milić[48],
Nebojša Banjac[49], Gordana Uščumlić[50]

KEYWORDS: lipophilicity; volume of distribution; plasma protein binding; in silico; drug design

INTRODUCTION:

Lipophilicity of drug candidates is used in quantitative structure–activity relationship (QSAR) studies, as molecular descriptor in ADME-tox predictions and as structural information about their biological effects. The distribution of drugs in the body depends mainly on their lipophilicity and their potential to bind to plasma proteins.

OBJECTIVES:

To analyze the influence of lipophilicity on the distribution of newly synthesized succinimide derivates in the human body based on in silico predicted volume of distribution and affinity to bind to the plasma proteins.

METHOD / DESIGN:

Thirteen newly synthesized 1-aryl-3-methyl succinimide derivates were studied by reversed chromatography and their anisotropic lipophilicity was determined. Precoated RP-18W/UV 254 plates (Macherey-Nagel GMBH and Co., Düren, Germany) was used as stationary phase while binary solutions of methanol and water with a varying volume fraction of organic solvent were applied as the mobile phase. The spots were detected at 254 nm with UV lamp. Software package i-lab 2.0 (https://ilab.acdlabs.com/iLab2/) was used for determining volume of distribution (Vd) while pkCSM (http://biosig.unimelb.edu.au/pkcsm/) was applied for predicting the volume of distribution in stationary state (Vdss) and the fraction of the drug unbound

48 *University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia. Corresponding author: m.dunja@hotmail.com*

49 *University of Belgrade, Faculty of Agriculture, Belgrade, Serbia*

50 *University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia*

to plasma protein (%unbound) based on the structure of the molecules. Finally, the percent of the drug bound to plasma proteins (PPB) was calculated by PreAdmet software (https://preadmet.bmdrc.kr/adme/) for all compounds observed.

RESULTS:

Retention constants, R_M^0 for 13 newly synthesized 1-aryl-3-methyl succinimide derivates obtained by using thin-layerv reversed-phase chromatography were applied as measurement of anisotropic lipophilicity. The values of the volume of distribution and plasma protein binding affinity varied depending on the software applied, but regardless of the applied software small distribution volumes and high protein binding affinity are expected for these compounds. Nevertheless, statistically significant parabolic correlation (r^2=0.968, $p<0.001$) was described between volume of distribution (i-lab 2.0 software) and anisotropic lipophilicity followed by statistically significant parabolic association (r^2=0.965, $p<0.001$) between volume of distribution in stationary state, Vdss, (pkCSM software) and experimentally determined anisotropic lipophilicity, RM0 for the analyzed compounds. Furthermore, the fraction of the drug unbound to plasma proteins (calculated with pkCSM software) was correlated with anisotropic lipophilicity of the analyzed compounds and parabolic association was obtained with high statistical quality (r^2=0.497, $p=0.013$). In addition, the percent of the drug bound to plasma proteins, PPB (PreAdmet software) was associated with anisotropic lipophilicity for observed series of succinimide derivatives with statistical significance (r^2=0.799, $p<0.001$).

CONCLUSIONS:

Lipophilicity is the primary underlying structural property that governs the distribution of 1-aryl-3-methyl succinimide derivates and their affinity to bind to plasma proteins. Introducing more lipophilic substituent in the 1-aryl-3-methyl succinimide core consequently results with increased volume of distribution followed by enhanced plasma protein affinity. One should be careful when making structural modifications that change lipophilicity in order to adjust an ADMET property since other properties that are affected by lipophilicity may be altered as well and should be monitored.

T3-P-13 Acrylamide treatment affects oxidative stress parameters in rat hepatocytes

Jelena Marković Filipović[51], Danijela Kojić[51], Marko Miler[51], Verica Milošević[52], Milica Matavulj[52]

KEYWORDS: acrylamide; hepatocytes; nitrite; gluthatione; lipid peroxidation

INTRODUCTION:

Acrylamide (AA) is industrial toxic substance with neurotoxic and reprotoxic effects. AA is a Maillard reaction product formed during processing of starchy food at high temperature.

OBJECTIVES:

The objective of our study was to determine whether acrylamide treatment disturbs redox balance by altering nitrite, glutathione (GSH), and malondialdehyde levels in rat hepatoma cell line (H4IIE).

METHOD / DESIGN:

Rat hepatoma cell line H4IIE was treated with 4 mM (IC_{20}) and 4.5 mM (IC_{50}) of AA for 24 h. The nitrite level in the medium was

51 *University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia. Corresponding author: jelena.markovic@dbe.uns.ac.rs*

52 *Department of Cytology, Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia*

analyzed as an indicator of NO production following the Griess reaction method. After ultrasonic cell lysis in 2.5% sulfocalicylic acid, supernatant was analysed for the content of gluthatione. Lipid peroxidation was evaluated using thiobarbituric acid reactive substance assay (TBARS).

RESULTS:

Detected nitrite, malondialdehyde and GSH levels in rat hepatoma cell line H4IIE after acrylamide treatment are shown in *Figure 1*.

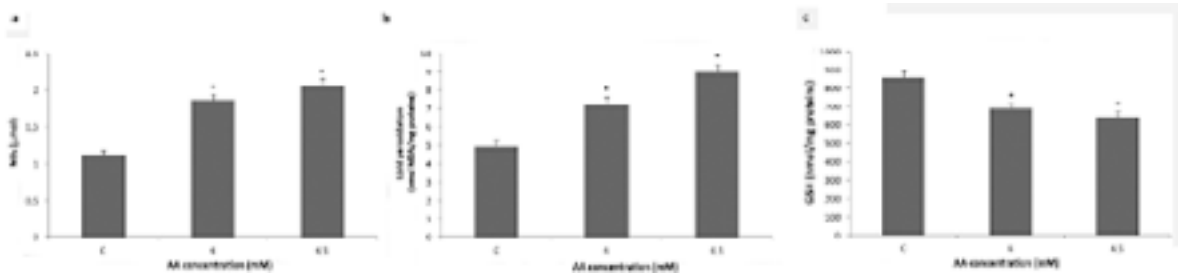


*Figure 1. Nitrite concentration (a), malondialdehyde (MDA) concentration (b), reduced glutathione (GSH) concentration (c) in H4IIE cells after treatment with 4 and 4.5 mM acrylamide (AA) for 24 h. Values in charts are means ± SEM of three experiments performed in triplicate. Mean values were significantly different from that of untreated control cells (*p<0.05).*

CONCLUSIONS:

In rat hepatoma cell line H4IIE, exposure to AA caused significant concentration-dependent increase of nitrite level and lipid peroxidation (*Fig. 1a, b*). On the other hand, GSH content significantly decreased in a concentration-dependent manner in H4IIE cells (*Fig.1c*). Obtained results indicate that AA disturbs redox status in hepatocytes.

T3-P-14 Multi-targeted anticancer activity of human amniotic membrane homogenate on various cancer cells

Aleksandar Janev[53], Taja Železnik Ramuta[53], Larisa Tratnjek[53], Žiga Sardoč[53], Hristina Obradović[54], Slavko Mojsilović[54], Milena Taskovska[55/56], Tomaž Smrkolj[55/56] and Mateja Erdani Kreft[53]

KEYWORDS: bladder cancer; human amniotic membrane; anticancer activity, 2D and 3D in vitro models

INTRODUCTION:

Based on the 2020 GLOBOCAN data, bladder cancer ranks as one of the ten most common cancer types throughout the world. Despite its increasing incidence and high recurrence rates, there have been no significant improvements in the standard treatment options of bladder cancer. Human amniotic membrane (hAM) is an innermost fetal membrane, which is associated with a wide range of biological properties such as anti-inflammatory, anti-fibrotic and anti-microbial activity. Furthermore, recent studies have underlined the possibility that human amniotic membrane (hAM) might also act as a promising anti-cancer agent.

53 *Institute of Cell Biology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia. Corresponding author: mateja.erdani@mf.uni-lj.si*

54 *Laboratory for Experimental Hematology and Stem Cells, Institute for Medical Research, University of Belgrade, Belgrade, Serbia*

55 *Department of Urology, University Medical Centre Ljubljana, Ljubljana, Slovenia*

56 *Chair of Surgery, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*

OBJECTIVES:

The aim of this study was to evaluate the anticancer effect of hAM homogenate on 2D and 3D cancer *in vitro* models.

METHOD / DESIGN:

Human muscle-invasive bladder cancer urothelial (T24) cells, papillary cancer urothelial (RT4) cells, normal porcine urothelial (NPU) cells, human mammary gland nontumorigenic (MCF10a) cells and low-metastatic breast cancer (MCF7) cells were treated with different hAM homogenate preparations at different time points. The effect of hAM homogenate on the desquamation of cancer cells, their attachment capacity, proliferation rate and spheroid architecture was evaluated.

RESULTS:

After 24-hour treatment with hAM homogenate, we observed gradual desquamation of cancerous cells, while normal cells remained firmly attached to the culture surface. We observed that hAM homogenate caused the highest desquamation of bladder cancer cells in a time-dependent manner. Additionally, we demonstrated that hAM homogenate not only caused desquamation but also significantly decreased the adhesion, growth and proliferation rate of human bladder invasive and papillary cancer cells, whereas it did not affect the normal urothelial cells. In addition, with the help of light and electron microscopy, we showed that hAM homogenate disrupted the 3D structure of bladder cancer spheroids.

CONCLUSIONS:

The obtained results further strengthen the idea for the potential clinical application of hAM in bladder cancer treatment. Nevertheless, additional in vitro and in vivo studies are needed to identify the main molecules inside the hAM that exert the anti-cancer effect and to elucidate their cell biological mechanism of action.

T3-P-15 Effects of Di(2-Ethylhexyl) Phthalate on primordial germ cells in zebrafish (*Danio rerio*) embryos

Biljana Tesic, Svetlana Fa, Dragana Samardzija Nenadov, Kristina Pogrmic-Majkic, Nebojsa Andric[57]

KEYWORDS: DEHP; zebrafish embryos; primordial germ cells; reproduction.

INTRODUCTION:

Di(2-Ethylhexyl)Phthalate (DEHP) belongs to the group of synthetic chemicals with ubiquitous exposures because of its use in plastics and cosmetics. It has been shown that reproduction and development of the reproductive system is highly sensitive to DEHP exposure. Development of the zebrafish reproductive system begins with migration of embryonic primordial germ cells (PGCs) to genital ridge, where PGCs govern the process of gonad formation. Zebrafish sex determination is also dependent on environmental influence. During embryogenesis, migration, number and proper function of PGC are crucial events in the development of the reproductive system. Zebrafish PGC migrate to genital ridge during the first 24 h of embryogenesis and the process can be traced by means of *sdf-1* and *cxcr-4b* genes, which navigate the process, as well as *nanos1* and *esr2a*. It was shown that an estrogen receptor encoded by *esr2a* mediates estradiol coordinated distribution of PGC. There are also other genes which are important for maintenance, proliferation and differentiation of PGC such as *dazl, vasa, nanos1 and piwil1*. The dominance of *amh* or *cyp19a1* expression indicates certain sex development in zebrafish, while disruption of ar expression increases the number of female offspring. *esr1* and *esr2a* genes by encoding estrogen receptors are involved in cellular responses to estrogens. It has been shown that DEHP exposure of zebrafish from hatching till adulthood

57 *Faculty of Sciences University of Novi Sad. Corresponding author: svetlana.fa@dbe.uns.ac.rs*

significantly disrupted ovarian development in females and spermatogonia and spermatocytes formation in males followed by the reduction of fecundity in both sexes. However, there is no data how DEHP affects PGCs in zebrafish which give rise to the future gametes and are crucial for a gonad formation.

OBJECTIVES:

Our study aims to investigate effects of DEHP on zebrafish PGC by evaluating the expression of genes crucial for migration (*sdf-1, cxcr-4b, esr2a, nanos1*), maintenance, differentiation and functioning of PGC (*piwil1, nanos1, dazl, vasa*) and hormonal homeostasis (*cyp19a1, amh, esr1, esr2a, ar*). Global DNA methylation as a screening tool of the DEHP induced global effects on the zebrafish genome was also analyzed.

METHOD / DESIGN:

Zebrafish embryos were treated with DEHP (0, 10^{-8} M, 10^{-7} M and 10^{-6} M) from 5 h post fertilization (hpf) until 5 days post fertilization (120 hpf). The control and the DEHP-treated embryos were collected at 24 hpf and 120 hpf. The relative mRNA of genes involved in migration, maintenance, differentiation and functioning of PGC and hormonal homeostasis was analyzed by relative quantification using the real-time PCR. The global DNA methylation was assessed in 120 hpf embryos by Whole Mount Immunostaining (Incubation with primary 5-mC antibody and secondary Alexa Flour 488 antibody).

RESULTS:

Our results revealed that in 24 hpf zebrafish embryos, DEHP inhibited the expression of *cxcr-4b* gene which encodes the receptor on PGC cell surface crucial for their migration to genital ridge. In 120 hpf embryos DEHP inhibited the expression of *dazl* (proper functioning of germ cells), *esr2a* and *amh* (hormonal homeostasis) genes. The results revealed that DEHP had no significant effect on the global DNA methylationin in 120 hpf embryos.

CONCLUSIONS:

Our results show that DEHP can disrupt mRNA expression of genes involved in regulation of migration, maintenance and functioning of PGCs as well as hormonal homeostasis during the early embryonic development of zebrafish. This may have lasting effects on their expression and can be reflected in the further development and functioning of the reproductive system.

T3-P-16 Chemical and biological properties of juice and wine made from Malvasia grape variety originating from Fruška Gora, Serbia

Diandra Pintać[58],
Marija Lesjak, Dejan Orčić, Neda Mimica-Dukić[59]

KEYWORDS: Malvasia; grape juice; wine; Fruška Gora; polyphenols

INTRODUCTION:

Malvasia is a grape variety belonging to an ancient and heterogenic grapevine group, most probably originating from Greece and growing in the Mediterranean basin. Although it is cultivated in the neighboring countries such as Croatia and Slovenia, where it is very popular, in Serbia Malvasia can only be found in a few vineyards, one of which is located in a famous winery region of Fruška Gora.

58 *University of Novi Sad Faculty of Medicine, Novi Sad, Serbia. Corresponding author: diandra.pintac@mf.uns.ac.rs*

59 *University of Novi Sad Faculty of Sciences, Novi Sad, Serbia*

OBJECTIVES:

Since the agroclimatic factors can greatly influence the chemical composition of grapes, and consequently wine, which in turn affects the organoleptic properties of grapevine products, the aim of this study was to determine the polyphenolic content of grape juice and wine of Malvasia variety originating from Serbia and evaluate their antioxidant and neuroprotective properties.

METHOD / DESIGN:

Malvasia grape juice and wine were obtained from a winery located in the Fruška Gora vineyard region during the year 2015. The samples were filtered and stored at -20°C before the assays. Total phenolic and flavonoid content was determined using standard spectrophotometric assays while a detailed quantitative analysis of 39 phenolic compounds was performed by the LC-MS/MS technique. Antioxidant potential was evaluated by measuring the reduction potential (FRAP) and free radical scavenging ability of the samples towards diphenylpicrylhydrazyl (DPPH•) and nitric oxide (•NO) radicals. Neuroprotective properties of the samples were tested by conducting the acetylcholinesterase (AChE) inhibition assay.

RESULTS:

Both samples showed a similar content of total flavonoids, while the wine had a higher total phenolic content. 21 compounds were detected and were more abundant in Malvasia wine compared to grape juice, with the exception of esculetin, kaempferol, kaempferol-3-*O*-glucoside, quercetin-3-*O*-glucoside and hyperoside. Wine contained large quantities of caffeic acid, as well as *p*-coumaric, ferulic and 2,5-dihydroxybenzoic acids. Both juice and wine exhibited good reduction potential, wine was better at scavenging DPPH•, juice at scavenging •NO, but the samples did not express inhibitory activity towards the enzyme AChE.

CONCLUSIONS:

This study characterized the phenolic profile and biological activity of Malvasia grape juice and wine originating from Fruška Gora, Serbia, in order to elucidate its properties that are, to the best of the authors' knowledge, missing in scientific literature. Wine expressed a better antioxidant activity and a much richer phenolic composition compared to grape juice. Juice contained higher content of a few flavonoids, while caffeic acid stood out in wine, along with some other phenolic acids. Further analyses of regional Malvasia grape products are needed to better define the unique properties that arise from specific agroclimatic factors and vineyard and winery practices of different winery regions.

T3-P-17 Potential mechanisms of Action of vitamin D affecting Sars-Cov-2

Milana Vuković, Jelena Jovičić Bata, Mladena Lalić Popović, Nemanja Todorović , Jelena Čanji Panić[60], Dunja Vesković[61]

KEYWORDS: vitamin D; COVID-19; pandemic; SARS-CoV-2; mechanism of action, angiotensin-converting enzyme 2.

INTRODUCTION:

Since the outbreak of the pandemic, vitamin D has been in focus as a potential molecule for prevention and treatment of COVID-19 because of its already known role in the immune response to pathogens, including respiratory viruses. The sup-

60 Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia. Corresponding author: milana.milankovic@mf.uns.ac.rs

61 Clinic for Dermatovenerology Diseases, Clinical Center of Vojvodina, Novi Sad, Serbia

plementation with vitamin D has been popularized in many countries to alleviate vitamin D deficiency, especially during lockdown.

OBJECTIVES:

The objective of this study was to present the potential mechanisms of action of vitamin D that were reported as potentially beneficial against SARS-CoV-2 and identify ongoing clinical trials regarding COVID-19 and vitamin D.

METHOD / DESIGN:

PubMed and Google Scholar databases were searched in order to identify articles published in 2020 and 2021 concerning the objective of this study. The registry ClinicalTrials.gov was searched using search terms "COVID-19" and "vitamin D" to identify registered clinical trials (October 13, 2021).

RESULTS:

The classical mechanisms reported to support antiviral effects of vitamin D are based on its ability to up-regulate antimicrobial peptides and induce antiviral cytokines to interfere the viral replicative cycle. Vitamin D promotes the production of antimicrobial and antiviral peptides like cathelicidin and β-defensin 2. Vitamin D induces the respiratory epithelial cells to produce cathelicidin LL-37, a peptide that competitively binds to SARS-CoV-2 S protein. That way cathelicidin LL-37 inhibits viral binding to the receptor angiotensin-converting enzyme 2 (ACE2) and most likely prevents viral entry into the cell.

Vitamin D has been shown to down-regulate the activity of T helper cells type 1 and 17 and promote differentiation of regulatory T cells. This leads to a decrease in the production of proinflammatory cytokines, including interleukin IL-6, IL-8, IL-12, tumor necrosis factor-α, interferon-γ and IL-17, thereby mitigating the cytokine storm syndrome in patients with COVID-19 with high inflammatory burden. It has been suggested that activation of the vitamin D receptor in the pulmonary stellate cells might play a role in suppressing inflammation and fibrotic changes in the lungs of patients with COVID-19.

Once the SARS-CoV-2 enters the body, it binds to the extracellular domain of ACE2 in nasal, lung, and gut epithelial cells through its spike glycoprotein subunit S1. ACE2 directly catalyzes angiotensin II, a natural peptide hormone in the renin angiotensin aldosterone system (RAAS) with strong vasoconstriction effect, thereby lowering its levels. COVID-19 infection down-regulates ACE2, which in turn could lead to excessive accumulation of angiotensin II. High levels of angiotensin II cause heightened pulmonary vasoconstriction and severity of COVID-19 and may also cause acute respiratory distress syndrome (ARDS), myocarditis with heart failure and acute kidney injury. The vitamin D analogue calcitrol induces expression of ACE2 in the lungs in experimental animals in specific experimental conditions. ACE2 thus expressed more as a consequence of vitamin D supplementation might reduce lung injury. Although vitamin D increases the expression of ACE2, which indeed promotes the binding of the virus, it prevents pulmonary vasoconstriction response in COVID-19 cases. ACE2 has potentially contradictory roles. On one hand, greater expression of it would be assumed as a potential risk since it is the receptor for SARS-CoV-2, but on the other hand, it has an important protecting role against acute lung injury and ARDS. Suppression of RAAS by vitamin D is also achieved by inhibition of cAMP response element-binding protein (CREB), a transcription factor key for the renin gene regulation.

All of the mentioned mechanisms of action are still an area of research and need to be clinically confirmed.

A total of 110 clinical trials regarding vitamin and COVID-19 were registered in the ClinicalTrials.gov registry, out of which 32 were completed, and 72 were active or had not yet started recruiting participants. Regarding the type, most of the trials were interventional (78/110), while there were 32/110 observational trials.

CONCLUSIONS:

The possible antiviral activity of vitamin D encompasses complex signaling pathways. As the exact molecular mechanisms are being clarified, new clinical trials are designed in order to confirm the link between vitamin D deficiency and COVID-19 severity and the potential benefits of vitamin D administration for prevention and treatment of COVID-19.

T3-P-18 Is microglia associated with glioblastoma its enemy or ally?

Jovana Drljaca[62/63], Dragica Bulajic[62/63], Aleksandra Popovic[63/64], Tihomir Dugandzija[65], Nebojsa Stilinovic[66], Aleksandra Isakovic[67], Slobodan Sekulic[68], Ivan Capo[63/69]

KEYWORDS: microglia; glioblastoma; tumor microenvironment; experimental animal model

INTRODUCTION:

Glioblastoma (GBM) is one of the deadliest primary brain neoplasm, heavily infiltrated with tumor-associated microglia/macrophages (TAM). Recently, the role of TAM in GBM progression has received a great deal of interest. Bearing in mind that the number of peripheral macrophages by the 14th day is negligible, in our study TAM were referred to as microglia.

OBJECTIVES:

Here we evaluated histopathological characterization of TAM, the kinetics of their infiltration and their impact on U87 orthotopic GBM, a commonly used model in preclinical research.

METHOD / DESIGN:

After placing Wistar rats on a stereotaxic frame (Stoelting®, USA), U87 cell suspension was injected into putamen. The inflammatory reaction against the human GBM cells was inhibited with dexamethasone subcutaneous injections (1 mg/kg) daily, starting on the day before inoculation. The animals were sacrificed 4, 7 and 14 days post-inoculation (n= six animals per time point). Following the tissue processing, tumor volume was determined, and immunohistochemical analyses were performed using anti-GFAP, anti-Ki67, anti-human nucleoli, anti-Iba1, and anti-CD34 antibodies.

RESULTS:

Our data demonstrated that the highest areal density of TAM was 7 days after GBM inoculation, with ability to proliferate early after initiation of GBM growth. In addition, the areal density of TAM within the tumor correlated with GBM growth and proliferation. Moreover, microglia underwent substantial morphological changes upon exposure to GBM cells. A transition from ramified morphology in peritumoral area to ameboid shape with larger soma and shortened, thick branches in the tumor core was observed during time. Tumor growth was accompanied by the higher areal fraction of blood vessels, which correlated with the areal density of TAM.

CONCLUSIONS:

Clarifying that microglia in this experimental GBM model showed similar morphological pattern and pro-invasive features as microglia in human GBM, these findings highlight the use of microglia in U87 experimental GBM as a potential target for manipulating GBM growth and a new strategy to fight with.

62 *Faculty of Medicine, University of Novi Sad, Serbia. Corresponding author: jovana.drljaca@uns.ac.rs*

63 *Center for Medical and Pharmaceutical Investigations and Quality Control, Faculty of Medicine, University of Novi Sad, Serbia*

64 *Department of Physiology, Faculty of Medicine, University of Novi Sad, Serbia*

65 *Departmentof Epidemiology, Faculty of Medicine, University of Novi Sad, Serbia*

66 *Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad, Serbia*

67 *Institute of Medical and Clinical Biochemistry, School of Medicine, University of Belgrade, Serbia*

68 *Department of Neurology, Faculty of Medicine, University of Novi Sad, Serbia*

69 *Department of Histology and Embryology, Faculty of Medicine, University of Novi Sad, Serbia*

T3-P-19 Defensive secretions of Millipedes *Megaphyllum unilineatum* (C. L. Koch, 1838), *Pachyiulus hungaricus* (Karsch, 1881) and *Cylindroiulus boleti* (C. L. Koch, 1847) (Diplopoda, Julida) as antimicrobial agents in the inhibition of biofilms of *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus*

Jelena Đorđević[70], Jelena Milovanović[72], Bojan Ilić[72], Aleksandra Stevanović[71], Anastasija Malešević[72], Slobodan Makarov[72], Branka Vuković-Gačić[72]

KEYWORDS: biofilm inhibition; antimicrobial agents; millipedes; *Pseudomonas aeruginosa* PAO1; *Staphylococcus aureus*

INTRODUCTION:

In recent years, the emphasis of the scientific community has been placed on the invention of new antimicrobial agents due to the increasing resistance of bacteria to antibiotics. However, serious global health concern is focused on bacterial biofilms, a complex structure of a microbiome made up of colonies of bacteria or individual bacterial cells in a group, attached to a surface. Bacterial biofilms are highly resistant to antimicrobial agents and grow on the surfaces of medical implants such as sutures, catheters, and dental implants. Given that plants and animals are a valuable source of natural biologically active products, they are a good basis for finding new antimicrobial and antibiofilm agents. Bacterial strains of *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus* are known for biofilm production and cause opportunistic and chronic infections in humans, some of which are due to biofilm production. Due to their characteristic way of life, millipedes (Diplopoda) are characterized by a diverse and complex defense against predators, which includes the secretion of various chemical compounds that are toxic, repellent, or tasteless to predators. Analyzes have shown that millipedes produce chemical compounds such as phenols, alkaloids, quinones, terpenoids, cyanogenic compounds, and fatty acid esters, which showed antimicrobial activity, among other. Representatives of the order Julida, which are frequent in Republic of Serbia, produce defense secretions that are chemically very complex (the most complex within Diplopoda) and exhibit antimicrobial, antioxidant, and neurodegenerative potential, so they represent a good basis for the invention of new antibiofilm agents.

OBJECTIVES:

Objectives are to determine the inhibition of biofilm formation and degradation of the formed biofilm of *P. aeruginosa* PAO1 and *S. aureus* by defense secretions of selected millipede species from the family Julidae as well as to determine their antimicrobial activity.

METHOD / DESIGN:

Biofilm formation was quantified by the crystal violet staining method, while antimicrobial activity was examined using the broth dilution minimum inhibitory concentration (MIC) test.

RESULTS:

Defensive secretions of *Megaphyllum unilineatum* (MUN), *Pachyiulus hungaricus* (PHU), and *Cylindroiulus boleti* (CBO) showed

70 University of Belgrade, Institute for Multidisciplinary Research, Belgrade, Serbia. Corresponding author: jelenadjo@imsi.rs

71 University of Belgrade, Institute of Botany and Botanical Garden "Jevremovac", Chair of Microbiology, Centre for Genotoxicology and Ecogenotoxicology, Faculty of Biology, Belgrade, Serbia

72 University of Belgrade, Institute of Zoology, Faculty of Biology, Belgrade, Serbia

antimicrobial activity against *S. aureus* with MIC values of 0.03, 0.06, and 0.06 mg/mL, respectively. On *P. aeruginosa* PAO1, defense secretions did not show antimicrobial activity even at the highest tested concentration of 1 mg/mL for MUN while for PHU and CBO the MIC was 1 mg/mL, which is most likely due to the high resistance of this bacterial strain. The antibiofilm effect was observed in all tested defense secretions and was more pronounced against *S. aureus* than against *P. aeruginosa* PAO1. The strongest biofilm inhibition of *S. aureus* was at the highest tested concentrations (2 × MIC) with percentages of inhibition of CBO: 88.6%, PHU: 73.7%, and MUN: 67.2%. Degradation of already formed *S. aureus* biofilm was shown at lower tested defensive secretions concentrations (MIC/4), about 40% of biofilm degradation for MUN and PHU and about 30% for CBO. The strongest inhibition of *P. aeruginosa* PAO1 biofilm formation was observed at the highest tested concentrations of defensive secretions, 1 and 0.5 mg/mL for PHU (82 and 54%), and CBO (64.3 and 38.5%) while MUN had the strongest activity at the lowest tested concentration of 0.06 mg/mL (34.3%). All examined defense secretions had similar degradation activity of *P. aeruginosa* PAO1 biofilm with stronger activity at lower tested concentrations (about 30%). Defensive secretions of MUN and PHU extracted in DMSO solvent showed a stronger antibiofilm effect compared to the same ethanol extracts.

CONCLUSIONS:
The defense secretions of MBO, PHU, and CBO show a good basis for further investigations of their use as antimicrobial agents, especially against *S. aureus*.

T3-P-20 Effect of metformin on AMPK/Akt/mTOR pathway against butyrate-resistant colorectal cancer spheroid cells

Kesara Nittayaboon, Surasak Sangkhathat, Raphatphorn Navakanitworakul[73]

KEYWORDS: Metformin; Butyrate-resistant cells; Colorectal cancer; Spheroid cells.

INTRODUCTION: Metformin, the anti-diabetic drug, has been studied as anti-cancer drug in various types of cancer, such as cervical, breast, prostate, and colorectal cancer. The meta-analysis study has been shown that metformin is associated with decreasing cancer incidence and mortality rate. However, there is no study on the effect of metformin on butyrate-resistant colorectal cancer cells. Normally, butyrate is an anti-cancer agent produced by colonic microbiota. However, the microbiota study reveals that *F. nucleatum*, a butyrate producing and inflammatory stimulator bacteria, was increased in colorectal cancer patients. The resistant-to-butyrate cell show a chemotherapy resistant phenotype which related to treatment failure and cancer recurrence.

OBJECTIVES: The aim of this study was evaluated the effect of metformin on butyrate-resistant colorectal cancer spheroid cells.

METHOD / DESIGN: HCT-116, and PMFko-14 colorectal cancer cells were induced with butyrate reagent at the final concentration of 3.2 mM. The resistant properties were determined by cell viability at IC50 of parental and resistant cells. Influx and efflux transporters were evaluated by qRT-PCR. The spheroids of parental and resistant cell were generated, then the effect of metformin was studied. Live/dead and caspase assays were used to examine the inhibitory effect of metformin on spheroid cells. Finally, molecular mechanisms of metformin were investigated by Immunoblotting analysis.

73 Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand, 90110.
Corresponding author: n_ruxapon@yahoo.com

RESULTS: After treatment of butyrate, butyrate-resistant (BR) cells showed higher cell viability than parental (PT) cells. The influx and efflux transporter genes were increased in BR cells. Both PT and BR cells could form spherical shape in a Poly-HEMA coating plate. Metformin shows an inhibition effect on spheroid cells in a dose-dependent manner by increase of dead cells and caspase activity. The results indicated that metformin could induce apoptosis of spheroid cells. Finally, metformin's actions on BR cells were induced through AMPK/Akt phosphorylation, resulting in mTOR and ACC inhibition leading to apoptosis.

CONCLUSIONS: Our findings can demonstrate that metformin showed a potential therapeutic utility by apoptotic induction on BR spheroid cells. This information may be used as alternative approach in colorectal cancer therapy improvement.

T3-P-21 Evaluating the potential effect of ethanol treatment on whey proteins digestibility

Charikleia Kyrkou, Konstantina Tentzidou, Asterios Stamkopoulos, Foteini Tsakoumaki, Garyfalia Charitou, Thomas Moschakis, Costas G. Biliaderis, Alexandra-Maria Michaelidou[74]

KEYWORDS: *In vitro* digestion, Whey protein isolates, Ethanol treatment, SDS-PAGE

INTRODUCTION:

Whey proteins (WP), although exhibiting high nutritional and functional attributes, demonstrate significant resistance to hydrolysis phenomena due to their globular structure (Sanchón et al., 2018; Hussein et al., 2020). Studies have indicated that, in comparison with the traditional heat treatment, ethanol can irreversibly and more effectively denature proteins and change their secondary structures (Nikolaidis and Moschakis 2018; Feng et al., 2021). However, to the best of our knowledge, data regarding the effect of ethanol on WP digestibility is scarce.

OBJECTIVES:

This study focuses on the comparison of the degradation pattern and evaluation of differences in digestibility of whey proteins under four different conditions, namely: in native WP isolates aqueous solution (N-WPI), in water-ethanol WP solutions with different ethanol concentrations (i.e., 10% and 50% w/v, E-10 WPI, and E-50 WPI, respectively) and in WP aqueous solution obtained after heating at 90°C for 10 min (H-WPI).

METHOD / DESIGN:

The protein content was measured with the Kjeldahl method according to ISO 8968-3:2007/IDF 20-3: 2007 (ISO 8968-3, 2007). To simulate the physiological digestion process (i.e., the oral (OP), gastric (GP), and intestinal phases (IP)), all substrates were subjected to the INFOGEST static *in vitro* digestion protocol (Brodkorb et al., 2019). The enzyme activities (pepsin and trypsin) were quantified prior to the implementation of the INFOGEST protocol as proposed by Minekus et al., (2014). A discontinuous polyacrylamide gel electrophoresis (disc-PAGE) was initially applied on the samples obtained from the *in vitro* digestion (4 treatments x 3 phases) (Andrews,1983). Furthermore, Sodium Dodecyl Sulphate PAGE (SDS–PAGE) was carried out following the method of Laemmli (1970) with some modifications. All experiments were conducted in triplicates and on a protein-equivalent basis.

74 Department of Food Science and Technology, School of Agriculture, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, Thessaloniki 541 24, Greece. Corresponding author: amichail@agro.auth.gr

RESULTS:

According to the Kjeldahl method, the mean protein content of undigested substrates, as well as OP, GP, and IP samples was 3.57% (±0.01), 1.84% (±0.07), 1.00 (±0.04) and, 0.55% (±0.02), respectively. During the application of the disc- PAGE, the OP samples gave, in all 4 substrates, bands with the same electrophoretic mobility (EM) as the bands corresponding to a-lactalbumin (a-la) and b-lactoglobulin (β-LG), ran on the same gel. At the end of the GP, the band with EM similar to a-la was not visible indicating that this band was quite susceptible to proteolysis by pepsin in all four matrices (Cheison and, Kulozik 2017). However, clear differences in the gastric proteolysis of the band with EM similar to β-LG were observed among the four treatments. Namely, this protein band remained pronounced, i.e., more pepsin resistant throughout the whole gastric phase, in N-WPI compared to E-10 WPI, E-50 WPI, and H-WPI.

A quite comparable proteolysis pattern was recorded by SDS-PAGE analysis. The following observations are of interest based on the data provided by the current literature (Nikolaidis and Moschakis 2018; Feng et al., 2021; Li, Ye and Singh, 2021):

1. In GP, a fainter band corresponding to intact β-LG was evident in E-10 WPI, E-50 WPI, and H-WPI, compared to the native system (N-WPI).
2. The greater degradation of β-LG in the above three denatured protein substrates (E-10 WPI, E-50 WPI, and H- WPI) was accompanied by the appearance of a set of lower molecular weight peptides (< 14.4 kDa). It should be highlighted that no such peptides were visualized in N-WPI.
3. Regarding the IP phase, the degradation patterns of E-10 WPI and H-WPI exhibited many similarities as opposed to the degradation profile of N-WPI and E-50 WPI. At this point it should be mentioned, that under the conditions of the study of Mao et al. (2019), the presence of ethanol influenced not only the hydrolysis of intact β-LG but also the hydrolysate profiles, resulting from the action of trypsin.

CONCLUSIONS:

Based on the preliminary data provided in this study, the structural modifications, induced by ethanol treatment, were shown to affect the release of the peptides generated during hydrolysis and consequently the *in vitro* digestibility of WP. Nevertheless, further investigation is required to confirm the aforementioned results and elucidate the underlying mechanisms of enzymic hydrolysis of ethanol treated WP preparations.

T3-P-22 Effect of lipinifolin on the proteome of multidrug-resistant *Enterococcus faecium*

Chutimon Promthong, Wipawadee Sianglum[75],
Sittirak Roytrakul[76], Nantiya Joycharat[77]

KEYWORDS: antibacterial; *Enterococcus faecium*; lupinifolin; multidrug resistance; proteomic analysis

INTRODUCTION:

Vancomycin-resistant *Enterococcus* (VRE) is one of the most important causes of nosocomial infection because of multidrug resistance (MDR) and its virulence factors associated with biofilm formation. Bioactive natural products are an important

75 Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand. Corresponding author: chris.chu2541@gmail.com

76 Proteomics Research Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), The National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand

77 Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand

source of novel antimicrobial drugs due to the increase in multiple resistance to antibiotics in current clinical use. Lupinifolin is important bioactive compound isolated from stem of *Albizia myriophylla* Benth. The compound showed high antibacterial activity against Gram-positive bacteria including *Enterococcus*. However, the mechanisms of its antibacterial action are still poorly understood.

OBJECTIVES:

The aim of this study was to reveal the effect of lupinifolin against *E. faecium* HTY0256 strain using proteomic analysis.

METHOD / DESIGN:

Antibacterial activity of lupinifolin against the enterococci was conducted using broth microdilution method and time-killing assay. Proteome analysis of the bacteria after exposure to 2 MIC (minimal inhibitory concentration) of lupinifolin was performed to identify the protein expression changes compared to untreated control cultures by LC-MS/MS.

RESULTS:

MIC and MBC (minimal bactericidal concentration) of lupinifolin against enterococci were ranged from 4 to 8 µg/ml. Proteomic analysis of VRE in the presence of lupinifolin revealed different expression of proteins between the control and treatment groups. Proteins associated with stress response, acid phosphatase, virulence factor, ribosome, and carbohydrate metabolism, and also some virulence factors were down-regulated. The significant induction after treatment was noticed in proteins associated with cell membrane integrity, cell division, peptidoglycan biosynthesis, ABC transport, phosphotransferase system (PTS), aminoacyl-tRNA biosynthesis, acid-resistance of membrane and DNA recombination.
Interestingly, biological processes responsible for cell wall modification and cell shape were disrupted. The results demonstrated that lupinifolin expresses antibacterial activity mainly by effecting on cell membrane of the bacteria and that certain proteins may be responsible for the specific response of VRE to lupinifolin.

CONCLUSIONS:

These findings provide further evidence to support therapeutic efficiency of lupinifolin which could lead to the development of a new effective drug for treatment of multidrug resistant infections.

T3-P-23 Bone marrow derived mesenchymal stem cells from five donors perceived by Raman spectroscopy at a single cell level

Tamara Kukolj[78], Jasmina Lazarević[79], Ana Borojević[80], Uroš Ralević[79], Dragana Vujić3[81], Slavko Mojsilović[78], Aleksandra Jauković[78], Diana Bugarski[78]

KEYWORDS: Mesenchymal stem cells; Raman spectroscopy; Biochemical characterization

INTRODUCTION:

Although mesenchymal stem cells (MSCs), as adult stem cells, hold great promise in the field of regenerative medicine, signif-

78 Laboratory for Experimental Hematology and Stem Cells, Institute for Medical Research, University of Belgrade, Serbia.

Corresponding author: tamara.kukolj@imi.bg.ac.rs

79 Center for Solid State Physics and New Materials, Institute of Physics Belgrade, University of Belgrade

80 Serbia Mother and Child Health Care Institute of Serbia Dr Vukan Cupic, Belgrade, Serbia

81 School of Medicine, University of Belgrade, Serbia

icant obstacle for their clinical application is the lack of standardized markers for their isolation and characterization. Raman spectroscopy is fast, non-invasive optical technique based on an inelastic scattering of the visible light on a probed material, providing a unique (bio)chemical information. Therefore, it is considered that the Raman spectrum is a unique imprint of the analyte, implying that Raman spectroscopy could be the method of choice for the analysis of MSCs properties on a single cell level.

OBJECTIVES:

Objective of this study was to isolate and characterize bone marrow MSCs (BMMSCs) from five donors using standard biological procedures and to detect their biochemical profile using micro-Raman spectroscopy with the goal to investigate the possibility to employ micro-Raman system to distinguish individual features of BMMSCs populations.

METHOD / DESIGN:

BMMSCs were isolated from samples (2 ml) aspirated from iliac bone during collection of bone marrow for allogenic transplantation. Five healthy donors (age 2-12 years) were analyzed in this study. All experiments were performed using BMMSCs of the 5th passage. To assess immunophenotype of isolated, adherent cells expression of positive mesenchymal cell surface markers (CD90, CD44, CD73, CD105) and negative markers (CD34, CD45 and HLA-DR), a flow cytometry was used. Multilineage differentiation capacity was investigated based on corresponding histochemical staining: alkaline phosphatase and alizarin red staining were used to detect early and late osteogenesis; chondrogenesis was detected based on Safranin O staining of proteoglycans, while Oil red was used to visualize lipid drops of differentiated adipocytes. Following these standard procedures, Raman spectroscopy was performed on methanol-fixed BMMSCs. Excitation was realized by Ar^+/Kr^+ ion laser at 514.5 nm. The beam was focused through the x50 long range microscope objective, NA=0.5 in the backscattering configuration setup. Thermal damage was prevented by keeping low power density absorbed by the sample. Acquisition time was 300 s per spectrum and every cell was probed on several randomly chosen positions. For every donor, 50 to 100 cells were analyzed.

RESULTS:

In accordance with the minimal criteria for defining MSCs of International Society for Cellular Therapy (ISCT) we successfully isolated bone marrow MSCs showing typical adherent, fibroblast-like morphology. All donors exhibit high expression of positive mesenchymal cell surface markers (CD90, CD44, CD73, CD105) without expression of negative markers (CD34, CD45 and HLA-DR), along with the multilineage differentiation capacity toward osteogenesis, chondrogenesis, and adipogenesis. Based on these standard biological tests, no differences between donors were detected. However, Raman spectroscopy coupled with multivariate statistical method – Principal component analysis – showed distinct clustering between donors, based on specific spectral features, implying that Raman spectroscopy could be a step forward in routine analyzes of BMMSCs.

CONCLUSIONS:

As Raman spectroscopy still paves a way towards wider application in biological/biomedical field, our results once more contribute to the achievement of that goal. This technique allows the collection of a large number of data on the biochemical composition at a single cell level in a short time interval, thus providing prompt and unambiguous interpretation of cell populations' features, which is not possible by performing available methods in cell biology. Although the significance of standard techniques should not be neglected, a comprehensive analysis can only be achieved by their association with Raman spectroscopy.

T3-P-24 The impact of cultivation method and soil contamination on polyphenol content and antioxidant activity of celery (*Apium Graveolens L.*)

Tanja Radisavljević[82], Ivana Nemeš[82], Neda Mimica-Dukić[82],
Slobodanka Pajević[83], Danijela Arsenov[83], Nataša Simin[82]

KEYWORDS: *Apium graveolens L.*; celery; phenolics; flavonoids; antioxdant activity

INTRODUCTION:

Celery (*Apium graveolens L.*) has been cultivated since ancient times for dietary use. Celery root, leaf and stalks are consumed in hot dishes (soup, broth), fresh salads or popular smoothie drinks, while dried leaf or root can be used as spices. In addition, this aromatic plant is rich in essential oil, which is used in the pharmaceutical industry and in the production of perfumes, creams and soap lotions. According to historical records, celery was also used as a medicinal plant in traditional medicine of many countries. Celery can be cultivated by organic and conventional methods. The effect of the cultivation method, as well as the impact of the presence of heavy metals in the soil, on the the chemical composition and biological activity of edible parts of celery is not well studied.

OBJECTIVES:

The objective of this study was to investigate the effect of organic and conventional cultivation methods on the phenolic and flavonoid contents of celery (*Apium graveolens L.*) roots and leaves, as well as on their antioxidant activity. In addition, the aim of this study was to examine the impact of cadmium contamination on the chemical composition and biological activity of cultivated celery plants.

METHOD / DESIGN:

The contents of total phenolics and total flavonoids in celery roots and leaves extracts were determined by standard spectrophotometric methods. Antioxidant capacity was estimated by determine the potential of the extracts to inhibit lipid peroxidation and neutralize DPPH radicals, as well as by FRAP assay.

RESULTS:

The leaf extracts contained higher amounts of total phenols and total flavonoids compared to root extracts. Consequently, the antioxidant activity of the leaf extracts was higher compared to root extracts. In general, plants grown by organic cultivation method had higher content of total phenols and flavonoids and showed stronger antioxidant activity compared to conventionally grown plants.

Low concentrations (3 mg/kg) of cadmium used for soil treatment induced an increase in total phenolic and total flavonoid content, while higher concentrations of cadmium (6 mg/kg) decreased the total phenolics content, total flavonoids content and antioxidant activity of the samples.

CONCLUSIONS:

Organic production contributes to the increase of phenolic compounds in celery and rises its antioxidant potential. Low concentration of cadmium in the soil induces an increase of the phenolics content in plants probably because these secondary

82 *Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Science, University of Novi Sad, Novi Sad, Serbia.*

Corresponding author: dh.tanja.radisavljevic@student.pmf.uns.ac.rs

83 *Department of Biology and Ecology, Faculty of Science, University of Novi Sad, Novi Sad, Serbia*

metabolites participate in the plant defense mechanisms. High concentrations of cadmium in the soil have toxic effect on celery plants and reduce the content of phenolic compounds and antioxidant activity.

T3-P-25 Effect of subacute Dibutyl Phthalate treatment on liver enzymes in female wistar rats

Ivana Ivelja, Jelena Karan, Nebojša Andrić, Jelena Marković Filipović[84]

KEYWORDS: Dibutyl phthalate; liver enzyme, rat

INTRODUCTION:

Dibutyl phthalate (DBP) is an organic compound often used as a plasticizer. It can be found in cosmetic products and food packaging. In addition, DBP can contaminate freshwater environments, and thus humans get exposed to it via water and food, such as fish. In some of the recent studies, DBP was considered as a chemical with endocrine disruptive activity and reproductive toxicity in rats. Consequently, DBP effect on health has become the main topic of interest.

OBJECTIVES:

Objective of our study was to investigate whether DBP can induce liver damage by affecting the activity of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in blood.

METHOD / DESIGN:

Twenty-four female Wistar rats were divided in 4 groups (6 per group) and treated subacutely (28 days) with 0, 100, 500 and 5000 mg DBP/kg diet, that correspond to DBP dose of 0, 8.54, 41.34 and 447.33 mg/kg BW/day. Activities of ALT, AST and ALP in plasma were determined by clinical chemistry analyser (Autolyser Dialab). Statistical analysis of obtained data was performed using STATISTICA® version 13.0 (StatSoft, Inc). Results from control and treated groups were compared using One-way analysis of variance (ANOVA) for multiple comparisons, followed by Tukey post-hoc tests.

RESULTS:

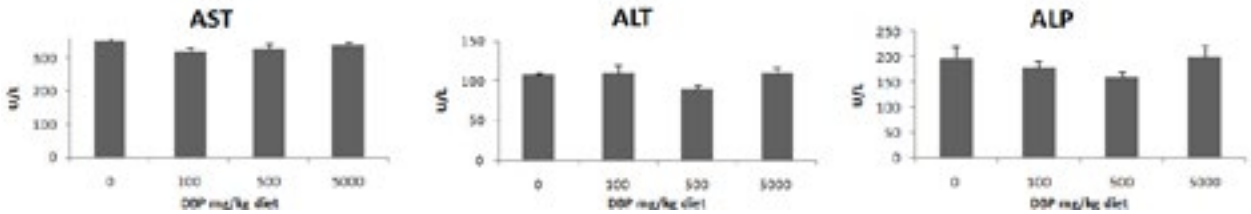


Figure 1. Mean values of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in blood samples of rats that were treated with 0, 100, 500 and 5000 mg/kg DBP in diet. Values in charts are means ± SEM; n = 10.

CONCLUSIONS:

Statistical analysis of AST, ALT and ALP activities revealed no significant difference between control and DBP-treated rats (*Fig. 1*). Our results indicate that DBP subacute treatment does not affect the activity of liver enzymes.

84 *University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia. Corresponding author: ivana.ivelja@dbe.uns.ac.rs*

T3-P-26 Improvement of functional properties of plum protein isolate by complexation with caffeic acid

Jelena Čakarević, Tea Sedlar, Ljiljana Popović[85]

KEYWORDS: protein isolates; phenolic acid; complexation; functional properties.

INTRODUCTION:

The oil industry generates large amounts of by-products which, besides use as animal feed and biofuels, can be used as sources of high-value compounds such as proteins. These proteins, in order to be attractive for various food formulations, should possess good functional properties, as well as significant bioactivity. In recent years, application of polyphenols for improving functional properties of proteins is increasingly applied. Interaction between proteins and polyphenols, yielding „protein-polyphenol complexes", have an impact on sensorial, functional and nutraceutical properties of protein products. In this article, mechanism of protein-polyphenol interactions as well as the functionalities and potential applications are investigated.

OBJECTIVES:

The objectives of this study were evaluation of properties of complexes obtained by interaction between protein and phenolic compound-caffeic acid (CA) in three different concentration and their characterization in terms of functional properties such as solubility and emulsification and the potential of their improvement.

METHOD / DESIGN:

The complexation of plum protein isolates (PI) and caffeic acid was prepared at pH 9 at room temperature for 24 h. Obtained complexes were characterized by FTIR and size exclusion chromatography (SEC). Functional properties, such as solubility and emulsification, were determined, too.

RESULTS:

Plum protein isolate that used in complexation with caffeic acid, was obtained from plum oil cake, which remained after oil extraction process. Protein content in the isolate is up to 90%. The FTIR spectrum of complexes showed decreased in the absorption near amide I and II confirming that the -NH_2 group of PI was involved in the reaction with CA. The elution profile of samples shows that all three complexes eluted earlier than PI. This phenomenon indicates that the conjugation of proteins with phenols results in complexes of higher molecular weights as results of cross-linking. Further, the solubility was increasing with the increase a concentration of CA in the complexes at alkaline pH. Moreover, emulsion properties also were improved by complexation with CA.

CONCLUSIONS:

Generally, binding of charged polyphenols changes the electrical properties of proteins, especially at the values of their isoelectric point, which significantly affects their solubility. Interaction of these components affects their functional properties and form the protein-phenol complexes that possess better functional properties and increase their potential application in different products. Obtained results showed that the complexation between plum protein isolate and caffeic acid made the conformational changes on the surface properties of protein that resulted in better solubility and emulsifying properties.

85 Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1. Corresponding author: jelena.cakarevic@uns.ac.rs

T3-P-27-ORAL The influence of glucokinase regulatory protein gene polymorphisms on lipid profile in Acute ischemic stroke patients

Jelena Bašić[86], Vuk Milošević[87], Milica Živanović[88], Jasen Kundalić[89], Milena Despotović[86], Tatjana Jevtović-Stoimenov[86], Ivana Stojanović[86]

KEYWORDS: Acute ischemic stroke; glucokinase regulatory protein; rs780094; rs1260326, lipid profile

INTRODUCTION:

Although single nucleotide polymorphisms (SNPs) rs780094 and rs1260326 in the glucokinase regulatory protein gene (*GCKR*) could be associated with lipid profile imbalance, their functional significance in acute ischemic stroke (AIS) patients has not yet been well established.

OBJECTIVES:

The aim of this study was to investigate the influence of *GCKR* rs780094 and rs1260326 SNPs on lipid profile parameters in patients with AIS, as well as to evaluate the association of these SNPs and the risk of AIS.

METHOD / DESIGN:

In a case-control study, a total of 148 subjects were screened for GCKR rs780094 and rs1260326 SNPs, using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Lipid profile was determined based on serum total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), and triacylglycerol (TG) concentrations.

RESULTS:

The frequencies of the minor rs780094A allele and the minor rs1260326T allele were significantly lower in AIS patients compared to controls (p=0.034, p=0.018, respectively). The rs780094AA genotype and the rs1260326TT genotype were associated with decreased risk of AIS compared to wild-type carriers (p=0.029, p=0.013, respectively). The mean values of TC, LDL-C, and TG in AIS patients were significantly higher in comparison to healthy subjects. On the other hand, HDL-C values were significantly lower in the patients compared to control ($p < 0.001$). There were no significant differences in the values of lipid profile parameters neither in AIS patients carriers of the rs780094 GG genotype compared to patients carriers of the rs780094 GA/AA genotypes nor between carriers of different genotypes (CC vs. CT/TT) of rs1260326 SNP.

CONCLUSIONS:

This is the first study implying that decreased risk of AIS in rs780094 and rs1260326 homozygous minor allele carriers may not be caused by dyslipidemia, but possibly by the lack of coagulation factors glycosylation.

86 University of Niš, Faculty of Medicine, Department of Biochemistry, Serbia. Corresponding author: jelena.basic@medfak.ni.ac.rs

87 Clinic of Neurology, University Clinical Center Niš, Serbia

88 Radiology Center, University Clinical Center Niš, Serbia

89 University of Niš, Faculty of Medicine, Serbia

T3-P-28 A comparative study of mycochemical composition, antioxidant and anti-acetylcholinesterase activity of *F. Fomentarius* (L.) Fr. 1849 from Balkan region

Milena Rašeta[90]*, Diandra Pintać[91], Sanja Berežni[90], Suzana Jovanović-Šanta[90]

KEYWORDS: bioactive compounds; *Fomes fomentarius*; oxidative stress; neuroprotective agents; phenolics

INTRODUCTION:

From the ancient times, mushrooms have been consumed by humans due to their taste, aroma and texture, being part of the normal diet as well as a delicacy. The interest in consumption of non-edible mushrooms is increasing in many countries, especially in the view of their medicinal value and importance. The non-edible and medicinal mushroom species *Fomes fomentarius* is a member of higher Basidiomycete, which is historically, from Hippocrates time, one of the first mushrooms used for wound cauterization. Iceman, a prehistoric mummy discovered in the Tyrolean Alps in 1991, carried some F. fomentarius pieces, probably used as tinder, first aid or in spiritual purposes. Hieronymus Bock in the 15th century recommended it as an emetic against mushroom poisoning. In many regions of Europe it was used by barbers, dentist, surgeons and in pharmacies. Potential in terms of medicinal use of *F. fomentarius*, which is rich in mycochemicals with antioxidant properties, and which can potentially contribute in reducing the risk of human diseases such as diabetes mellitus, different type of cancers, heart diseases, as well as Alzeimer´s disease, has not yet been extensively analyzed.

OBJECTIVES:

The present study was designed in order to evaluate the potential application of different types of extracts (chloroform, water, ethanol and methanol) of *F. fomentarius* from three different localities from Balkan region, as a new source of valuable bioactive compounds with positive effect on health.

METHOD / DESIGN:

In order to thoroughly evaluate phenolic profile of the extracts, quantitative analysis of 45 phenolic compounds was performed using LC-MS/MS technique, while total phenolic content (TP) was determined according to Folin-Ciolcateu procedure. The antioxidant potential of extracts was determined using several assays: DPPH, ABTS and NO scavenging ability, Ferric Reducing Antioxidant Power (FRAP) and lipid peroxidation inhibition (LP). Anti-acetylcholinesterase assay (anti-AChE) was used with the aim to determine potential application in treatment of some neurological diseases, such as Alzeimer´s.

RESULTS:

To the best of our knowledge, this is the first report of the mycochemical characterization and biological activities of *F. fomentarius* from Croatia (FC) and Bosnia and Herzegovina (FB), while there are some reports for the same species from the territory of the Republic of Serbia (FS). The LC-MS/MS analysis of the selected extracts showed the presence of some bioactive phenolic compounds and some of them were quantified for the first time (amentoflavone, baicalein, chrysoeriol, esculetin and scopoletin). The water and the ethanol extracts of FC and FS exhibited higher amounts of TP than the other analyzed extracts. These extracts and all methanol extracts showed high antioxidant activity, and, therefore, *F. fomentarius* polar extracts could be used as potential natural source of antioxidant additives. These results, as well as anti-AChE activity expressed

90 University of Novi Sad, Faculty of Sciences, Department of Chemistry,Biochemistry and Environmental protection, Trg D. Obradovića 3, Novi Sad, Serbia.

**Corresponding author: milena.raseta@dh.uns.ac.rs*

91 University of Novi Sad, Faculty of Medicine, Department of Biochemistry, Hajduk Veljkova 3, Novi Sad, Serbia

in vitro, could support the medicinal interest of the analyzed ethanol and methanol extracts. In comparison to usually used commercially available synthetic antioxidant and anti-acetylcholinesterase substances, analyzed extracts showed moderate activity.

CONCLUSIONS:

According to the obtained results, it can be concluded that primarily polar extracts of *F. fomentarius* from Balkan region are a valuable source of natural antioxidants, which could indicate their potential application for therapeutic purposes in the form of functional ingredients, preferably for chronically diseases which are associated with oxidative stress.

T3-P-29 Changes in metabolome and proteome of cancer cells after treatment of novel promising ligands of human sterol hydroxylases

Yaraslau Dzichenka[92], Michail Shapira[92], Anton Kavaleuski[92], Alexei Yantsevich[92], Ljubica Grbović[93], Marina Savić[93], Sergei Usanov[92], Suzana Jovanović-Šanta[93]

KEYWORDS: cytochrome P450; bile acids; androstane derivatives; HPLC-MS

INTRODUCTION:

Analysis of the cell "-ome" (metabolome, proteome etc.) is of particular interest from the point of view of the influence of various bioregulators on the structural and functional properties of targets which are used in modern drug development. Cytochrome P450 (CYP) enzymes are often used as such targets. CYPs are heme-containing proteins that participate in oxidation of numerous endogenous and exogenous compounds and play an important role in the metabolism of steroid hormones, bile acids, cholesterol, unsaturated fatty acids, phenolic metabolites, as well as in neutralizing xenobiotics (drugs, poisons). It is established that these enzymes are involved in the metabolism of more than 60% of all drugs that get into the human body, which clearly states the conclusion that disruption of the functioning of CYPs in most cases can lead to serious consequences, including death.

OBJECTIVES:

In the frame of the presented research we evaluated impact of the novel perspective ligands of human sterol hydroxylases CYP7B1 and CYP17A1 on metabolome and proteome of human epithelial colorectal adenocarcinoma and lung carcinoma cell lines in order to identify mechanisms of action of these compounds on molecular and cell level.

METHOD / DESIGN:

Cytotoxicity evaluation against selected cell lines was performed for test compounds **1, 2** and **3** (bile acid, estrogen and androgen hormone derived modified steroids) using XTT test. Next, cells were treated with test compounds (final concentration was 25 μM). Isolation of metabolome (polar and nonpolar fractions) and proteome was performed simultaneously using methanol-water-chloroform extraction approach. The quadrupole time-of-flight mass-spectrometer Q-TOF 6550 ("Agilent") equipped with an electrospray ionization source (APESI+), was used for the metabolomic and proteomic analysis of samples. Chemometric analysis was performed by using MassProfiler Professional Software (metabolome analysis) and Peaks Studio 8.5 (proteome analysis).

RESULTS:

92 *Institute of Bioorganic Chemistry NAS of Belarus, Minsk, Belarus. Corresponding author: suzana.jovanovic-santa@dh.uns.ac.rs*

93 *University of Novi Sad Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Novi Sad, Serbia*

Analysis of cytotoxicity of the tested compounds showed that estrane derivative **2** expressed significant cytotoxic effect against A549 cells, while bile acid derivative **1** and D-secoandrostane **3** did not show significant effect. In case of Caco-2 cells, bile acid and D-secoandrostane derivatives **1** and **3** increased proliferation (up to 1.7 times), while estrane derivative **2** did not produce changes of the cells viability. It is interesting that Caco-2 is an epithelial colorectal adenocarcinoma cell line and the highest positive effect among tested compounds was found for bile acid derivative **1**. Chemometric analysis of polar and nonpolar fractions of metabolome allowed to identify 371 (A549 cells) and 294 (CaCo-2 cells) compounds in Metlin database. PCA analysis of the data allowed dividing samples into clusters depending on the compound used for cells treatment. Relative level of metabolites significantly increased, in general, after treatment of A549 cells by bile acid derivative **1**, while changes after treatment by compounds **2** and **3** were lower. In case of CaCo-2 cells relative changes in metabolites level were quite similar in samples grown with compounds **1** and **2**. As opposed to A549 cells, higher level of metabolites downregulation was detected for CaCo-2 cells, especially after treatment by bile acid and estrane derivatives (**1** and **2**). Analysis of cell proteome after cell lines treatment with modified steroids showed that in case of CaCo-2 cells treated with estrane-derived compound **2**, level of mitochondrial heat shock protein (75 kDa) was increased. Similar was in case of A549 control sample, but not in experimental A549 samples. Further, 14-3-3 sigma protein was detected in A549/compound **2** sample. According to the literature, the expression level of the protein correlates with effectiveness of the cancer therapy, which is in good agreement with our metabolome data. The higher differences between protein levels were detected for CaCo-2 cells, treated with androstane-derived compound **3**.

CONCLUSIONS:

Analysis of human cancer cells metabolome and proteome changes after treatment with modified steroids showed that significant changes in proteins and small bioregulators levels were detected. Deeper view can shed light on the mechanism of action of novel promising ligands of human sterol-hydroxylases on the cell level.

This study was supported by Belarus-Serbia bilateral project (No. X20SRBG-004 / 337-00-00612/2019-09/04) which is being realized between IBOCh of NAS of Belarus and University of Novi Sad Faculty of Sciences.

T3-P-30 One-pot synthesis of 4,5-Disubstituted 2-amino-1*h*-imidazoles from Mannich precursors

Zsófia Makra, Dr. László G. Puskás, Dr. Iván Kanizsai[94]

KEYWORDS: 2-aminoimidazole; Mannich-type substrate; ring cleavage; one-pot process; Groebke-Blackburn-Bienaymé reaction.

INTRODUCTION:

The 2-aminoimidazole (2-AI) core is general motif occurring in marine alkaloids isolated from different species of sponge, in addition, excellent precursor for drug discovery studies[95]. Depending on the substitution pattern, some 2-AI analogues possess wide range of biological activities.[96][97] Although numerous synthetic methodologies are known for the preparation

94 *Avidin Ltd., Alsó Kikötő Sor 11/D, Szeged, H-6726, Hungary. Corresponding author: i.kanizsai@avidinbiotech.com*

95 *R. G. S. Berlinck, M. H. Kossuga, Nat. Prod. Rep. 2005, 22, 516–550*

96 *N. Zidar, S. Montalvão, Ž. Hodnik, D. A. Nawrot, A. Žula, J. Ilaš, D. Kikelj, P. Tammela, L. P. Mašič, Mar. Drugs 2014, 12, 940–963.*

97 *A. S. Hogendorf, A. Hogendorf, R. Kurczab, J. Kalinowska-Tłuścik, P. Popik, A. Nikiforuk, M. Krawczyk, G. Satała, T. Lenda, J. Knutelska, et al., Eur. J. Med. Chem. 2019, 179, 1–15.*

of novel structures, but condensation processes have priority, in which the vinyl-azids or 2-bromo-2-alkenones have been treated by cyanamide or guanidine analogues, or reductive cleavage protocol of the imidazo[1,2-*a*]pyrimidine ring in the presence of hydrazine derivatives or sec-amines.[9899]

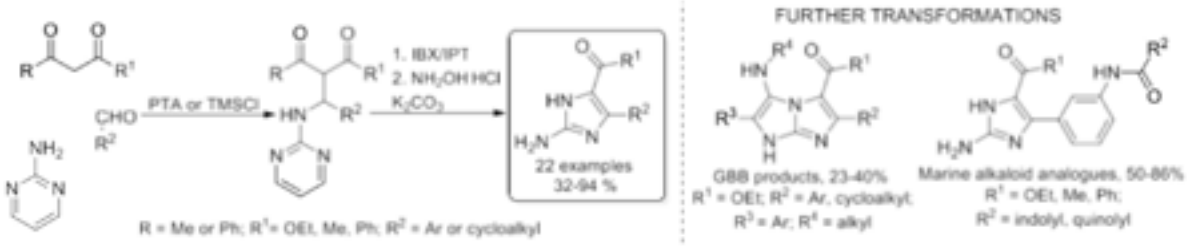
OBJECTIVES:

Herein, we disclose an efficient, sequential, one-pot synthetic protocol towards sophistically decorated 4,5-disubstituted 2- AI scaffolds including Mannich three-component reaction (Mannich-3CR), intramolecular oxidative annulation and ring-cleavage sequence.

METHOD / DESIGN:

The PTA or TMSCl mediated Mannich-3CR was accomplished starting from β-ketoesters/diketones, 2-aminopyrimidine and aldehydes to yield our precursors for sequential one-pot, two-step synthetic procedures. The construction of target 4,5-disubstituted 2-AI were carried out including IBX/IPT mediated oxidative cyclization of Mannich substrates and $NH_2OH.HCl$/K_2CO_3 induced ring-cleavage sequence. Further transformations were also demonstrated presenting $HClO_4$- catalysed Groebke-Blackburn-Bienaymé (GBB-3CR) reaction and preparation of sponge alkaloid analogues.

RESULTS:



CONCLUSIONS:

We demonstrated a convenient, sequential one-pot access towards the preparation of highly diverse C4/C5-functionalised 2-AI structures as valuable fragment-like heterocycles, utilisation of Mannich precursors and exploiting the IBX/IPT- mediated intramolecular oxidative annulation as well as hydroxylamine-induced ring cleavage sequence. Further modifications have also been performed including the GBB-3CR process towards unique 1H-imidazo[1,2-*a*]imidazoles with four diversity points. Other efforts to synthesize pharmacophores such as structurally modified marine alkaloid (Oroidin) analogues were also achieved.

T3-P-31 Assessment of loop-mediated isothermal amplification assays for *Escherichia Coli* detection

Mila Djisalov, Ljiljana Šašić Zorić, Ljiljana Janjušević, Teodora Knežić, Ivana Gadjanski[100]

KEYWORDS: *Escherichia coli*; Colorimetric LAMP; WarmStart LAMP; malB gene

98 *A. Žula, D. Kikelj, J. Ilaš, J. Heterocycl. Chem. 2016, 53, 345–355*

99 *E. V. Babaev, Stud. Nat. Prod. Chem. 2017, 52, 69–113.*

100 *University of Novi Sad, BioSense Institute, Dr Zorana Đinđića 1, 21000 Novi Sad, Serbia. Corresponding author: mila.djisalov@biosense.rs*

INTRODUCTION:

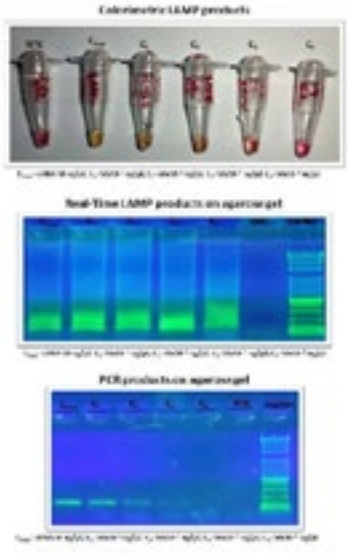
As of lately, loop-mediated isothermal amplification (LAMP) has become a powerful alternative to the polymerase chain reaction (PCR) in the field of molecular diagnostics, especially for pathogen detection in clinical and food samples. LAMP is a powerful and new method of nucleic acid amplification, with the ability to detect DNA at a very low level. In comparison to other molecular methods such as PCR and Real-Time PCR, LAMP eliminates the need for sophisticated thermal cyclers. High efficiency of DNA amplification by LAMP significantly shortens the whole amplification process.

OBJECTIVES:

This study aims to assess the efficacy of two different LAMP kits, WarmStart (Real-Time LAMP) and Colorimetric LAMP, for detection of *Escherichia coli*. In present study, kits were tested and optimized for detection of *E. coli* ATCC® 25922 directly from bacterial suspension and indirectly using bacterial genomic DNA (gDNA). LAMP results were then compared with the results of *E. coli* detection using standard PCR methodology.

METHOD / DESIGN:

For use in LAMP detection of E. coli directly from the bacterial suspension, eight bacterial dilutions in concentrations ranging from $1.5x10^{8}$ - $1.5x10^{1}$ CFU/mL were prepared. All bacterial dilutions were then used for LAMP detection using WarmStart Colorimetric LAMP 2X Master Mix (New England BioLabs) and WarmStart LAMP Kit (New England BioLabs). Real-Time LAMP reactions were done on the Genie® II, an instrument for isothermal nucleic acid amplification. LAMP primers (F3, B3, FIP, BIP, LF, BF) for the malB gene are taken from the literature. For indirect detection, genomic DNA from E. coli using GeneJET Genomic DNA Purification Kit (Thermo Scientific) was extracted. Extracted gDNA with the concentration 50 ng/µl was used for preparation of ten-fold dilutions (10^{-1} - 10^{-4}), and these dilutions were used in both Colorimetric and WarmStart LAMP assays in order to compare limits of detection with and without DNA extraction. Finally, E. coli genomic DNA serial dilutions for standard PCR, using F3 and B3 primers for *malB* gene, were applied.



RESULTS:

The results showed that both LAMP methods (WarmStart and Colorimetric LAMP) enabled the detection of *E. coli* directly, in suspension (without prior DNA isolation) for bacterial concentration of $1.5x10^{8}$ CFU/mL. Additionally, the results of Colorimetric LAMP using serial dilutions of *E. coli* gDNA showed detection in all tested concentrations, except the lowest. On the other hand, the WarmStart LAMP kit enabled *E. coli* gDNA detection in all tested gDNA dilutions. The conventional PCR methods failed to detect *E. coli* malB gene product for the lowest DNA concentration. Additionally, DNA bends on agarose gels were more intensive in the LAMP products compared to those obtained with PCR products.

CONCLUSIONS:

Presented research showed the incredible power of the LAMP methodology (both, Colorimetric and Real-Time) to detect *E. coli* even without prior isolation of bacterial gDNA. Also, LAMP enabled *E. coli* detection even at an extremely low concentration of tested gDNA (50×10^{-4} ng/µl), while with conventional PCR that was not the case. PCR managed to detect bacterial gDNA up to a concentration of 50×10^{-3} ng/µl. According to all the above, LAMP was proved to be more sensitive than PCR, with a large capacity to be used in molecular diagnostics. Also, LAMP is easier to handle since it is more time efficient and involves the use of portable small devices suitable for on Point-of-Care (POC) research.

T3-P-32 Quantitative structure – ADME properties relationship of boeravinones A-J

Nataša Milošević[101], Maja Milanović[101], Nebojša Pavlović[101], Larisa Đurić[101], Nunzio Antonio Cacciola[102], Francesca Borrelli[103], Nataša Milić[101]

KEYWORDS: medicinal herb; QSPR; drug design; lipophilicity; polarity

INTRODUCTION:

Boeravinones A-J, rotenoids extracted from Indian ayurvedic herb, *Boerhaavia diffusa* are identified as potent antioxidants, spasmolytics as well as inhibitors of the BCRP multidrug transporter and their structure has been confirmed by 1H NMR and 13C NMR spectroscopy. However, most of the drug candidates do not meet the expected strict criteria for new drugs and only 4.3% of them proceed from the preclinical stage to the Phase III trial with a positive outcome. The main causes for high attrition rates are toxicity, inadequate pharmacokinetics and low bioavailability. Therefore, it is crucial to optimize the ADME (absorption, distribution, metabolism and excretion) profile of the new drug candidates and to understand how structural changes can affect their pharmacokinetic/pharmacodynamic relationship.

OBJECTIVES:

To conduct *in silico* analysis of physico-chemical properties and ADME behaviour for ten boeravinones.

METHOD / DESIGN:

In silico analysis of molecular properties and pharmacokinetic characteristics of ten boeravinones (A-J) was conducted based on their structure. Molecular properties of the analysed boeravinones as fraction of sp3 hybridized carbon atoms (Fsp3) and polar surface area (PSA) were determined by SwissADME software. Software pkCSM was applied for the calculation of lipophilicity (logP), the percent of intestinal absorption (%IA), volume of distribution in stationary state (Vdss), fraction of unbound compound (FU) for plasma proteins, permeation through blood brain barrier (log BB) and skin permeability (logKp).

RESULTS:

The percent of intestinal absorption (%IA) of boeravinones A-J can be presented as a linear function (r^2=0.803, p<0.001) of lipophilicity expressed as logP. Distribution parameters such as volume of distribution in stationary state (Vdss) and fraction of drug unbound to plasma proteins (FU) were statistically significant associated to molecular flatness expressed as fraction of sp3 hybridized carbon atoms (Fsp3). Both Vdss and FU can be described as parabolic function of Fsp3 (r2=0.920, p<0.001 and r^2=0.661, p=0.009) for boeravinones A-J. The permeability through different membranes was governed by the polarity of boeravinones A-J quantified as PSA. Additionally, the permeability through the brain-blood barrier (logBB) and the skin (logKp) were correlated with PSA with high statistical quality (r^2=0.816, p=0.001 and r^2=0.729, p=0.004) and the parabolic functions were also obtained.

CONCLUSIONS:

Pharmacokinetic behaviour of boeravinones A-J based on *in silico* analysis is strongly affected by their physico-chemical characteristics. Lipophilicity governs the intestinal absorption of the analysed boeravinones, while the distribution process

101 University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia. Corresponding author: natasa.milosevic@mf.uns.ac.rs

102 University of Naples Federico II, Department of Veterinary Medicine and Animal Productions, Naples, Italy

103 University of Naples Federico II, School of Medicine and Surgery, Department of Pharmacy, Naples, Italy

is depended mainly on their flatness. Polarity of the observed boeravinones controls their permeability through different membranes, and limits their permeation through both the blood-brain barrier and the skin. The obtained results are applicable only for investigated structurally homogenous series of boeravinones A-J and should not be taken for general predictions.

T3-P-33 *In silico* prediction of boerhaavia diffusa rotenoids potential to inhibit breast cancer resistance protein (BCPR)

Nataša Milošević, Maja Milanović, Nebojša Pavlović, Nataša Milić[104]

KEYWORDS: medicinal herb; QSAR; drug design; BCPR; *in silico*

INTRODUCTION:

Breast cancer resistance protein (BCRP) is a member of the ATP-binding cassette (ABC) transporter family. BCRP transporter extracts the chemotherapeutics from the tumour cells and thus reduces the therapeutic efficiency of chemotherapeutics. It was found on the membranes of various carcinoma cells including fibrosarcoma, glioblastoma, myeloma, colon breast and gastric carcinoma. Nonprenylated rotenoids from *Boerhaavia diffusa* (boeravinones and coccineones) were experimentally identified as possible BCRP inhibitors. Their structure has been confirmed by 1H NMR and 13C NMR spectroscopy.

OBJECTIVES:

To investigated the interaction profile of thirteen compounds (boeravinones A-J, coccineones B and E, and 6-O-demethylboeravinone H) as BCRP transporter inhibitors.

METHOD / DESIGN:

Vienna LiverTox Workspace online tool was applied to investigate the interaction of thirteen rotenoids from *B.diffusa* with BCRP and to predict whether these molecules inhibit BCRP or not. A score close to 1 indicates a high probability of being an inhibitor while a score close to 0 indicates a high probability of not being an inhibitor. Molecular flatness for the analyzed rotenoids expressed as fraction of sp3 hybridized carbon atoms (Fsp3), polar surface area (PSA) and lipophilicity (XlogP3, WlogP, MlogP) were determined by SwissADME software.

RESULTS:

Only boeravinone B, E and F were predicted as BCRP inhibitors by Vienna LiverTox Workspace online tool. However, previously published results indicated that boeravinones A, B, C, E, G, H, I, J, coccineones B and E as well 6-O-demethylboeravinone H are BCPR inhibitors. Experimentally obtained results for BCPR inhibition published previously and *in silico* derived predictions were consistent only for boeravinones B and E. Boeravinone F was predicted to be BCPR inhibitor via Vienna LiverTox Workspace which is inconsistent with experimentally obtained results. Moreover, nine more rotenoids were experimentally proven to accumulate mitoxantrone that was not predicted by the Vienna LiverTox Workspace. Vienna LiverTox Scores for analysed thirteen rotenoids were linearly related with the flatness (Fsp3) of the molecules with statistical significance (r^2=0.334, p=0.023). Also, polarity was correlated with Vienna LiverTox Scores for all observed rotenoids and parabolic function was obtained with high statistical quality (r^2=0.611, p=0.003). However, no association was obtained between Vienna LiverTox Scores and lipophilicity (XlogP3, WlogP or MlogP) of the investigated rotenoids.

104 University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia. Corresponding author: natasa.milosevic@mf.uns.ac.rs

CONCLUSIONS:

Based on the obtained results rotenoinds extracted from *B.diffusa* should be considered as potential BCPR inhibitors. Further studies involving the proper *in silico* strategy applied with complement experiments could enable the development of deep learning algorithms and independent validation datasets that would select promising rotenoid with favourable biological potential. Computational modelling reduces the time and the cost involved in drug discovery process. The combination of computer-aided drug development with good designed experiments allows understanding of the complex interrelation between molecular properties of a compound and its biological effect, which cannot be accomplished by single approach.

T3-P-34 Determination of ginseng saponins by a standardized reference extract method

Andrey Stavrianidi[105], Aleksandra Cvetanovic[106], Oleg Shpigun[105]

KEYWORDS: HPLC-MS; method validation; ginsenosides; reference extract.

INTRODUCTION:

Quality control methods for herbal medicines and food supplements are always evolving. It is challenging to control multiple secondary metabolites, which is important for a more comprehensive evaluation of chemical compositions of such products. Also, the limited availability of high-purity (>98%) commercial standards is a major bottleneck for such methods. Roots of *Panax ginseng*, *Panax quinquefolius*, and *Panax notoginseng* are highly valuable herbal materials that contain triterpene glycosides (ginsenosides) that are known to be responsible for a number of beneficial pharmacological effects on the central nervous system, cardiovascular system, and antidiabetic and anticancer activities. There are hundreds of ginsenosides identified in the extracts from these roots, while there are only about 20-30 individual standards that are commercially available and their prices are very high.

OBJECTIVES:

The aim of this work was to study the possibilities of using standardized reference extract methodology to close the gap of the limited supply and expensiveness of individual ginsenosides standards. It was also important to validate the proposed procedure in order to compare its performance with the simple external standard method.

METHOD / DESIGN:

Ginseng infusion samples were purchased from different drug stores and were manufactured by six pharmaceutical companies. For all the samples there was the same alleged composition: 100 g of a ginseng root in 1 L ethanol:water (70:30, v:v). The infusion samples were diluted 10 and 200 times with methanol:water (20:80, v:v) to obtain the appropriate saponin peak areas for minor and major ginsenosides, respectively. All of the sample solutions were filtered through a 0.45 μm Millipore filter prior to HPLC analysis. An LCMS-8060 Shimadzu (Japan) system coupled on-line with a triple quadrupole mass spectrometer with an electrospray ionization source was used. The separation was performed on a Hypersil Gold PFP (150 × 2.1 mm; 3 μm) from «Thermo Scientific» (USA). Detection conditions in MS in SIM mode (ion source temperatures and gas flows, interface voltage, dwell time and entrance potentials in the Q-array and desolvation line) were selected by using the response surface methodology and separately in a series of one-factor experiments.

105 Lomonosov Moscow State University, Leninskie gory 1/3, 119991 Moscow, Russia. Corresponding author: stavrianidi.andrey@gmail.com

106 Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21 000 Novi Sad, Serbia

RESULTS:

The contents of 23 ginsenosides in reference extract (RE) material of American ginseng (*Panax quinquefolius*) were standardized by the proposed HPLC-MS method with a PFP column. Similar small positive matrix effects (about 115-126 %) for the ginsenosides were observed for the reference extract and ginseng infusion solutions, which shows that the developed RE-based approach will potentially be more accurate in determination of ginseng saponins. The measured repeatability was less than 6%, intra-day and inter-day precision expressed as RSD were not exceeding 8,5 and 12,8, respectively. The RE and standard mixture solutions were stable for 72 h at room temperature. With no correction to the matrix effect, the calculated mean method differences between RE and external standard methods were below 10 %, which shows that the proposed and validated approach is applicable to the quality control of investigated ginseng root infusions.

CONCLUSIONS:

A novel plant extract-based quantification methodology was applied in HPLC-MS analysis of ginseng infusions. The method was tested in the analysis of 11 ginseng infusions. The mean differences between the values obtained by the use of developed method and external standard method were less than 10% for all analytes, except ginsenoside Rg5. According to the results of our study, it is possible to use several REs and conduct quality control of complex herbal products and traditional medicines.

ACKNOWLEDGEMENTS:

The investigation is a part of the postdoctoral research of Aleksandra Cvetanović and she is grateful for financial support of Serbian Ministry of Education, Science and Technological Development (Contract number: 451-03-820/2019-14). Authors are also grateful for support from Project number 451-03-68/2020-14/200161.

T3-P-35 Anti-D immunoglobulin prevents hemolytic disease of fetus and wewborn; significance and consumption

Dejana Bajić[107], Nemanja Todorović[108], Mladena Lalić Popović[108], Jelena Čanji[108], Andrea Zubnar[109], Dragica Bulajić[110]

KEYWORDS: anti-D immunoglobulin; hemolytic disease; D antigen

INTRODUCTION: The Rh blood group system is the most polymorphic of the human blood groups, consisting of at least 45 independent antigens and, next to ABO, is the most clinically significant. The RhD protein expresses the D antigen (large protein on the surface of red blood cells with immunogenic properties). Approximately 85% of population is a RhD positive with expressed D antigen and remainder (15% of population) don't have this protein on erythrocytes. If the mother is RhD negative and fetus is RhD positive, her immune system "detect" fetus red blood cells as foreign and produce antibodes againist them. Incompatibility of the Rh blood group between the mother and fetus is major cause of the hemolytic disease of the fetus and newborn (HDFN), which can be associated with serious morbidity or mortality. All RhD negative pregnant women with RhD positive fetus should be given anti D immunoglobulin at 28 weeks. Anti D Ig should be administered as

107 Department of Biochemistry, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21137 Novi Sad, Serbia.

Corresponding author: dejana.bajic@mf.uns.ac.rs

108 Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21137 Novi Sad, Serbia

109 Department of Physiology, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21137 Novi Sad, Serbia

110 Department of Histology and Embriology, Faculty of Medicine, University of Novi Sad

soon as possible and always within 72 hours of a potentially sensitizing event (normal delivery, miscarriage, termination of pregnancy, ectopic pregnancy, amniocentesis, cordocentesis, abdominal trauma with fetal-maternal haemorrhage…).

OBJECTIVES: Implementation of programs for antenatal and postnatal anti-D immunoglobulin (Ig) prophylaxis during last decades achieved important reduction in the frequency of D alloimmunization and following fetal/neonatal complications. The aim of this research is to compare data about anti-D immunoglobulin consumption during period 2009- 2019 in Serbia.

METHOD / DESIGN: Consumption data are taken from the annual reports on the medicines consumption issued by the Medicines and Medical Devices Agency of Serbia (ALIMS). Reports for 10-year period from 2009 to 2019 can be found on the official ALIMS website. Consumption was observed as the total number of medicines from ATC groups J06BB01 (anti-D (Rh) immunoglobulin), regardless of pharmaceutical form, dose and packaging. Data on the number of live births were taken from the official website of the Statistical Office of the Republic of Serbia.

RESULTS: According to data obtained from website for Medicines and Medical Devices Agency of Serbia for the period from 2009 to 2019, it is noticed variable number of consumed medicines. Consumption ranged between 6768 (2012) and 12354 (2009) medicines per year, noting a consumption trend in the observed period that might be considered declining. Also, the trend in the number of live births in the same period could be considered declining. The number of live births varied in the range from 70299 (2009) to 63975 (2018).

CONCLUSIONS: Consumption of anti-D (Rh) immunoglobulin in Serbia in the period from 2009 to 2019 has a declining trend. This may be related to a reduced number of births. Incomplete agreement between the number of consumed medicines and the number of live births indicates the existence of several factors that affect consumption, and that it does not depend only on the number of pregnancies. Further studies should explain the variability of anti-D (Rh) immunoglobulin consumption.

T3-P-36 Optimization of a hemolymph protein extraction method from native polyacrylamide gel

Teodora Knežić[111], Miloš Avramov[112], Željko D. Popović[112], Ljiljana Janjušević[111], Mila Djisalov[111], Ivana Gadjanski[111]

KEYWORDS: insects; hemolymph proteins; native PAGE; protein isolation; proteomics

INTRODUCTION:

Although there are indications that insect-based proteins may have potential biomedical applications (anticancer and antimicrobial), as well as in cellular agriculture (food and feed), they have not been sufficiently investigated. The hemolymph of insect larvae is protein-rich, particularly in storage proteins that are involved in amino acid metabolism and protein synthesis. In order to characterize these proteins, the first step is their successful isolation. Using diapausing 5th instar larvae of the economically important European corn borer moth (ECB) *Ostrinia nubilalis* (Hbn.) as a model system, in this study we optimized a method for isolating individual native hemolymph proteins from polyacrylamide gels and we performed initial tests of isolated proteins bioactivity.

111 University of Novi Sad, BioSense Institute, Serbia. Corresponding author: teodora.knezic@biosense.rs

112 University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Serbia

OBJECTIVES:

The main objective in this study was to optimize an easy and affordable method for isolation of individual hemolymph proteins in the native state, without the use of chemicals that would affect their structure and function (e.g. sodium dodecyl sulfate, SDS). This allows further testing of these proteins for biomedical and application in cellular agriculture, and further work with isolated proteins in downstream *in vitro* proteome research, which will bring new knowledge and directions for different *in silico* proteome research.

METHOD / DESIGN:

Hemolymph was collected from diapausing 5th instar ECB larvae, after which hemocytes were removed from the hemolymph by centrifuging the samples for 30 min. at 16 000 g. Hemolymph proteins were separated by native polyacrylamide gel electrophoresis (PAGE) on a customized discontinuous gel without a well comb, using the BIO-RAD Mini-PROTEAN® Tetra cell. In order to determine the position of protein fractions of interest on the gel after electrophoresis, thin vertical strips were cut from the sides of the polyacrylamide gel and stained with Coomassie Brilliant Blue, after which the same gel strips were destained. The strips were placed next to the original gels and 5 protein fractions were cut from the unstained part of the polyacrylamide gel, chopped and transferred to microtubes. Ultrapure water was added to the tubes and they were placed on the Biometra TSC ThermoShaker overnight at 30°C to elute the proteins from the gels. After elution, the protein samples were centrifuged for 15 min. at 10 000 g. The concentration of isolated proteins was determined by measuring the absorbance at 230 nm using the Shimadzu BioSpec-nano, with a serial dilution of bovine γ-globulin used as the protein standard. To confirm that the proteins were well isolated, the individual fractions were run in duplicate wells on discontinuous native PAGE using the BIO-RAD Mini-PROTEAN® 3 Cell, after which the gels were stained, destained and imaged. Finally, the effect of successfully isolated proteins on MRC-5 cell viability was examined using an MTT assay.

RESULTS:

Five distinct protein fractions were detected after the first native PAGE (P1-P5). After elution from the gel, these fractions and the method for their isolation were validated with a second native PAGE (*Fig. 1*). Regarding the testing of isolated protein bioactivity, the results of the MTT assay indicate an antiproliferative effect of all 5 protein fractions, especially in the P4 fraction.

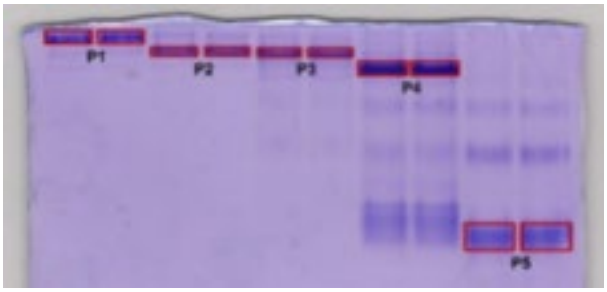


Figure 1: Five distinct protein fractions isolated from the hemolymph of diapausing 5th instar ECB larvae

CONCLUSIONS:

The insect hemolymph protein extraction method optimized in this study proved to be simple and successful and could potentially be applied to other insect species as well. Also, the structure and function of the proteins remained intact during the isolation process, which allows further use of the isolated proteins in downstream in vitro proteome research, the results of which will contribute to protein identification and *in silico* proteome research based on different bioinformatics tools (e.g. protein-protein interaction analysis, *in silico* bioactivity analyses, etc.). Finally, since the isolated proteins showed antiproliferative effects on the selected cell line, their anticancer and antimicrobial activity will be further tested.

T3-P-37 PCR - based detection methods as a tool for identification of *Aspergillus* species

Nataša Ćurčić[113], Jelena Krulj[113], Aleksandra Bočarov Stančić[114], Marija Bodroža Solarov[113]

KEYWORDS: *Aspergillus* species; ITS region; β tubulin; PCR-RFLP; aflatoxin biosynthesis genes

INTRODUCTION:

Aspergillus species are one of the most important producers of aflatoxin that can contaminate wide range of agricultural and food commodities. Molecular methods have been widely applied in the identification of different fungi species. Due of its reproducibility, speed, high sensitivity and specificity, PCR based methods have been used to identify the most important *Aspergillus* species.

OBJECTIVES:

The aim of this study was to validate PCR-RFLP based method to discriminate *Aspergillus* at the species level. PCR were performed to successfully amplify the ITS1-5.8S rDNA-ITS2 region, parts of β tubulin and calmodulin gene. One of the goals of this study was to identify the presence of genes (*afl*S, *afl*R, *afl*D and *afl*Q) in 8 examined *Aspergillus* species.

METHODS:

Aspergillus isolates were identified in the level of species by using molecular methods. Genomic DNA was isolated from mycelia using DNeasy Plant Mini Kit (Qiagen), according to the manufacturer's instruction. PCR amplifications were carried out with gene-specific primers. Products of PCR reaction obtained after amplification with primers (ITS1/ITS4) were incubated with restriction enzymes H*haI* and M*woI*, while PCR products obtained after amplification with primer pair (Bt2a/Bt2b) were digested with A*lwI* (BspPI) restriction enzyme.

The presence of structural genes (*afl*D and *afl*Q) and regulatory genes (*afl*S and *afl*R) were evaluated by PCR using 4 primer pairs. Amplified PCR products (before digestion) and restriction fragments (after digestion) were separated by agarose gel electrophoresis.

RESULTS:

Universal primer pair ITS1 and ITS4 was able to successfully amplify the ITS1-5.8S rDNA-ITS2 region of all tested *Aspergillus* species. Amplification of the 560-610 bp fragment followed by *Hha*I and *Mwo*I restriction in RFLP analysis produced different patterns of fragments among the examined species, revealing genetic variability.

Using the calmodulin primer pair (cmd5/cmd6), a 475-595 base pair fragment was successfully amplified in tested *Aspergillus* species. Amplification of a part of the β tubulin gene was performed by using the primer pair (Bt2a/Bt2b) and generated PCR product ranging in size from 415 to 580 bp. This PCR product was digested with restriction enzyme A*lwI* (BspPI). The RFLP pattern of A*lwI* for tested *Aspergillus* was species-specific and none of the examined species generated fragments with similar sizes. PCR test on β tubulin gene generated unique patterns for eight examined *Aspergillus* species (*Aspergillus flavus, A. ochraceus, A. nidulans, A. versicolor, A. candidus, A. tamari, A. fumigatus and A. niger*).

The presence of genes (*afl*S, *afl*R, *afl*D and *afl*Q) involved in aflatoxin biosynthesis pathway were detected only in *Aspergillus flavus*.

113 Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21 000 Novi Sad, Serbia. Corresponding author: natasa.curcic@fins.uns.ac.rs

114 Institute for Application of Science in Agriculture, Bulevar despota Stefana 68b,11 000 Belgrade, Serbia

CONCLUSIONS:

PCR/RFLP on β tubulin gene provided rapid identification of the most important species of *Aspergillus*. The presence of structural genes (*afl*D and *afl*Q) and regulatory genes (*afl*S and *afl*R) detected in *Aspergillus flavus*, could be considered as a quick and reliable method for the detection of aflatoxigenic *Aspergillus*.

Ministry of Education, Science and Technological Development, Republic of Serbia (Institution: Institute for Food Technology, Novi Sad) (MESTD - 451-03-68/2020-14/200222).

T3-P-38 Cannabinoids in non-alcoholic and alcoholic hemp-based beverages: development of analytical method and health risk assessment

Katarina Bijelić, Blagoje Prpa, Marija Perić, Dejan Kusonić, Branislava Srđenović Čonić, Biljana Božin, Nebojša Kladar[115]

KEYWORDS: hemp; *Cannabis sativa*; GC-MS; health risk assessment

INTRODUCTION:

Cannabis sativa L. Cannabaceae is a species with a long history of use. It has been utilized in the textile, food, and pharmaceutical industries. *Cannabis* species are characterized by the presence of numerous classes of secondary metabolites, while the most important are terpenophenolic compounds - cannabinoids. The best studied cannabinoids are Δ9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD) and cannabinol (CBN). The two mostly grown *Cannabis* subspecies are subsp. *sativa* and subsp. *indica*, which differ from each other in terms of Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) content. Subspecies *sativa* is mostly used in the food industry due to the significantly lower Δ^9-THC content and is commonly recognized by the name hemp. On the other hand, subsp. *indica* is known to exhibit psychoactive effects, and is usually utilized for the isolation of Δ^9-THC or abused as narcotic. The huge increase in presence of hemp-based food products on the market has highlighted the question of safety of such products. Regulations regarding the content of cannabinoids in food products are generally not harmonized. European Food Safety Authority (EFSA) and The German Federal Institute for Risk Assessment (BfR) define an acute reference dose for Δ^9-THC of 1 µg/kg bw.

OBJECTIVES:

The aims of conducted study were development and validation of gas chromatography–mass spectrometry analytical method (GC-MS) for the simultaneous determination of cannabinoids (CBD, "total" Δ^9-THC and CBN) in beverages based on industrial hemp, safety assessment of these products, as well as the assessment of health risk related to consumption of these products.

METHOD / DESIGN:

The chemical standard substances of CBD, Δ^9-THC and CBN were analyzed by GC-MS technique for the purpose of analytical method development and validation. Five samples of hemp teas purchased on the market of Serbia, Austria and Slovenia were analyzed, as well as one sample of beer based on industrial hemp purchased on the Serbian market. The collected samples of hemp-based teas were prepared as decoctions, further extracted with hexane in a separatory funnel, evaporated to dryness and reconstituted in internal standard solution (ketamine hydrochloride in acetonitrile, 0.2 µg/mL). The hemp-based beer sample was directly extracted with hexane and prepared in the same way as tea decoctions. The products were ana-

115 *University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Hajduk Veljkova 3, Serbia. Corresponding author: bijelick5@gmail.com*

lyzed for cannabinoid content followed by exposure assessment of adults to "total" Δ^9-THC (based on the results that were analytically determined, as well as based on the principle of maximum transfer).

RESULTS:

The GC-MS based analytical method was developed and validated in terms of selectivity, linearity, precision, accuracy, matrix effect, limits of detection and quantification. In the analyzed beverages, the content of "total" Δ^9-THC ranged from 0.34-946.50 µg/kg of ready to drink beverage, while the exposure of the adults (>18 years) to "total" Δ^9-THC ranged from 2.42-2704.30 ng/kg bw. The content of CBD ranged from 22.84-16844.45 µg/kg of ready to drink beverage, and the content of CBN ranged from 3.16-75.02 µg/kg of ready to drink beverage. By applying the principle of maximum transfer, which is often recommended by regulatory authorities, consumer exposure to "total" Δ^9-THC ranged from 2330.00 ng/kg bw to 11919.00 ng/kg bw.

CONCLUSIONS:

The results show that the amounts of cannabinoids in hemp-based beverages vary significantly. In three of the six evaluated samples, the concentration of "total" Δ^9-THC can represent a threat to the health of consumers. Considering the principle of maximum transfer, the results obtained for the estimated content of cannabinoids in beverages prepared from teas are significantly higher than those obtained by analytical determination. Although the results obtained by applying the principle of maximum transfer differ significantly, this principle is justified because it reflects the maximum health risk of persons consuming products based on industrial hemp.

T3-P-39 Disulfiram and metformin coadministration exhibits anticancer effect on fibrosarcoma in hamsters

Dušica J. Popović[116], Kosta J. Popović[117], Dejan Miljković[116], Dušan Lalošević[116], Ivan Čapo[116], Jovan K. Popović[118]

KEYWORDS: disulfiram; metformin; fibrosarcoma; hamsters; anticancer effects

INTRODUCTION:

We investigated the effect of dısulfıram and metformin on fibrosarcoma in hamsters. Aldehyde dehydrogenase (ALDH) is a cancer stem cell marker, associated with chemoresistance. Disulfiram, an alcohol aversion agent, is a well known ALDH and proteasome inhibitor. Disulfiram inhibits growth of various cancer cell lines and is a candidate for repurposing in oncology.

OBJECTIVES:

Objective of the research was to prove that coadministration of disulfiram and metformin exhibits anticancer effects *in vivo* on fibrosarcoma inoculated to hamsters.

METHOD / DESIGN:

The 40 Syrian golden hamsters of approximately 90 g, both sexes, were randomly allocated in 3 experimental and 1 control groups of 10 animals in each. 2 x 10^6 BHK-21/C13 cells in 1ml were injected subcutaneously on the back of animals in

116 Department of Histology and Embryology, Faculty of Medicine, University of Novi Sad.

Corresponding author: jovan.popovic@mf.uns.ac.rs; jovapopmf@gmail.com

117 Department of Pharmacy, Faculty of Medicine, University of Novi Sad

118 Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad, Novi Sad, Republic of Serbia

4 groups. The first experimental group started peroral treatment with metformin 500 mg/kg daily, second with disulfiram 200 mg/kg daily and third with combination of metformin 500 mg/kg and disulfiram 200 mg/kg daily, via gastric probe 3 days before tumor inoculation. After 19 days, when tumors were approximately 2-3 cm in control group, all animals were sacrificed, blood collected for glucose and other analyses, tumors excised, weighed, diameters measured, tumor samples pathohistologically (HE) and immunohistochemically (Ki-67, CD 31, COX IV, GLUT-1, iNOS) assessed (Figure) and main organs toxicologically analyzed, including control animals receiving metformin and disulfiram. Tumor volume was determined using the water displacement method and formula $LxS^2/2$, L - the longest, S - the shortest diameter. Ki-67-positive cells in the tumor samples were quantified, images were taken and processed by software UTHSCSA Image Tools for Windows Version 3.00. Statistical significances were determined by the one way ANOVA.

RESULTS:

The combination of disulfiram and metformin inhibited fibrosarcoma growth in hamsters without toxicity.

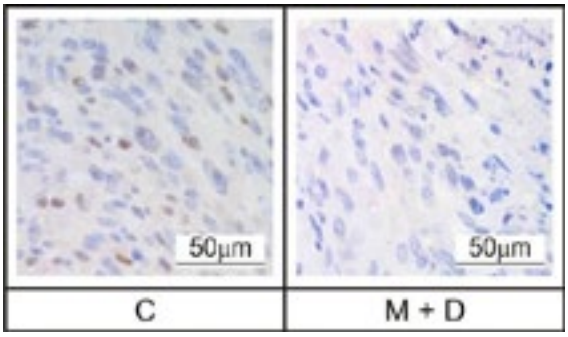


Figure: Illustration of experimental methodology. BHK fibrosarcoma immunohistochemical assessment of Ki-67 - proliferation marker protein: C – control group, M+D – group treated with combination of metformin and disulfiram.

CONCLUSIONS:

Administration of disulfiram with metformin might be an effective and safe approach in novel nontoxic adjuvant anticancer treatment and relapse prevention antitumor therapy.

T3-P-40 Antioxidant effects and salicin content of bark extracts of seven willow species

Emilia Gligorić[119], Ružica Igić[120], Ljiljana Suvajdžić[119], Branislava Teofilović[119], Nevena Grujić[119]

KEYWORDS: willow bark; *Salix*; antioxidant; salicin

INTRODUCTION:

Willow bark (*Salix sp.*, Salicaceae) is traditionally used to treat pain, fever and inflammation. The antioxidant activity of willow bark extracts is closely related to their anti-inflammatory effect. Salicin is regarded as one of the main active agents found in willow bark, responsible for its pharmacological effects.

OBJECTIVES:

The objective of this paper was to evaluate the antioxidant effects and salicin content of bark extracts of seven different willow species.

119 University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, 21000 Novi Sad, Serbia.

Corresponding author: emilia.gligoric@mf.uns.ac.rs

120 University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 3, Novi Sad, Serbia

METHOD / DESIGN:

Willow bark was extracted with 70% (v/v) ethanol for 48h. Antioxidant activity of extracts was assessed spectrophotometrically by their ability to inhibit 2,2-diphenyl-1-pycrylhydrazyl (DPPH) and hydroxyl radicals (OH). Salicin content was determined by High Performance Liquid Chromatography.

RESULTS:

Concentrations of extracts that inhibited 50% of DPPH radical ranged from 1.83±0.09 to 7.79±0.21 μg/ml and OH radical from 22.2±0.36 to 51.69±0.31 μg/ml. Differences in antioxidant activities between species were statistically significant ($p<0.05$). The highest DPPH scavenging activity was observed for *S. alba* bark extract, while *S. fragilis* bark inhibited OH radical the most. Salicin content among species varied from 2.6±0.057 to 8.29±0.092 mg/g of dried bark. The highest amount of salicin was found in the bark of *S. amplexicaulis*.

CONCLUSIONS:

The obtained results indicate that bark extracts of all investigated willow species exhibited strong antioxidant activity in both DPPH and OH radical scavenging assays. Results also showed that *Salix* species other than those in commercial use, contain significant or even greater amounts of salicin and could be used as valuable sources of this bioactive compound.

T3-P-41 Molecular docking studies of salicin, a major constituent of willow bark, as cox-1 and cox-2 inhibitor

Emilia Gligorić[121], Ružica Igić[122], Ljiljana Suvajdžić[121], Branislava Teofilović[121], Nevena Grujić[121]

KEYWORDS: salicin; molecular docking; COX

INTRODUCTION:

Salicin is considered the major active compound of willow bark, responsible for its anti-inflammatory, analgesic and antipyretic properties. Inhibition of cyclooxygenase (COX) is one of the possible mechanisms involved in its anti-inflammatory action.

OBJECTIVES:

The aim of this study was to elucidate the interaction and binding affinity of salicin toward COX-1 and COX-2 using molecular docking.

METHOD / DESIGN:

Chemical structures of ligands were taken from the PubChem database, while 3D crystallographic structures of COX-1 and COX-2 from Protein Data Bank. Molecular docking was conducted using AutoDock 4.2.3. program, by Lamarckian Genetic Algorithm, with standard docking procedure for rigid receptor and flexible ligand. Discovery Studio Visualizer 4.5. was used to visualize the results.

121 University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, 21000 Novi Sad, Serbia.

Corresponding author: emilia.gligoric@mf.uns.ac.rs

122 University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 3,Novi Sad, Serbia

RESULTS:

Salicin had similar affinity toward COX-1 and COX-2. In comparison with acetylsalicylic acid, salicin had similar affinity toward COX-2, but lower toward COX-1. Salicin showed hydrogen bonding and hydrophobic interactions with important amino acid residues of the active sites of COX-1 and COX-2. Interactions of salicin with most of residues at the active site of COX-2 have also been reported for compounds showing strong inhibition of COX-2 and correspond to the active binding site of non-steroidal anti-inflammatory drugs.

CONCLUSIONS:

Lower affinity of salicin toward COX-1 might partially explain why willow bark extract does not damage the gastrointestinal mucosa in contrast to acetylsalicylic acid. Salicin exhibited a number of strong hydrogen bonds and hydrophobic interactions with significant amino acid residues of the active site of COX-2 which could explain anti-inflammatory potency of this compound.

T3-P-42 Phytochemical composition of the essential oil and hydrolates of *Veronica Officinalis L.* and *Veronica Urticifolia* Jacq.

Marija Nazlić[123], Ivana Katavić[124], Dario Kremer[125], Marko Randić[126], Valerija Dunkić[123]

KEYWORDS: *Veronica*; free volatile compounds; hexadecanoic acid, hexahydrofarnesyl acetone; phytol

INTRODUCTION:

Plants of the genus *Veronica* (family Plantaginaceae) are used in traditional medicines in countries around the world. This sparked interest in studying these plants in terms of their chemical composition and biological activity. *Veronica officinalis L.*, and *Veronica urticifolia* Jacq. which are the subject of this study, are traditionally used in the medicine of Balkan peoples.

OBJECTIVES:

The objective of this study was to investigate free volatile compounds (FVCs) of *Veronica urticifolia* and. *V. officinalis* species especially in the terms of comparing FVCs from essential oil (EO) and from water residues (hydrolates, HY) and also discussing differences and similarities in the composition considering different plant material collection locations. Hydrolates are condensed water vapours containing dissolved molecules of EOs and more water-soluble (polar) FVCs.

METHOD / DESIGN:

For the purposes of this research, EOs and HY were isolated by water distillation of dry plant material of the species *V. urticifolia* and *V. officinalis* in a Clevenger type apparatus. These plant species were collected in different areas in the territory of the Republic of Croatia. The phytochemical composition of FVCs was determined by GC and GC-MS analysis of isolated EO and HY, and the relative percentage of the identified compounds were calculated. The individual peaks for all samples were identified by comparison of their retention indices of n-alkanes to those of authentic samples and literature.

123 Faculty of Science, University of Split, Croatia, 21000 Split, R. Boškovića 33. Corresponding author: marija.nazlic@pmfst.hr; dkremer@pharma.hr; marko.randic@ju-priroda.hr; dunkic@pmfst.hr; ive.katavic@gmail.com

124 Ljekarna Joukhadar, Farkaševac 43, Croatia 10344 Farkaševac

125 Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia, 10000 Zagreb, A. Kovačića 1

126 Biology Public Institution "Priroda", Croatia, 51000 Rijeka, Grivica 4

RESULTS:

The most abundant compound In EO of *V. officinalis* collected in Lokve - Mali raj (Site 1) was hexadecanoic acid (49.5%) whereas in the material collected in Zagreb (Site 2) heptacosane (25.1%), hexahydropharnesyl acetone (phyton) (21.8%) and phytol (17.1%) were the main components. The most common compounds in HY of *V. officinalis* from the Sites 1 and 2 were hexadecanoic acid (34.1%; 30.3%), phytol (17.3%; 23.3%) and phyton (14.5%: 18.5%), respectively.

In the EO of *V. urticifolia* collected in Mala Kapela (Site 1) and Zelin Crnoluški (Site 2) the most common compounds were hexadecanoic acid (28.3%; 30.3%), phyton (20.2%; 18.5%), heptacosan (17.9%, 17.2%) and phytol (15.4%; 20.3%), respectively. The most common compounds in the HY of this species from the mentioned localities were phyton (35.9%; 40.3%), hexadecanoic acid (22.9%) and phytol (20.3%; 21.1%), respectively.

CONCLUSIONS:

It can be concluded that in these *Veronica* species the same compounds predominate in the highest percentages (hexadecanoic acid, hexahydropharnesyl acetone and phytol) in both types of isolates (EOs and HY). We can also conclude that HYs also can have their application. For both isolates, further investigations of potential biological activities are needed in the future.

T3-P-43 Characteristics of vaccines on Serbian market in terms of presence of potentially harmfull excipients

Jelena Čanji, Nemanja Todorović, Nebojša Pavlović, Gorana Puača, Milana Vuković, Nataša Milošević[127], Mladena Lalić-Popović[127/128]

KEYWORDS: sodium; thiomersal; ethanol; sorbitol; excipients with known effect.

INTRODUCTION: Vaccine use is often reduced due to concerns for their safety. In addition to the active pharmaceutical ingredient (API), the question of safety of all other ingredients that make up the pharmaceutical-technological formulation of vaccines is raised. Preservatives are the most common group of excipients that can be potentially dangerous, but it is now known that other classes of excipients can cause non-API related adverse drug reactions (ADR).[129]

OBJECTIVES: The aim of this study was to identify potentially harmful excipients that are part of vaccines that marketing authorization received from the Medicines and Medical Devices Agency of Serbia (ALIMS).

METHOD: This study was conducted in October of 2021. Qualitative content of vaccines registered in Serbia was observed from Summaries of product characteristics (SmPC) available at official website of ALIMS. Sections 2. and 6.1. were obsereved. Excipients were considered potentially harmful if they were listed as excipients with known effect (EKE) in European regulation, Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use'[130] from official website of European Medicines Agency (EMA).

RESULTS: A total of 64 SmPCs were analyzed. Each of analyzed SmPC showed presence of at least one EKE in vaccine for-

127 University of Novi Sad, Faculty of Medicine Novi Sad, Department of Pharmacy, Novi Sad, Serbia. Corresponding author: jelena.canji@mf.uns.ac.rs

128 University of Novi Sad, Center for Medical and Pharmaceutical Investigations and Quality Control, Novi Sad, Serbia

129 Geier DA, Jordan SK, Geier MR. The relative toxicity of compounds used as preservatives in vaccines and biologics. Med Sci Monit. 2010;16(5):SR21-7

130 European Commission.Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' (SANTE-2017-11668) [Internet]. European Medicines Agency 2019 [cited 2021 October 11]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/annex-european-commission-guideline-excipients-labelling-package-leaflet- medicinal-products-human_en.pdf

mulation. **Sodium** was present in 61 of 64 analyzed formulations, in form of sodium chloride, sodium hydroxide, sodium bicarbonate, sodium borate, sodium citrate, sodium dihydrogen phosphate, sodium hydrogen phosphate, disodium adipate, edetate disodium. **Potassium** was present in 22 analyzed vaccine formulations either as potassium chloride or potassium hydrogen phosphate and potassium dihydrogen phosphate as part of the phosphate buffer. **Phenylalanine** was found in 19 formulations, in 16 of those as part of *Hanks medium 199* which also contains **glucose**. In one other SmPC was glucose observed, making it a total of 17 formulations containing glucose. Ethanol was present in 13 analyzed formulations, sorbitol in 7 and thiomersal in 4. Also, in two SmPCs amino acids are listed as excipients, which could potentially include phenylalanine. **Sucrose** was found in 17 analyzed SmPCs, however, EMA recognized it as EKE only for oral route of administration. Nevertheless, ALIMS did label it as EKE in section 2. in three vaccine SmPCs, even stating quantitative content. In section 2. of SmPCs ALIMS also singled out as EKE ethanol in 10 analyzed SmPCs, sorbitol in 7 (same as EMA), phenylalanine in 10, sodium in only one, and thiomersal in 4 (same as EMA). Most of the analyzed SmPCs did not contain information about quantitative content of excipients, EKE included.

CONCLUSIONS: The analysis of SmPC documents showed that all vaccines on the Serbian market contain at least one EKE. Particular caution should be exercised with thiomersal-containing vaccines as this EKE may cause the most severe ADR of all detected EKE - allergic reactions. Also, EKEs that interact with other drugs (ethanol in 13 vaccines) and that indicate contraindications for the use of vaccines (phenylalanine in 19 vaccines) have been detected. The effect of EKE is in most cases dose-related, and as these data are often not available in SmPC documents, no definitive conclusions can be drawn about the potential harm. All detected EKEs are completely safe for the vast majority of patients.

T3-P-44 Needs and opportunitues for compounding of medicines for bioidentical hormone replacement therapy in Serbia

Jelena Čanji, Nemanja Todorović, Nebojša Pavlović, Dejan Kusonić, Milana Vuković[131],
Dejana Bajić[132],
Mladena Lalić-Popović[131/133]

KEYWORDS: progesterone; estriol; estradiol; extemporaneous medicines; magistral medicines.

INTRODUCTION: Around 75% of women in menopause experience some symptoms and approximately one-third of these experience severe symptoms which affect their quality of life.[134] Postmenopausal hormone replacement therapy (HRT) is an effective, well-tolerated treatment for women with menopausal symptoms. Nationwide, a lot of women use compounded hormone replacement therapy. According to a study, usage of these compounded medicines made in pharmacy for an individual patient is increasing each year.[135]

OBJECTIVES: The aim of this study was to determine needs and opportunities for bioidentical hormone replacement therapy (HRT) in Serbia.

131 University of Novi Sad, Faculty of Medicine Novi Sad, Department of Pharmacy, Novi Sad, Serbia. Corresponding author: jelena.canji@mf.uns.ac.rs

132 University of Novi Sad, Faculty of Medicine Novi Sad, Department of Biochemistry, Novi Sad, Serbia

133 University of Novi Sad, Center for Medical and Pharmaceutical Investigations and Quality Control, Novi Sad, Serbia

134 Newson L, Rymer J. The dangers of compounded bioidentical hormone replacement therapy. Br J Gen Pract. 2019;69(688):540-1

135 MacArthur RB, Mattison D, Parker RM. Compounded bioidentical hormone products, a path forward. J Am Pharm Assoc. 2021;9:S1544- 3191(21)00346-0

METHOD / DESIGN: Data about consumption of approved bioidentical monocomponent HRT medicines was obtained from official website of Medicine and Medical Devices Agency of Serbia in September 2021 for period of 2011-2019. Consumption was observe d as defined daily dose (DDD) per 1000 inhabitants per day. Opportunities for compounding of extemporaneous hormone replacement medicines were considered according to Serbian Law on Medicines and Medical Devices and following regulations.

RESULTS: At the moment of obtaining these results, in Serbia there were 13 formulations registered with either progesterone, estradiol or estriol as the only active pharmaceutical ingredient. There are 8 formulations with progesterone (2 vaginal gels, 1 gel, 1 solution for injection and 4 soft capsule formulations). Estradiol is present in 2 transdermal spray formulations and one transdermal patch. Estriol is registered as one vaginal cream and one vagitoria. Data about consumption are presented in *Figure 1*. Consumption trend in the observed period can be considered to be increment, with the exception in year 2014. During this year oral progesterone was not licensed in Serbia. Serbian drug law provides the possibility of compounding magistral medicines in situations like these, when an adequate medication is not available in the market. These medicines are proscribed by physicians and prepared in community pharmacies for each individual patient.

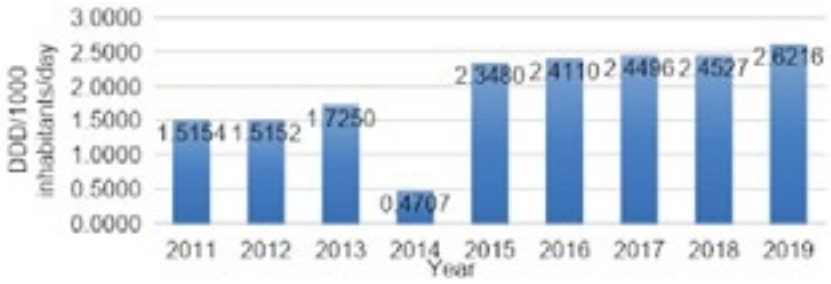


Figure 1. Consumption of approved monocomponent bioidentical hormone replacement medicines

CONCLUSIONS: In the observed period there is increasing trend in consumption of approved bioidentical hormones. Extemporaneous medicines can be appropriate in situation when there is shortage of approved medicines or there are opportunities for individualization of HRT therapy concerning dose or specific combinations of bioidentical hormones. However, caution is advised as neither safety nor efficacy of compounded bioidentical hormone formulations has been proven.

T3-P-45 Biocompatibility of three pulp-capping materials on human deciduous dental pulp stem cells

Dragica Bulajić[136/137], Jovana Drljača[137/137], Aleksandra Popovic[138139], Ivan Čapo[137/140], Slobodan Sekulić[141], Dejana Bajić[142], Branislav Bajkin[136/143]

KEYWORDS: SHED; NeoMTA; MTA repair; biodentine; cytotoxicity

INTRODUCTION:

Direct pulp capping procedures involve the application of a medicament, dressing, or dental material to the exposed pulp in cases of pin-point pulp exposure, in an attempt to preserve its vitality. Calcium silicate cements, like Biodentine and mineral trioxide aggregate (MTA), are dental biomaterials with the ability to raise the number and odontogenic differentiation of human dental pulp cells *in vitro*. Standard MTA cement had some drawbacks due to the poor handling characteristics, potential discoloration of dental tissue and the long material setting time. Therefore, all future developed MTA- based materials are designed to overcome these weaknesses.

OBJECTIVES:

Here we assessed and compared the biocompatibility of various pulp capping materials- NeoMTA Plus (Avalon Biomed), ProRoot MTA (Dentsply Tulsa Dental Specialties), and Biodentin (Septodont) on human deciduous dental pulp stem cells (SHEDs).

METHOD / DESIGN:

SHEDs were isolated and their phenotypes were evaluated by flow cytometry. Subsequently, they were cultured in the eluates of the above-mentioned pulpotomy materials (aged 24h, 7 and 14 days) for 24h. Cell viability was determined by Thiazolyl Blue Tetrazolium bromide assay (MTT).

RESULTS:

The appearance of the first adherent cells from explant tissue were observed after four days of cell culture. The analysis of characteristic surface antigens CD73, CD105, CD90, CD34, CD45, and CD235a showed that SHEDs were negative for hematopoietic stem cell markers and positive for mesenchymal stem cell markers. All tested materials and groups showed cell viability mathematically similar to the control group, at all time points. Generally, the materials displayed excellent biocompatibility on SHEDs, indicating that all three materials could potentially serve as a suitable substrate for bone regeneration.

136 Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia. Corresponding author: dragica993db@gmail.com

137 Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia. Corresponding author: dragica993db@gmail.com

138 Center for Medical and Pharmaceutical Investigations and Quality Control, Faculty of Medicine, University of Novi Sad, Serbia

139 Department of Physiology, Faculty of Medicine, University of Novi Sad, Serbia

140 Faculty of Medicine, Department of Histology and Embryology, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia

141 Faculty of Medicine, Department of Neurology, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia

142 Department of Biochemistry, Faculty of Medicine, University of Novi Sad, Serbia

143 Dentistry Clinic of Vojvodina, Hajduk Veljkova 12, Novi Sad, Serbia

CONCLUSIONS:

Our present results indicated that all studied materials showed low cytotoxicity on SHED. Furthermore, looking at long-term results (extracts aged 14 days) all tested materials showed similar biocompatibility. Overall, future *in vitro* and *in vivo* studies should be conducted to add more information about ProRoot MTA, Biodentin and NeoMTA plus.

T3-P-46 19-modified steroidal D-homoandrost-4-EN-3-ones: synthesis, *in silico* ADME and *in vitro* antitumor potential

Ivana Kuzminac[144], Andrea Nikolić[144], Marina Savić[144], Jovana Ajduković[144], Sofija Bekić[144], Anđelka Ćelić[145], Dimitar Jakimov[146], Tijana Šestić[144], Marija Sakač[144]

KEYWORDS: lactone, SwissADME, cytotoxicity, estrogen receptors, androgen receptors

INTRODUCTION:

Breast cancer is the most commonly occurring form of cancer in women and the second most common cancer overall. The growth of around 80% of breast cancers is stimulated by estrogens that bind to receptors in tumor cells. For the treatment of these cancers, antiestrogens and inhibitors of the enzyme aromatase are used. Some of these pharmaceuticals have steroid structures and were used in this work for the design of novel steroid derivatives as potential antitumor compounds.

OBJECTIVES:

In this paper, we report the synthesis of two new 19-modified steroidal derivatives (*Figure 1*). *In silico* ADME properties of these compounds were tested using SwissADME online tool (*Figure 2*). Novel steroid derivatives were tested for relative affinity to the ligand-binding domains of estrogen receptors (ERα and ERβ) and androgen receptor (AR). In addition, these compounds were tested for their cytotoxic activity against six human tumor cell lines and one healthy human cell line (*Table 1*).

METHOD / DESIGN:

- Five-step synthesis from 3β-acetoxy-5α-bromo-6β-hydroxy-17-oxa-17a-homoandrostan-16-one;
- *In silico* ADME - Analysis of Bioavailability Radars and the BOILED-Egg model;
- Receptor binding - a fluorescence assay in yeast;
- Cytotoxicity - MTT assay, cell lines: MCF-7 (human breast adenocarcinoma ER+), MDA-MB-231 (human breast adenocarcinoma ER-), PC-3 (prostate cancer AR-), HeLa (cervical cancer), HT-29 (colon cancer), A549 (lung adenocarcinoma), MRC-5 (healthy fetal lung fibroblasts).

144 University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia. Corresponding author: email ivana.kuzminac@dh.uns.ac.rs

145 University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

146 Oncology Institute of Vojvodina, Faculty of Medicine, University of Novi Sad, Put Dr Goldmana 4, 21204 Sremska Kamenica, Serbia

RESULTS:

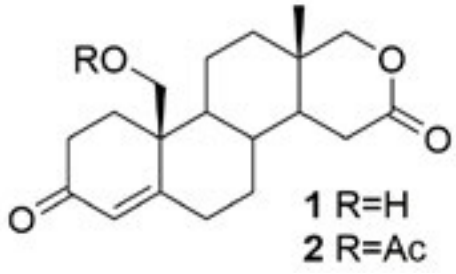


*Figure 1: Compounds **1** and **2***

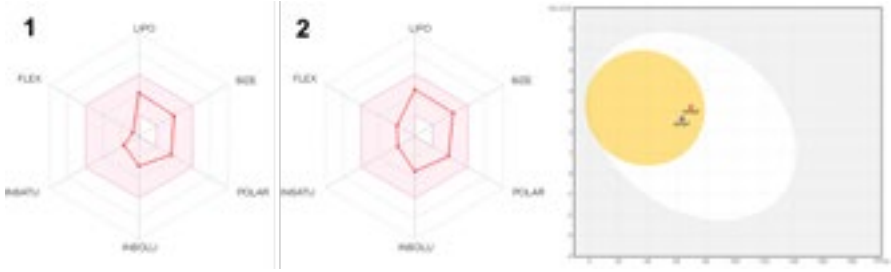


*Figure 2: In silico ADME properties of compounds **1** and **2***

None of the tested compounds showed affinity for ERα, ERβ, and AR.

Comp.	IC_{50} (μM)						
	MCF-7	MDA-MB-231	PC-3	HeLa	HT-29	A549	MRC-5
1	20.27	55.90	>50	>50	>50	15.62	>50
2	1.71	>50	>50	>50	>50	>50	>50
cisplatin	1.60	2.64	4.56	2.10	4.10	3.20	0.24
formestane	>50	19.61	26.37	3.36	>50	38.59	>50

Table 1: Cytotoxic activity of compounds 1 and 2

CONCLUSIONS:

Compounds **1** and **2** have shown good results in *in silico* ADME testing, meaning that they possess drug-like properties. Compound **2** showed high cytotoxicity and selectivity for the MCF-7 cell line.

The authors acknowledge financial support of Provincial Secretariat for Higher Education and Scientific Research of the Autonomous Province of Vojvodina [Project: Development of steroid derivatives of potential biomedical importance, No. 142-451-2309/2021-01].

T3-P-47 Effect of subacute dibutyl phtalate treatment on the levels of estradiol and progesterone in female wistar rats

Jelena Karan, Ivana Ivelja, Nebojša Andrić, Jelena Marković Filipović[147]

KEYWORDS: dibutyl phtalate; female reproductive hormones; endocrine disruptor; steroidogenesis; ovarian toxicology

INTRODUCTION: Dibutyl phthalate (DBP), one of the two most abundant phthalates, is used worldwide as plasticizer in many consumer products. Phthalates are known endocrine-disrupting chemicals that can directly target the ovary, potentially causing defects in ovulation and fertility.

OBJECTIVES:

The objective of our study was to determine whether DBP treatment affects estradiol and progesterone levels in female rat.

147 University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia. Corresponding author: jelena.karan@dbe.uns.ac.rs

METHOD / DESIGN:

Female Wistar rats, aged 40 days at the beginning of the experiment, were divided in 4 groups, and subacutely (28 days) treated with DBP added to the diet in concentrations: 0, 100, 500, 5000 mg DBP/kg diet, that correspond to 8.58, 41.34 and 447.33 mg/kg BW/day. After treatment termination, plasma was collected in vacutainer tube from rats that were in the diestrus (6 rats per group). Estradiol and progesterone concentrations were determined on a Roche Cobas e411 analyzer. Statistical analysis was performed using STATISTICA® version 13.0 (StatSoft, Inc). Data from control and treated rats were compared using One-way analysis of variance (ANOVA) for multiple comparisons, followed by Tukey post-hoc tests.

RESULTS:

DBP treatment in a doses of 100 and 500 mg/kg diet led to decrease in estradiol level, while 5000 mg DBP/kg diet induced increase of estradiol concentration when compared to control. On the other hand, DBP treatments led to increase of progesterone concentrations comparing to control, with the most prominent result in group treated with 100 mg DBP/kg diet. However, statistical analysis revealed that there is no significant difference in estradiol and progesterone concentrations between control and DBP-treated rats (*Figure 1*).

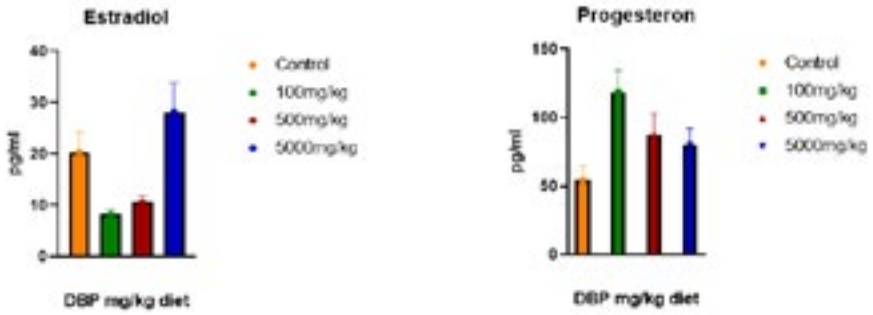


Figure 1. The effect of DBT treatment to the estrogen and progesterone level in the plasma of rats treated with 0, 100, 500, 5000 mg DBP/kg diet. Values in charts are means ± SEM; n = 6.

CONCLUSIONS:

Based on these data we can conclude that, in these experimental conditions, subacute DBP treatment has no significant effect on progesterone and estradiol level in female rats.

T3-P-48 Synthesis and *in silico* testing of novel androstane 1,3,4-thiadiazolines

Tijana Šestić, Jovana Ajduković, Ivana Kuzminac, Andrea Nikolić, Marina Savić[148]

KEYWORDS: heterocycle; thiosemicarbazone; 17a-homo lactone; 17α-picolyl derivative; ADME

INTRODUCTION:

It is widely known that *N-*, *O-* and *S*-heterocycles are important structural units present in many drugs, natural and synthetic products with broad spectrum of pharmacological activities. Among them, the 1,3,4-thiadiazoline nucleus is one of the most studied, and its potency is demonstrated by drugs that are currently in clinical use, such as cefazolin, megazol or acetazolamide.[149]

148 Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia. Corresponding author: jovana.ajdukovic@dh.uns.ac.rs

149 G. Serban, O. Stanasel, E. Serban, S. Bota, Drug Design, Development and Therapy 12 (2018) 1545.

OBJECTIVES:

Bearing this in mind, we have synthesized androstane thiosemicarbazone derivatives in 17a-homo lactone and 17α-picolyl (**1A** and **1B**, *Figure 1*) series, which were further subjected to ring closure reactions, affording 1,3,4-thiadiazolines (**2A, 2B** and **3A, 3B**, *Figure 1*) in good yields. In addition, the physicochemical properties of the obtained compounds were predicted using the web tool SwissADME and compared with Lipinski, Veber, Egan, Ghose and Muegge criteria.[150] Parameters for all compounds were within the optimal range and further studies on biological activity are planned.

METHOD / DESIGN:

All new compounds were obtained according to established synthetic procedures and characterized by IR and NMR spectroscopic data. *In silico* ADME profile was determined as well, allowing a first insight into a drug-likeness of the compounds, while the BOILED-Egg model provided information about gastrointestinal absorption and brain penetration.

RESULTS:

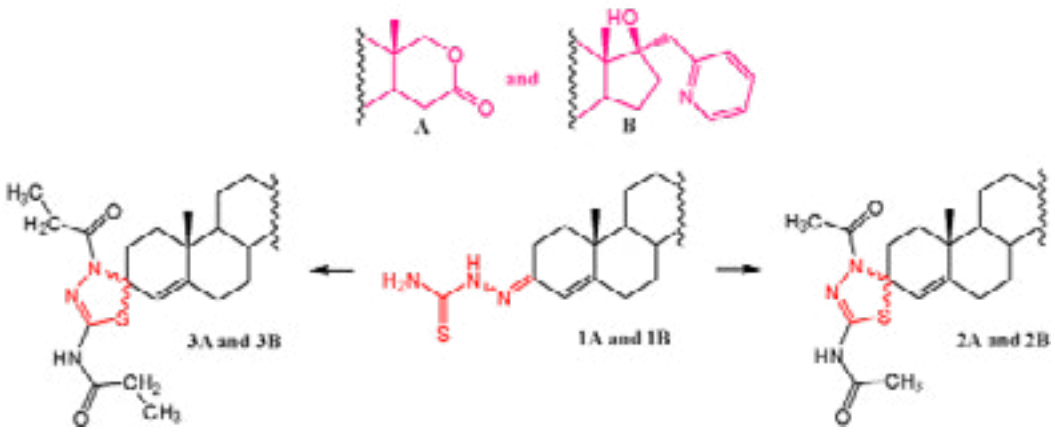


Figure 1. Synthesis of new androstane 1,3,4-thiadiazolines.

CONCLUSIONS:

A suitable synthesis of new thiosemicarbazone and thiadiazoline derivatives was performed, starting from the corresponding 4-en-3-one androstanes. According to *in silico* ADME physicochemical properties, all compounds possess drug-like qualities which are required for Lipinski, Veber, Egan, Ghose and Muegge criteria. The bioavailability radars indicated that all compounds are in the optimal range for lipophilicity, polarity, solubility, saturation and flexibility, with a slight deviation in compounds **2B** and **3B** due to higher molecular weight. The BOILED-Egg model indicated that these compounds could be absorbable by the intestines (with the exception of **3B**), but couldn't penetrate the brain.

From the obtained results it can be concluded that all compounds are good candidates for further *in vitro* studies.

The authors acknowledge financial support of Provincial Secretariat for Higher Education and Scientific Research of the Autonomous Province of Vojvodina [Project: Development of steroid derivatives of potential biomedical importance, No. 142-451-2309/2021-01].

150 A. Daina, O. Michielin, V. Zoete, Scientific Reports 7 (2017) 42717.

T3-P-49 Synthesis, *in silico* admet properties and virtual screening of newly *O*-substituted derivatives of dehydroepandrosterone (16e)-oxime

Andrea Nikolić, Jovana Ajduković, Marina Savić, Ivana Kuzminac[151]

KEYWORDS: steroids; *O*-substituted oximes; ADMET

INTRODUCTION:

The steroid scaffold is present in many FDA-approved drugs for the treatment of various diseases such as inflammation, allergic reaction, heart disease, cancer, and metabolic disease. The steroid skeleton is a favorable scaffold for the design and development of novel agents with pharmacological activities because even a small change in steroid moiety can lead to changes in the properties and biological activity of the compound. Introduction of a H-bond-donating oxime group into a hydrophobic steroid skeleton can increase their ability to interact with cell membranes which is important for the biological activity of such molecules. This approach proved as an excellent strategy for obtaining potent cytotoxic agents. *O*-Alkyl derivatives of oximes containing the H-bond-accepting group can change activity against cancer cells.

OBJECTIVES:

The aim of this work was the synthesis of new steroid *O*-substituted (16E)-oximes with and without H-bond-donating group as well as *in silico* assessment of their physicochemical properties and pharmacokinetics.

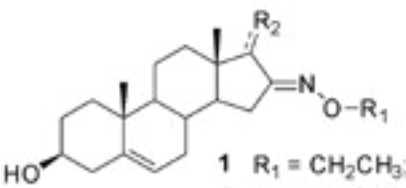
METHOD / DESIGN:

The structures of synthesized compounds were determined based on their spectral data. *In silico* ADMET properties were studied for all synthesized compounds using SwissADME and ProTox-II web tools.

RESULTS:

Newly steroid *O*-substituted (16E)-oximes were prepared from dehydroepiandrosterone (16E)-oxime which was obtained by oximination of dehydroepiandrosterone.

The Bioavailability Radar, which takes into account six physicochemical properties: lipophilicity, size, polarity, solubility, flexibility, and saturation, showed that all parameters of the synthesized compounds were in the optimal range. The drug-likeness evaluation revealed that synthesized compounds fulfill the requirements of the five different rule-based filters (Lipinski, Weber, Egan, Ghose, and Muegge). Prediction of pharmacokinetic behavior showed that tested compounds could be absorbed by the human intestine but couldn't cross the blood-brain barrier (except **1**). Androstane derivatives **1, 2, 5,** and 6 did not show inhibition effect on five major isoenzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4) involved in the metabolic drug elimination. Compounds **2-6** were found to bear a low risk of being mutagenic and carcinogenic, while carcinogenicity was predicted for compound **1**.



1 $R_1 = CH_2CH_3$; $R_2 = O$
2 $R_1 = CH_2COOH$; $R_2 = O$
3 $R_1 = CH_2COOCH_2CH_3$; $R_2 = O$
4 $R_1 = CH_2COOCH_2CH_3$; $R_2 = 17\beta$-OH
5 $R_1 = CH_2CH_2OH$; $R_2 = 17\beta$-OH
6 $R_1 = CH_2CONHNH_2$; $R_2 = 17\beta$-OH

151 Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia. Corresponding author: jovana.ajduković@dh.uns.ac.rs

CONCLUSIONS:

In silico ADMET analysis showed that synthesized derivatives of dehydroepiandrosterone (16*E*)-oxime possess drug-like properties, and compounds **2-6** have better safety profile than **1**.

The authors acknowledge financial support of Provincial Secretariat for Higher Education and Scientific Research of the Autonomous Province of Vojvodina [Project: Development of steroid derivatives of potential biomedical importance, No. 142-451-2309/2021-01].

T3-P-50 Maternal Covid-19 may negatively influence trophoblast cell function

Milica Jovanović Krivokuća, Aleksandra Vilotić, Danica Ćujić[152]

KEYWORDS: SARS-CoV-2; HTR-8/SVneo; trophoblast migration; MMP

INTRODUCTION:

COVID-19, caused by SARS-CoV-2 virus is ongoing global problem. It has been shown that pregnant women are at greater risk for a severe illness. Moreover, the infection may lead to the massive placental damage i.e. trophoblast necrosis and inflammation. SARS-CoV-2 infection is also proposed to be a risk factor for early pregnancy loss. Extravillous trophoblast cells are specific cells of the placenta that invade maternal uterine tissue and are in direct contact with maternal circulation. Trophoblast invasion is essential process for the establishment and maintenance of pregnancy, while matrix metalloproteinases (MMP) -2 and -9 are among crucial molecular mediators.

OBJECTIVES:

This pilot study was designed to investigate the possible effects of maternal sera obtained within three months from COVID-19 on the trophoblast cell function. Sera from eight women of reproductive age positive for IgM towards SARS-CoV-2 were used to treat extravillous trophoblast cell line HTR-8/SVneo. Sera from healthy age-matched women were used as control.

METHOD / DESIGN:

HTR-8/SVneo cells were treated for 24 h with 5% IgM- or IgM+ serum. Cell viability was determined by MTT assay. Cell migration was assessed by "wound healing" scratch assay. The expression of MMP-2 and MMP-9 at mRNA level was assessed by qPCR, while relative protein levels of MMP-2 and MMP-9 in HTR-8/SVneo cell lysates were determined by SDS-PAGE gelatin zymography.

RESULTS:

Treatment with 5% IgM+ sera did not affect HTR-8/SVneo cell viability. However, HTR-8/SVneo cell migration was significantly decreased by the treatment to 77% of control ($p<0.05$). This was followed by a significant decrease in MMP-9 mRNA level to 60% of control ($p<0.05$), while MMP-2 mRNA showed the same tendency, but without statistical significance. At the protein level, the treatment significantly inhibited the production of both MMP-2 and MMP-9 to 64% and 70% ($p<0.05$) of control, respectively.

CONCLUSIONS:

The results of this pilot study suggest the potential negative effect of maternal COVID-19 on the trophoblast cell function

152 Institute for Application of Nuclear Energy (INEP), University of Belgrade, Serbia. Corresponding author: milicaj@inep.co.rs

and hence on the placentation process which could lead to pregnancy loss or intrauterine growth restriction. However, these findings need further verification using a larger number of sera and a confirmation *in vivo*.

T3-P-51 Inhibition of tumor growth in disulfiram treatment of fibrosarcoma inoculated to hamsters

Kosta J. Popović[153],
Dušica J. Popović, Dejan Miljković, Dušan Lalošević, Ivan Čapo[154],
Jovan K. Popović[155]

KEYWORDS: disulfiram; hamster fibrosarcoma; anticancer effects

INTRODUCTION:

We investigated the anticancer effect of a clinically used anti-alcoholism drug disulfiram on an in vivo solid tumor model of fibrosarcoma in hamsters. Disulfiram inhibits growth of various cultured cancer cell lines.

OBJECTIVES:

Objective of the research was to prove that disulfiram inhibits growth of fibrosarcoma inoculated to hamsters.

METHOD / DESIGN:

20 Syrian golden hamsters of both sexes (10 males and 10 females), weighing approximately 70 g, were randomly allocated to the experimental and control group (10 hamsters/group). 2 x 10^6 BHK-21/C13 cells in 1 ml were injected subcutaneously into the animals' back in both groups. The experimental group started peroral treatment with disulfiram 200 mg/kg daily via a gastric probe 3 days before tumor inoculation. After 19 days, when the tumors were approximately 2-3 cm in the control group, all animals were sacrificed. The blood was collected for glucose and other analyses. The tumors were excised and weighed and their volume (by water displacement method) and diameters were measured (*Figure*). The tumor samples were histologically and immunohistologically assessed and the main organs toxicologically analyzed. Tumor volume was also determined using the formula $LxS^2/2$, where L was the longest and S the shortest diameter. Ki-67-positive cells in the tumor samples were quantified; images were taken and processed by software UTHSCSA Image Tools for Windows Version 3.00. Statistical significance of differences in tumor weight, volume, number of Ki-67-positive cells and other parameters were determined by the one way ANOVA.

RESULTS:

Disulfiram inhibited fibrosarcoma growth in hamsters without toxicity and without influence on blood analyses.

CONCLUSIONS:

Inhibition of proteasome activity by disulfiram as an anti-tumor strategy might be an effective and safe therapeutic approach in novel nontoxic therapies and relapse prevention for human cancers.

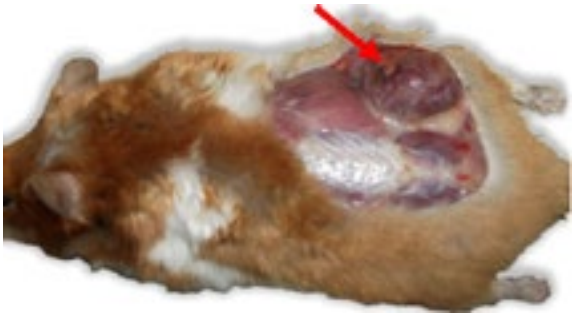


Figure: BHK fibrosarcoma: subcutaneous localization in a hamster

153 Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Novi Sad, Republic of Serbia.

Corresponding author: jovan.popovic@mf.uns.ac.rs; jovapopmf@gmail.com

154 Department of Histology and Embryology, Faculty of Medicine, University of Novi Sad, Novi Sad, Republic of Serbia

155 Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad, Novi Sad, Republic of Serbia

T3-P-52 Microwave-assisted synthesis and *in silico* ADMET properties of bile acids lactones

Ksenija Pavlović[156], Ljubica Grbović[156], Bojana Vasiljević[157], Srđan Bjedov[156], Denis Uka[156], Andrea Nikolić[156], Marija Sakač[156]

KEYWORDS: microwave-assisted synthesis; bile acid; lactone; ADMET

INTRODUCTION:

In modern synthetic chemistry, alternative energy sources are increasingly relevant, and one of them are microwaves. The use of microwave energy is considered as an acceptable technique in the principles of "green chemistry", but it is also a useful approach to syntheses because it enables more efficient and selective reactions. Baeyer-Villiger oxidation is the most commonly used method for converting ketones to lactones by oxidizing agents.

OBJECTIVES:

In our previous work, we synthesized a series of bile acids with lactone function in the steroid skeleton and examined their hydrophobicity and self-association properties (micellization).[158] The aim of this work was to modify the conventional method of Baeyer-Villiger oxidation used for synthesis of lactones (Figure 1). We performed the reaction in a microwave reactor with significantly shortened duration of the reaction. We tested *in silico* ADMET properties of compounds **1–4**.

METHOD / DESIGN:

The syntheses were performed in a Discover Bench Mate microwave reactor (CEM), in a closed system under pressure. The structures of all the obtained compounds (**1–4**) were confirmed by IR and NMR spectral data. *In silico* ADMET profiles were predicted using SwissADME and ProTox-II online tools.

RESULTS:

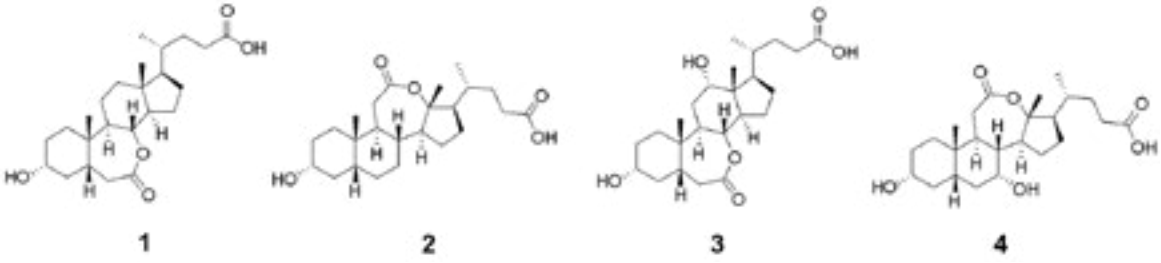


*Figure 1: Structures of compounds **1–4***

Based on the values of molecular descriptors of oral radar for bioavailability, it can be seen that all tested compounds meet all the stated empirical criteria without deviations, i.e. to fulfill the theoretical precondition for adequate bioavailability in the organism, and thus to possess appropriate biological potential. The BOILED-Egg model indicated that these compounds could be absorbable by the human intestine but couldn't penetrate the brain barrier. Toxicity prediction results showed that compounds **1–4** do not possess mutagenic, carcinogenic and cytotoxic potential.

156 Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia. Corresponding author: ksenija.pavlovic@dh.uns.ac.rs

157 Vinča Institute of Nuclear Sciences, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Vinča, Beograd

158 Poša M., Tepavčević V., Grbović Lj., Mikulić M., Pavlović K., Journal of Physical Organic Chemistry 34 (2021) 4133

CONCLUSIONS:

The Baeyer-Villiger oxidation of oxo derivatives of bile acids performed in a microwave reactor is up to 300 times faster compared to the same reaction that use conventional methods of heating. The syntheses were performed on a small scale, using the pressure of 20 mbar. *In silico* ADMET analysis showed that all synthesized lactone derivatives of bile acids (**1–4**) possess desirable drug-like properties.

T3-P-53 Galectin-8 increases extravillous trophoblast cell migration

Janko Legner, Žanka Bojić-Trbojević, Milica Jovanović Krivokuća[159]

KEYWORDS: galectin-8; extravillous trophoblast; migration; matrix metalloproteinases

INTRODUCTION:

During the first trimester of pregnancy extravillous trophoblast cells undergo differentiation which leads to the invasion of trophoblast cells into the maternal decidua and subsequent remodeling of the uterine spiral arteries. This complex and controlled physiological process at the fetomaternal interface involves various molecules including galectins. Galectin-8 is expressed by extravillous trophoblast cells throughout the invasive pathway of trophoblast differentiation, where it may play a role in the organization of extracellular matrix and the modulation of cell adhesion. No direct data, however, are yet available regarding its involvement in trophoblast function.

OBJECTIVES:

This study was conducted to investigate the effects of exogenously added rhGal-8 on extravillous trophoblast cell line HTR-8/SVneo cell viability, cell migration and production of crucial molecular mediators of trophoblast invasion - matrix metalloproteinases (MMP)-2 and -9.

METHOD / DESIGN:

HTR-8/SVneo cell viability was determined by MTT assay and adherent cell number by crystal violet assay, following a 24h incubation with rhGal-8 (10, 50, 100, 200 and 500 ng/ml). Cell migration was assessed using a „wound healing" scratch assay after 24h of treatment with rhGal-8 (50, 100 and 200 ng/ml). Levels of produced MMP-2 and MMP-9 in conditioned media from HTR-8/SVneo cells treated with rhGal-8 (10-500 ng/ml) for 24h, were determined by SDS-PAGE gelatin zymography.

RESULTS:

Treatment with a wide range of rhGal-8 concentrations (10-500 ng/ml) did not affect HTR-8/SVneo cell viability nor adherent cell number. On the other hand, rhGal-8 (200 ng/ml) significantly increased HTR-8/SVneo migration capacity to 133% of control ($p<0.05$). Production of MMP-2 and MMP-9 was significantly increased as well. RhGal-8 stimulated MMP-9 to 187% ($p<0.05$) and 198% ($p<0.01$) of control when cells were treated with 50 ng/ml and 100 ng/ml of rhGal-8, respectively. MMP-2 was increased to 141% ($p<0.05$) and 142% ($p<0.05$) of control by the same concentrations applied.

CONCLUSIONS:

Exogenously added rhGal-8 enhanced migration of HTR-8/SVneo cells *in vitro*, but had no effect on cell viability and adherent cell number. Based on these findings it can be proposed that rhGal-8 might exert stimulatory effect on trophoblast cell migration mediated at least in part through the increase of MMP-2 and MMP-9 gelatinolytic activity.

159 Institute for Application of Nuclear Energy (INEP), University of Belgrade, Serbia. Corresponding author: milicaj@inep.co.rs

T3-P-54-ORAL The effects of *Plantago L.* water extracts on mRNA expression of enzymes involved in cyclooxygenase pathway of arachidonic acid metabolism

Ljiljana Milovanović, Tatjana Majkić, Bojana Milutinović, Kristina Bekvalac, Ivana Beara[160]

KEYWORDS: *Plantago L.*; acteoside; plantamajoside; metabolism of arachidonic acid; anti-inflammatory activity

INTRODUCTION:

Plantain (*Plantago L.*) species are used worldwide in traditional medicine or as a food. Also, they are accepted in the modern medicine, and several studies confirmed that certain plantains have significant biological activity such as anti-inflammatory, antioxidative or immunoregulatory.

OBJECTIVES:

The aim of this study was to evaluate the potential of six *Plantago* species and two phenylethanoids distinctive for *Plantago* genus, acteoside and plantamajoside, to modulate metabolism of arachidonic acid, one of the most important processes in inflammation. Two of these *Plantago* species are renowned (*P. lanceolata L.* and *P. major L.*), while others have been poorly investigated (*P. altissima L, P. argentea Chaix, P. holosteum Scop., P. media L.*).

METHOD / DESIGN:

The water extracts were prepared according to the standard procedure of tea preparation. The total polyphenols, flavonoids and tannins contents were investigated by spectrophotometric methods. Detailed chemical characterization included quantitative analysis of 47 compounds by LC/MS-MS and 4 compounds by HPLC-DAD technique. Human monocytes (U937 cell line) were used as an *in vitro* model system to examine anti-inflammatory activity of plantain extracts, acteoside and plantamajoside. qPCR was applied to measure mRNA expression of enzymes involved in cyclooxygenase pathway of arachidonic acid metabolism (cPLA2α, COX-1, COX-2, mPGES-1/2-, cPGES, TXAS).

RESULTS:

The results showed that *Plantago* species were good sources of polyphenols, iridoids and phenylethanoids. Quantitative, rather than qualitative differences were observed among extracts, while acteoside and aucubin were dominant compounds in all species. Considering anti-inflammatory potential, plantain extracts and standards showed notable potential to modulate mRNK expression of enzymes involved in cyclooxygenase pathway of arachidonic acid metabolism.

CONCLUSIONS:

Obtained results reveal one aspect of the possible mechanisms of anti-inflammatory activity of *Plantago* species. Moreover, the results showed that underinvestigated species *P. altissima* can be pointed out as a new, prospective anti-inflammatory agent.

160 Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia.

Corresponding author: tatjana.majkic@dh.uns.ac.rs

T3-P-55-ORAL Wine against obesity – *Cabernet Sauvignon* wine as inhibitor of panceratic lipase

Tatjana Majkić[161], Ljilja Torović[162], Ivana Beara[161]

KEYWORDS: Obesity; *Cabernet Sauvignon* wine; polyphenols; pancreatic lipase

INTRODUCTION:

According to the World Health Organization, worldwide obesity has almost tripled since 1975, and in 2016, more than 650 million adults were obese. One of the strategies to reduce this number is to support and promote healthy diet habits and regular physical activity. On the other hand, a great number of epidemiological studies pointed out the Mediterranean diet, rich in beverages with high content of polyphenols, as one of the healthiest diets.

OBJECTIVES:

The aim of this study was to compare the polyphenol profile of *Cabernet Sauvignon* wines from five European countries (France, Italy, Macedonia, Spain and Serbia) and their potential to inhibit pancreatic lipase, the enzyme involved in lipid metabolism. Inhibition of pancreatic lipase could reduce the hydrolysis and consequently absorption of fats, which could be one of the mechanisms to reduce obesity.

METHOD / DESIGN:

The content of total polyphenols, flavonoids, tannins and monomeric anthocyanins was measured by spectrophotometric methods. Comprehensive evaluation of the polyphenolic profile of wine samples was performed by HPLC-UV/VIS technique, including quantitative analysis of nine phenolic acids, six flavonoids, two stilbenes and five anthocyanin glucosides. Lipase inhibition assay was performed using porcine pancreatic lipase and 4-nitrophenyl palmitate as a substrate. After the end of the reaction, the absorbance of yellow-colored p-nitrophenol was measured. Orlistat, a standard inhibitor of lipase, was used as a positive control.

RESULTS:

In all analysed samples, gallic acid was the dominant phenolic acid (18.3-102.7 mg/L), catechin was the most abundant flavonoid (13.2-56.7 mg/L), while the malvidin-3-*O*-glucoside was the dominant anthocyanin. In general, moderate qualitative and quantitative differences among samples were found. All wine samples showed some potential to inhibit lipase, and IC50 values ranged from 4.76-10.2 mg/mL. However, in comparison with orlistat, standard inhibitor of lipase and well-known drug, wines have low activity.

CONCLUSIONS:

Obtained results confirmed that examined wines are a rich source of polyphenols and that wines from Serbia could be compared with renowned wines, such as French ones. Although examined wines did not strongly inhibit lipase, they could, at least partially, contribute to overall health benefits.

161 Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia.

Corresponding author: tatjana.majkic@dh.uns.ac.rs

162 Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

T3-P-56 Stress-induced glucocorticoids alter the Leydig cells` timing and steroidogenesis-related systems

Marija Lj Medar, Silvana A Andric, Tatjana S Kostic[163]

KEYWORDS: Clock genes; Glucocorticoids; Leydig cell; Testosterone; Stress

INTRODUCTION:

Two systems, stress and the circadian clock are crucial for adaptation and maintenance of dynamic equilibrium continuously challenged by environmental cues. Both systems involved in synchronizing with the environment regulate body physiology differently.

OBJECTIVES:

The study aimed to analyze the time-dependent consequences of stress on gene expression responsible for diurnal endocrine Leydig cell function connecting them to the glucocorticoid-signaling.

METHOD / DESIGN:

Three *in vivo* and one *ex vivo* experimental approaches were applied: (1) to examine the effect of Imobilization sress (IMO) on Leydig cell activity, IMO was applied from ZT0-3 followed by the expressional/functional study in hours after IMO (ZT3, ZT11, ZT17, and ZT23); (2) to prove that glucocorticoids mimic IMO effects rats were treated orally with Dexason at ZT0 and effects were analyzed after treatment in ZT3, ZT11, ZT17, and ZT23 respectively; (3) to estimate glucocorticoids contribution in the effects of stress, testes of IMO rats were treated by RU486 (glucocorticoid receptor blocker) (4) to obtain information about direct effect of glucocorticoids on clock and steroidogenesis in Leydig cells, *ex vivo* experiments were performed using primary cultures of purified Leydig cells.

RESULTS:

In the first 24h after the stress event, a daily variation of blood glucocorticoids increased, and testosterone decreased; the reduced testosterone/corticosterone detected were lowest at the end of the stress session overlapping with inhibition of Leydig cells' steroidogenesis-related genes (*Nr3c1/GR, Hsd3b1/2, Star, Cyp17a1*) and changed circadian activity of the clock genes (the increased *Bmal1/BMAL1* and *Per1/2/PER1* and decreased *Cry1* and *Rev-erba*). The glucocorticoid-treated rats showed a similar response. The PCA displayed an absence of significant differences between treatments especially *Per1* and *Rev-erba*. This observation was confirmed by the in vivo blockade of the testicular glucocorticoid receptor during stress and *ex vivo* treatment of the Leydig cells with hydrocortisone and glucocorticoid receptor blocker.

CONCLUSIONS:

In summary, stressful stimuli can entrain the clock in the Leydig cells through glucocorticoid-mediated communication.

163 The University of Novi Sad, Faculty of Sciences Novi Sad, Department of Biology and Ecology, Laboratory for Chronobiology and Aging, Laboratory for Reproductive Endocrinology and Signaling, Novi Sad, Serbia. Corresponding author: tatjana.kostic@dbe.uns.ac.rs

T3-P-57 *In vitro* and *in silico* investingation of antimicrobial activity of essential oils from two *Pastinaca Sativa* subspecies

Ljuboš Ušjak[164], Milica Drobac[164], Marija Ivanov[165], Marina Soković[165], Marina T. Milenković[166], Marjan Niketić[167], Silvana Petrović[164]

KEYWORDS: *Pastinaca sativa subsp. sativa*; *Pastinaca sativa subsp. urens*; essential oils; microdilution method; molecular docking.

INTRODUCTION:

Cultivated parsnip (*Pastinaca sativa* subsp. *sativa L.*, Apiaceae) root is a well-known vegetable, common ingredient of soups, stews, salads, casseroles etc. Besides, its leaves and young shoots can be added to soups and fruits are used as a condiment. Furthermore, young shoots of wild-growing parsnips, e.g. *P. sativa* subsp. *urens* (Req. ex Godr.) Čelak., are consumed pickled or in salads or soups and it is considered that their essential oil acts as a natural preservative.

OBJECTIVES:

To investigate and compare the antimicrobial activity of the essential oils obtained from the roots, leaves, stems, flowers and fruits of cultivated *P. sativa* subsp. sativa and wild-growing *P. sativa* subsp. urens collected in Serbia. Furthermore, the most active essential oil constituents (against the most susceptible microorganisms) were predicted *in silico*.

METHOD / DESIGN:

Minimum inhibitory concentrations (MICs) and minimum bactericidal/fungicidal concentrations (MBCs/MFCs) of the essential oils (isolated by hydrodistillation using Clevenger-type apparatus) were determined by microdilution method against three Gram-positive bacteria: *Staphylococcus aureus* ATCC 11632, *Bacillus cereus* clinical isolate and Listeria monocytogenes NCTC 7973, three Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Salmonella Typhimurium* ATCC 13311 and *Enterobacter cloacae* ATCC 35030, three *Candida* standard strains: *C. albicans* ATCC 10231, *C. tropicalis* ATCC 750 and *C. parapsilosis* ATCC 22019, and three *Candida* isolates from oral cavity: *C. albicans* 475/15, *C. krusei* H1/16 and *C. glabrata* 4/6/15. Pharmacokinetic properties of the compounds present in at least one oil in the quantity ≥ 1% (determined by GC-FID and GC-MS) were initially evaluated using SwissADME web tool and molecular docking was performed using AutoDock Vina 1.1.2 (interactions were visualized using Discovery Studio Visualizer 2019).

RESULTS:

All the investigated essential oils of the two Pastinaca sativa subspecies were able to reduce the growth of different tested *Candida* strains (MIC range 0.25-2 mg/mL; MFC range 0.5-4 mg/mL). The most promising activity was observed for both root oils (MIC range 0.25-1 mg/mL; MFC range 0.5-2 mg/mL). Among investigated *Candida* strains, *C. parapsilosis* strain was the most sensitive to these essential oils (MIC range 0.25-1 mg/mL; MFC range 0.5-2 mg/mL). The antibacterial activity of the test-

164 Department of Pharmacognosy, University of Belgrade - Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia.

Corresponding author: ljubos.usjak@pharmacy.bg.ac.rs

165 Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade, Bulevar Despota Stefana 142, Belgrade, Serbia

166 Department of Microbiology and Immunology, University of Belgrade - Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia

167 Natural History Museum, Njegoševa 51, Belgrade, Serbia

ed essential oils was lower compared to their anticandidal potential (MIC range 1-4 mg/mL; MBC range 2-8 mg/mL). Thirty compounds were present in at least one oil in the quantity ≥ 1%. Estimation of pharmacokinetic properties using SwissADME tool suggested that 23 of these compounds are inhibitors of some of the cytochrome P450 system isoenzymes. This fact led to assumption that they could also act against fungal sterol 14α-demethylase (CYP51), which is a common target of antifungal drugs (e.g., ketoconazole). Thus, the compounds (3D structures downloaded from PubChem) were docked to the active site of this enzyme (downloaded from Protein Data Bank, PDB code 5TZ1). The highest affinities were predicted for sesquiterpenes caryophyllene oxide, (E)-caryophyllene, germacrene D, α-copaene, β-bourbonene and δ-cadinene (free binding energies from -9.4 to -8.7 kcal/mol; ketoconazole -11.6 kcal/mol). These compounds were present in somewhat lower quantities in the essential oils (≤ 9.9%). For dominant compounds of the tested essential oils, e.g. myristicin, γ-palmitolactone and octyl butanoate a bit lower affinities were predicted (free binding energies from -7.3 to -5.8 kcal/mol). Tested compounds mostly docked near the heme of the enzyme and formed hydrophobic interactions with the amino acid residues of the active site. According to SwissADME tool, four of five most active compounds have low absorption from gastrointestinal tract and higher skin permeation value, while caryophyllene oxide and three dominant compounds have high absorption and lower skin permeation value (similarly to ketoconazole).

CONCLUSIONS:

Investigated parsnips represent sources of essential oils and compounds with anticandidal activity.

ACKNOWLEDGEMENTS: Ministry of Education, Science and Technological Development of Republic of Serbia (Grant numbers: 451-03-9/2021-14/200161 and 451-03-9/2021-14/200007).

T3-P-58 Comparative chemical analysis of essential oils from different organs of three *Pastinaca Taxa*

Ljuboš Ušjak, Milica Drobac, Marjan Niketić, Silvana Petrović[168]

KEYWORDS: three *Pastinaca taxa*; five plant organs; essential oils; GC-FID and GC-MS; multivariate statistics

INTRODUCTION:

Pastinaca sativa subsp. *sativa L.*, Apiaceae (parsnip) is cultivated mainly in the temperate regions of the world because of its edible root. The roots of the best quality are obtained from the plants from the first year, in which this biennial plant usually forms only leaf rosette. In the second year, flowering stems emerge (the plant is cultivated for two years in order to obtain fruits for reproduction). Wild-growing P. sativa subsp. *urens* (Req. ex Godr.) Čelak. is widely distributed in Europe and *P. hirsuta* Pančić is endemic in the central part of the Balkan Peninsula (east Serbia, North Macedonia and south and west Bulgaria).

OBJECTIVES:

To investigate and compare the composition of the essential oils obtained from roots, leaves, stems, flowers and fruits of cultivated *P. sativa* subsp. sativa (from the first and/or the second year) and wild-growing *P. sativa* subsp. urens and *P. hirsuta* from Serbia.

168 Department of Pharmacognosy, University of Belgrade - Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia, 2Natural History Museum, Njegoševa 51, Belgrade, Serbia. Corresponding author: ljubos.usjak@pharmacy.bg.ac.rs

METHOD / DESIGN:

Essential oils were isolated from dried and comminuted plant material by hydrodistillation using Clevenger-type apparatus for 2.5 h. The composition of essential oils was determined by GC-FID and GC-MS and analyzed using multivariate statistical methods: principal component analysis (PCA), non-metric multidimensional scaling (nMDS) and unweighted pair-group arithmetic averages clustering (UPGMA).

RESULTS:

Twenty-nine parsnip essential oils were investigated: 11 *P. sativa subsp. sativa* oils (from four localities; the oils of roots and leaves from both the first and the second year, and of the other organs from the second year), 10 *P. sativa* subsp. *urens* oils (from two localities) and eight *P. hirsuta* oils (from one locality, collected in two different years). *Pastinaca sativa* subsp. sativa roots from the first year provided the highest oil yields (0.51-0.77%, w/w). The roots of this taxon from the second year (0.02%), and the roots of other investigated *Pastinaca taxa* (0.10-0.14%) had notably lower oil yields. Regarding other plant organs, high oil yields were generally obtained for fruits (1.40-3.90%) and flowers (0.43-0.93%), and low oil yields for leaves (0.07-0.16%) and stems (0.03-0.13%).

In general, 13 to 53 compounds were identified in the essential oils; identified components accounted for 89.4-98.6% of the oils. Phenylpropanoid myristicin was the most abundant in the root essential oils of both investigated *P. sativa* subspecies (39.7-82.5%). It is interesting to note that the oil of *P. sativa* subsp. sativa roots from the first year also contained high amounts of terpinolene (14.8-28.7%), which significantly decreased in the oil of this taxon from the second year (1.2%). In *P. hirsuta* root oils another phenylpropanoid apiole (30.9 and 25.8%) was dominant and the quantities of myristicin were somewhat lower (11.6 and 20.3%). Cultivated *P. sativa* subsp. sativa leaf essential oils were dominated by myristicin (42.8 and 41.4%) and in the leaf oils of wild-growing parsnips (*P. sativa* subsp. *urens* and *P. hirsuta*), γ-palmitolactone was the most abundant (22.6-47.5%). Additionally, the leaf oils of both investigated *P. sativa* subspecies contained significant amounts of sesquiterpenes (36.0-46.2%), e.g. (E)-β-farnesene (13.8-22.4%). Compared with the leaf oils, corresponding stem oils were qualitatively similar. However, in the stem oils, the contents of sesquiterpenes were lower [e.g. (E)-β-farnesene 4.9-14.4% in *P. sativa* oils] and the contents of myristicin (64.9 and 63.3% in *P. sativa* subsp. *sativa* oils) and γ-palmitolactone (50.6-60.4% in wild-growing parsnips oils) were higher. The flower and fruit essential oils were dominated by aliphatic esters. The most abundant in *P. hirsuta* oils were hexyl butanoate (61.9% in the flower oil; 22.0 and 58.4% in the fruit oils) and hexyl hexanoate (17.0% in the flower oil; 59.8 and 29.1% in the fruit oils), and in the oils of both *P. sativa* subspecies the dominant was octyl butanoate (26.1-31.4% in the flower oils; 53.6-79.0% in the fruit oils). In PCA and nMDS analyses of the oils (except fruit oils), the separation of all three investigated *Pastinaca taxa* was noticed. The same relations were observed in UPGMA analyses of the leaf, stem and flower oils. In the case of statistical analysis of the fruit oils and UPGMA analysis of the root oils, the samples of two subspecies of *P. sativa* were grouped together.

CONCLUSIONS:

Wild-growing parsnips are equally interesting sources of essential oils as cultivated parsnip. Locality and year of collection did not significantly influence relations among taxa observed in multivariate statistical analysis.

ACKNOWLEDGEMENTS: Ministry of Education, Science and Technological Development of Republic of Serbia (Grant No: 451-03-9/2021-14/200161).

T3-P-59 Significance of microscopic characters in quality control of herbal teas

Blagoje Prpa, Katarina Bijelić, Marija Perić, Dejan Kusonić, Branislava Srđenović Čonić, Nebojša Kladar, Biljana Božin[169]

KEYWORDS: Herbal teas; quality control; microscopic analysis; macroscopic analysis; foreign matter

INTRODUCTION:

Phytotherapeutics, are plants and plant-derived products represented by wide spectra of agents, which are used for treatment or prevention of various pathological conditions. Nowadays, the usage of phytotherapeutics increased, as evidenced by the fact that 80% of the world's population applies some form of herbal medicine. Of all the phytopreparations, herbal teas are the most commonly used, due to the fact that they are widely present on the market. It is very important that herbal teas meet proscribed quality and safety standards. The quality of tea blends can be observed from two points of view: the first relates to adherence to the declared ingredients content, and the second to meeting the needs of users. Herbal tea´s quality can be assessed by using microscopic, macroscopic and foreign matter analysis.

OBJECTIVES:

The aim of the conducted study was to evaluate the authenticity of five commercially available tea samples that are suggested for use by patients suffering from diabetes type 2, anxiety and tension, intestinal problems and hypertension by application of microscopic and macroscopic methods of analysis and quality control of tea mixture for blood sugar lowering by foreign matter analysis.

METHOD / DESIGN:

All the samples have been analyzed by microscopic and macroscopic methods in order to identify raw herbal material in tea samples. The samples used in this research were tea mixtures suggested for application in case of: upper respiratory tract problems (Bronhivit®), digestive problems (Gastrovit®), cardiovascular system problems (Kardiovit®), nervousness and anxiety (Relaksan®) and type 2 diabetes (Herbal Tea "Sugar lowering"®). Macroscopic identification of herbal material was based on the observation of the shape, size, color, surface properties, texture and other properties of pieces of herbal drug samples. Microscopic analyses involved examination of the drug anatomical characteristics. Prior to microscopic examination, the samples were prepared by immersion in a mixture of glycerol and ethanol (1:1) to soften the firmer consistency drugs. After softening, a section of the drug was prepared, which was subsequently fixed on a microscope plate, stained with histochemical reagents and observed with a magnifying apparatus. The analyses were performed on binocular microscopes Carl Zeiss AxioLab1 and Stemi 508, both equipped with camera AxioCam Erc 5s. Histochemical reagent used in this experiment was Tucakov's general reagent. The foreign matters share was determined by physically separating impurities of plant material spread in a thin layer on filter paper using tweezers, with the application of magnifying glass and an anatomical needle. Five samples of these mixtures were subjected to microscopic and macroscopic analyzes, and one of the samples was analyzed on foreign matter content, which was possible due to the properties of the sample (grinding degree). After microscopic and macroscopic identification of drugs within the tea mixture, a list of herbal drugs that are part of the tea mixture was compiled. The list of plants was compared with the list available on the declaration, and in addition, the correctness of the indications stated in the packaging, which are also related to the content.

169 University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Hajduk Veljkova 3, Serbia. Corresponding author: blagoje.prpa@gmail.com

RESULTS:

The degree of matching between determined and declared content (in term of herbal drugs presence) for four tea blends was 100%, while for one tea blend this rate was significantly lower and equals 40%. Furthermore, the analysis of foreign matter in the only evaluated sample has shown that the share of impurities equals 10.4%. Among the impurities, it was possible to identify pine needles (*Pini folium*) and leaves of plants from the Poaceae family (*Graminis herba*). As stated in the Regulation on the quality of tea, herbal tea and their products, the degree of impurities in the product of such characteristics must not exceed 1%.

CONCLUSIONS:

The composition of four of the analyzed tea mixture samples corresponded to the content of ingredients declared on the samples' packing. One of the five analyzed samples did not meet the quality requirement because it contained herbal drugs not declared on the product's packing, but also did not contain drugs declared to be present. The degree of impurities determined in this tea sample was five times higher than the value proscribed by Regulation on the quality of tea, herbal tea and their products.

T3-P-60 Deficiency of the insulin-like growth factors signaling disturbs the androgen phenotype but increases aromatase activity in mouse leydig cells

Sava M. Radovic Pletikosic[170], Isidora M. Starovlah[170], Dejan Miljkovic[171], Dragana M. Bajic[170], Ivan Capo[171], Serge Nef[172], Tatjana S. Kostic[170], Silvana A. Andric[170]

KEYWORDS: estradiol, feminization, insulin/IGF1 receptors, Leydig cell, testosterone

INTRODUCTION:

A growing body of evidence pointed correlation between insulin-resistance, testosterone level and infertility, but there is scarce information about mechanisms.

OBJECTIVES:

The aim of this study was to identify the possible mechanism linking the insulin-resistance with testosterone-producing Leydig-cells functionality.

METHOD / DESIGN:

We applied *in vivo* and *in vitro* approach. The *in vivo* model of functional genomics is represented by insulin-resistant- testosterone-producing Leydig cells obtained from the prepubertal (P21) and adult (P80) male mice with insulin and IGF1 receptors deletion in steroidogenic cells (*Insr/Igf1r*-DKO). The *in vitro* model of insulin-resistant-cell was mimicked by blockade of insulin/IGF1 receptors on primary culture of P21 and P80 Leydig cells.

170 Laboratory for Reproductive Endocrinology and Signalling, Laboratory for Chronobiology and Aging, CeRES, DBE, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia. Corresponding author: silvana.andric@dbe.uns.ac.rs, sava.radovic@dbe.uns.ac.rs

171 Center for Medical-Pharmaceutical Research and Quality Control, Department for Histology and Embryology, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

172 Department of Genetic Medicine and Development, Medical Faculty, University of Geneva, Geneva, Switzerland

RESULTS:

Leydig-cell-specific-insulin-resistance causes the loss of androgen phenotype, but induce the development of estrogenic characteristics of progenitor Leydig cells in prepubertal mice and mature Leydig cells in adult mice. Level of androgens in serum, testes and Leydig cells decrease as a consequence of the dramatic reduction of steroidogenic capacity and activity as well as all functional markers of Leydig cell. Oppositely, the markers for female-steroidogenic-cell differentiation and function increase. The physiological significance is the higher level of estradiol in double knock-out mice of both ages. Intriguingly, the transcription of pro-male sexual differentiation markers *Sry* and *Sox9* increased in P21-Leydig-cells, questioning the current view about the antagonistic genetic programs underlying gonadal sex determination.

CONCLUSIONS:

The results provide new molecular mechanisms leading to the development of the female phenotype in Leydig cells from *Insr/Igf1r*-DKO mice and could help to better understand the correlation between insulin resistance, testosterone and male (in)fertility.

T3-P-61 Bile acid derivatives as promising glucocorticoid receptor ligands

Srđan Bjedov[173], Ksenija Pavlović[173], Sofija Bekić[174], Anđelka Ćelić[174], Marija Sakač[173]

KEYWORDS: Glucocorticoids; Bile acid; Glucocorticoid receptor; Synthesis

INTRODUCTION:

Glucocorticoids (GC) are among the most powerful medications for treatment of inflammation and autoimmune diseases. The first used for successful treatment of severe cases of COVID-19 in 2020.[175] was glucocorticoid dexamethasone. However, prolonged usage of these molecules has serious side effects, such as osteoporosis, diabetes, Cushing's syndrome, high blood pressure. Glucocorticoids are necessary drugs in pharmacological arsenal and there is an urgent need for new, safer GC.

OBJECTIVES:

Our objective is synthesis of new GC compounds with good activity and less side effects. Glucocorticoids exert their physiological role by interacting with glucocorticoid receptor (GR). The first part toward reaching our objective was synthesis of potential GCs and testing their affinity for GR.

METHOD / DESIGN:

Starting from easily accessible bile acids, using contemporary oranic chemistry techniques we have designed and synthesized a series of compounds and tested their affinity for GR ligand binding domain.

RESULTS:

The synthesized compounds are shown in *figure 1*., and their relative binding affinity for the ligand binding domain of GR using a yeast-based fluorescent assay is shown in *figure 2*.

173 Department of Chemistry, Biochemistry and Environmental Protection, Trg D. Obradovića 3, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia. Corresponding author: srdjan.bjedov@dh.uns.ac.rs

174 Department of Biology and Ecology, Trg D. Obradovića 2, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

175 https://doi.org/10.1038/d41586-020-01824-5

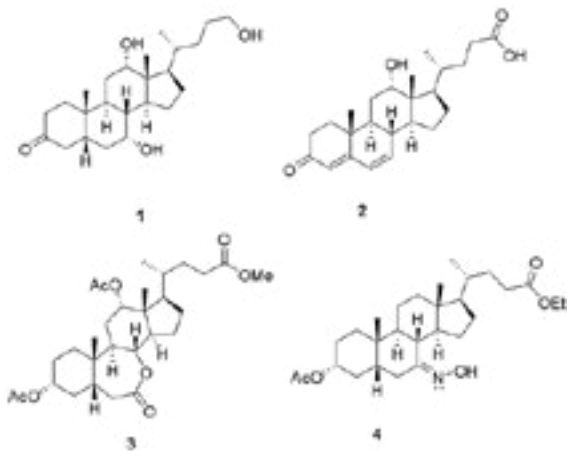


Figure 1

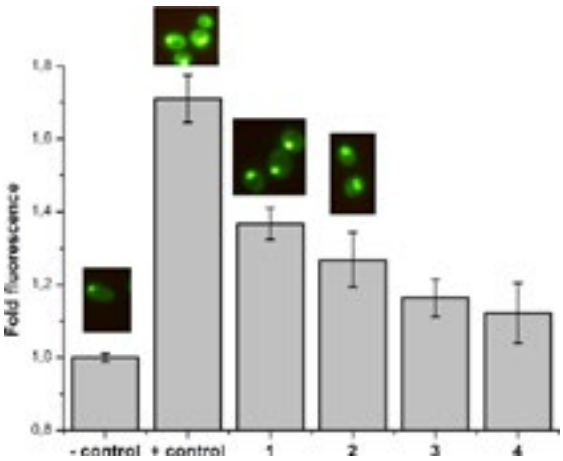


Figure 2

CONCLUSIONS:

New bile acid derivatives **1** and **2** showed moderate affinity for the ligand binding domain of glucocorticoid receptor, while binding of compounds **3** and **4** was weak. The obtained results gave us important guidelines towards the design of new GR ligands, potentially new GCs.

The authors acknowledge the financial support of the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-9/2021-14/ 200125).

T3-P-62 Extracellular hemoglobin of xenogeneic origin modulates functional characteristics of mesenchymal cells *in vitro*

Ana Stančić[176], Vesna Ilić[176], Branko Bugarski[177], Irina Maslovarić[176], Dragana Marković[176], Marijana Kovačić[176], Diana Bugarski[178], Ivana Drvenica[176]

KEYWORDS: porcine and bovine hemoglobin; mesenchymal cells; viability; migration; differentiation capacity

INTRODUCTION:

In addition to its highly conserved role in the transport of oxygen within erythrocytes, hemoglobin can also perform numerous functions when it is found in the extracellular environment[179]. Several studies have shown that extracellular hemoglobin affects activation, migration and metabolism of various cells which express Toll-like receptors[180]; all these effects of extracellular hemoglobin were demonstrated in studies using allogeneic human or rodent hemoglobin, aiming to simulate its extracellular presence *in vivo*. On the other hand, xenogeneic hemoglobins, besides being a valuable model for testing effects of extracellular hemoglobin on the cell functions, are also important topics of biotechnological research, like their usage as

176 Group for Immunology, Institute for Medical Research, National Institute of Republic of Serbia, University of Belgrade, Serbia.

Corresponding author: ivana.drvenica@imi.bg.ac.rs

177 Department of Chemical Engineering, Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia

178 Group for Hematology, Institute for Medical Research, National Institute of Republic of Serbia, University of Belgrade, Serbia

179 I.K. Quaye, Front. Physiol. 6 (2015) 1–13, https://doi.org/10.3389/fphys.2015.00096

180 Z. Loomis et al., PLoS One 12 (2017) 1–21, https://doi.org/10.1371/journal.pone.0171219

additives for organ and tissue preservation[181].

OBJECTIVES:

This study aimed to obtain data on the influence of extracellular hemoglobin on the functional properties of mesenchymal cells *in vitro*. Xenogeneic porcine (PHb) and bovine (BHb) hemoglobin isolated from slaughterhouse blood were used as abundant sources of vertebrate hemoglobin, which show a high degree of homology with human hemoglobin. Human peripheral blood mesenchymal stem cells (PB-MSCs) were selected as adequate cell model systems since extracellular hemoglobin may encounter these cells during intravascular hemolysis. In addition, the effects of hemoglobin were also tested on three cell lines ATDC5, MC3T3-E1, 3T3-L1, as more uniform model systems compared to PB-MSCs to study chondro-, osteo-, and adipogenesis.

METHOD / DESIGN:

Hemoglobin samples were isolated from porcine and bovine erythrocytes originating from slaughterhouse blood, as described by Kostić and co-workers[182], and purified and characterized as reported in Drvenica et al.[183] and Stančić et al[184]. We have investigated the effect of PHb and BHb in the concentration of 0.1, 1, and 100 μM on cells' proliferation, cycle, clonogenic and migratory potential, by using MTT test, Hoechst 33258 staining, propidium-iodide staining and flow cytometry, CFU-F and Scratch assay, respectively [6]. The cells multilineage differentiation capacity in the presence of 0.1 μM PHb and BHb was evaluated after induced differentiation, by histochemical staining and by RT-PCR analysis of the expression of specific genes specific for chondrogenic, adipogenic and osteogenic lineages.

RESULTS:

The obtained results show that extracellular hemoglobin has a significant influence on the viability and motility of MSCs and that its influence depends on the type of organism from which hemoglobin was isolated, the hemoglobin concentration and the type of cells on which the effect was examined. Furthermore, at a concentration of 0.1 μM extracellular hemoglobin had the effect on viability and migratory capacity of MSCs and inhibited their differentiation toward chondro-, osteo-, adipogenic lineages, modulating the expression of specific gene markers. Observed finely tuned differences in the effects of PHb and BHb on MSCs functional characteristics may be attributed to differences in primary protein structure, higher levels of protein organization or some differences in the level and type of contaminating proteins and phospholipids in isolated hemoglobin samples. These contaminants, although present in low amounts, represent an inevitable side component due to the preparation method used.

CONCLUSIONS:

These findings contribute to a better understanding of the manifestation of extracellular hemoglobin on MSCs in *in vivo* conditions, such as in an intravascular hemolysis. At the same time, these results are important as a basis for biotechnological research on the use of porcine and bovine hemoglobin as dietary supplements or additives for cell culture media.

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181 D. Dutheil et al., http://www.freepatentsonline.com/y2014/0113274.html

182 I.T. Kostić et al., Colloids. Surf. B Biointerfaces 122 (2014) 250–259, https://doi.org/10.1016/j.colsurfb.2014.06.043

183 I.T. Drvenica et al., J. Serb. Chem. Soc. 84 (2019) 1105-1117. https://doi.org/10.2298/JSC190513067D

184 A.Z. Stančić et al., Int. J. Biol. Macromol. 144 (2020) 909-920. https://doi.org/10.1016/j.ijbiomac.2019.09.167

T3-P-63 Stress reduces spermatozoa functionality through adrenergic-mediated disturbance of mitochondrial dynamics markers

Isidora Starovlah, Sava Radovic Pletikosic, Tatjana Kostic, Silvana Andric[185]

KEYWORDS: acute/repeated psychological stress; mitochondrial biogenesis markers; mitochondrial fusion/architecture markers; mitochondrial fission markers; mitophagy; spermatozoa functionality.

INTRODUCTION:

A growing body of evidence, states the increasing rate of male infertility in humans, an increasing number of unexplained cases of infertile males in the peak of the reproductive period, and a decrease of the fertility rate in men younger than age 30. Many studies discussed the correlation between stress and/or stressful life and male (in/sub)fertility. Yet, the mechanisms are not described.

OBJECTIVES:

Here we investigate the stress-signaling responsible for the effects of acute/repeated psychological stresses (the most common stresses in human society) on spermatozoa number and functionality, as well as the transcriptional profile of mitochondrial dynamics markers by using the in vivo and ex vivo approaches.

METHOD / DESIGN:

In the search for the possible mechanism(s) causing the reduced spermatozoa functionality during/after psychological stress, two approaches (*in vivo* and *ex vivo*) were applied. The *in vivo* approach was designed to mimic the situations in the human population exposed to acute as well as repeated psychological stress, the most common stress in human society, by using the immobilization of the adult male rats. The *ex vivo* approach was performed on epididymal spermatozoa isolated from the undisturbed adult male rats and exposed to stress hormones, adrenaline and hydrocortisone, and the agonists/antagonists of their receptors.

RESULTS:

Acute and repeated stress inhibit spermatozoa functionality (acute–>3.2-fold, repeated–>2.5-fold), while only repeated stress reduces the spermatozoa number (1.7-fold). Stress hormones mimic these effects and decrease the spermatozoa functionality (adrenaline: 10 μM–>2.4-fold, 100 μM–>2.8-fold; hydrocortisone: 50 pM–>2.7-fold, 500 pM–>8.5-fold). They also significantly disturb the transcriptional profile of all main mitochondrial dynamics markers in spermatozoa. *Ex vivo* manipulation of stress signaling in spermatozoa reveals that most of these effects are mediated through α1-and/or-β-adrenergic receptors.

CONCLUSIONS:

Stress-hormones-triggered changes in the transcriptional profile of mitochondrial dynamics markers, as well as adrenergic receptors and adrenergic receptors kinases are important molecular markers of spermatozoa functionality representing an adaptive mechanism regulated by stress signaling and does not only correlate-with but also are essential for spermatozoa functionality, being all events depend on the same regulators. The stress mimetics disturb (mostly increase) sixteen out of nineteen mitochondrial dynamics markers in spermatozoa with adrenergic signaling being more effective, suggesting the

185 Laboratory for Reproductive Endocrinology and Signaling, Laboratory for Chronobiology and Aging Center of Excellence for Reproductive Endocrinology Faculty of Sciences, University of Novi Sad. Corresponding author: isidora.starovlah@dbe.uns.ac.rs

importance of these spermatozoa markers in response on high energy demand during stress. Accordingly, the above mentioned molecular markers can be used as a test for spermatozoa functionality and for a better understanding of the correlation between stress as well as any other life-style-environmental-one-health-factors and male (in)fertility.

T3-P-64 Investigation of enzymatic effect on isolation of proteins fromagricultural waste

Tea Sedlar, Jelena Čakarević and Ljiljana Popović[186]

KEYWORDS: enzyme-assisted extraction; vegetable by-products; leaf proteins; protein characterization;

INTRODUCTION:

In recent years there has been growing interest in plant proteins as a substitution for animal proteins. This can be attributed to various factors, such as health problems, vegetarianism and religious restrictions. Scientific interest is focused on finding plant materials suitable for isolation of proteins with specific functional and biological activities, which can be further used in food production. Leaves of cauliflower and broccoli, which present a waste material in agricultural vegetable production, could be good potential sources of new proteins.

OBJECTIVES:

The first objective of this study was to adjust enzyme-assisted method for effective extraction of proteins from cauliflower and broccoli leaf waste. The second objective was to characterize physical and functional quality of obtained proteins in order to determine their potential utilization in food industry.

METHOD/DESIGN:

Waste material (leaves) from cauliflower and broccoli, was collected from a agricultural field located in the northern part of Serbia. The proteins were obtained from leaves with enzyme-assisted alkaline extraction at pH 10-11, and their isoelectric precipitation at pH 4. The enzymatic pretreatment was carried out with cellulityc and pectolytic complexes (Viscozyme®L and Vinozyme®) used in three different enzyme to substrate ratio (E/S) (0.2%, 2.5% and 4.8%). Simultaneously, extraction without enzymes was carried out as control sample. The efficiency of the enzymatic pretreatment was monitored by measuring protein yield (%) Obtained proteins were characterized by FTIR spectroscopy and SDS-PAGE electrophoresis. In addition, protein solubility, as one of the most important functional properties, was determined for both, control and enzymatic treated (at E/S 4.8%) samples, over the pH range from pH 2 to pH 11.

RESULTS:

The cell wall of a plant cell consist of a complex structure which makes it difficult to extract proteins. Therefore, after pre-treatment with cell wall degrading enzymes, significant improvements in protein yields, of more than 10%, were observed for both leaf sources. Also, it has been shown that protein yields correlate with applied amount of enzymes, the highest yields, for both sources, were achieved with the E/S ratio of 4.8%.

FTIR spectroscopy is an efficient technique used to assess the secondary structure of a protein. The characteristic infrared absorption band of proteins obtained from waste leaves of broccoli and cauliflower mainly includes amide A band (3270 cm^{-1}), amide B band (2920 cm^{-1}), amide I band (1632 cm^{-1}), amide II band (1516 cm^{-1}), and amide III band (1218 cm^{-1}). As the amount of enzyme complex increased, the characteristic peaks of the amide A, amid B, amide I, II, III bands showed

186 Tehnološki fakultet, Bulevar Cara Lazara 1, 21101 NoviSad, Srbija. Corresponding author: tea88todorovic@gmail.com

higher absorption numbers.

SDS-PAGE protein profiles of all investigated samples consist of same fractions with major subunits of 20 kDa, 25 kDa, 30 kDa and 45 kDa. Furthermore, the bands have the highest intensity in samples obtained by the highest enzyme concentration, in which the protein yield is also the highest.

The solubility profile of all protein samples was characterized by low solubility in acidic environment (pH 2 - pH 6) with a linear increase in alkaline environment (pH 8 - pH 11). The highest solubility for the enzymatically pretreated sample of broccoli leaves was achieved at pH 11 (29.4 mg/ml) and it was 25% higher compared to the control sample. In the case of cauliflower, the highest solubility was achieved at pH 10 (36.4 mg/ml) and was also improved in comparison with the control sample.

CONCLUSIONS:

Enzyme-assisted extraction leads to a significant increase in the yield of extracted proteins. Moreover, solubility of these proteins was significantly improved within alkaline pH values. Therefore, it can be expected that the improved solubility can potentially affect the improvement of other functional properties, thereby increasing the potential of these proteins as future food ingredients.

T3-P-65 Antioxidant potential of sweet basil (*Ocimum Basilicum L.*) extract in rats

Branislava Teofilović, Ana Tomas, Nebojša Stilinović, Nevena Grujić, Emilia Gligorić, Aleksandar Rašković[187]

KEYWORDS: basil; free radicals; oxidative stress; antioxidant enzymes.

INTRODUCTION:

The interest in a natural and healthy lifestyle has moved the functional food under the spotlight. Basil (*Ocimum basilicum L.*) is one of the most important industrial and pharmaceutical crop species from Lamiaceae family having a major application in the food, pharmaceutical and cosmetic industries. It exhibits strong antioxidant activity due to high content of phenolic and flavonoid compounds. It is most commonly used in the form of teas, essential oil, liquid extracts, as a spice and has an important application in the food, pharmaceutical and cosmetic industries.

OBJECTIVES:

The aim of this research was to examine the effects of pre-treatment with basil water extract (infusum) on acetaminophen-induced acute liver injury in rats.

METHOD / DESIGN:

Total phenolic and flavonoid contents were tested by spectrophotometric methods. For the chemical characterization of basil extracts, an appropriate high performance liquid chromatography (HPLC) method was applied. Effects of basil extract on oxidative stress parameters were determined in an in vivo model of acetaminophen-induced liver injury in 24 Wistar rats.

RESULTS:

Total phenolic content was 52.61±1.35 mg GAE/g of DE and flavonoid content was 0.5±0.2 mg QE/g of DE. Basil extracts contain chlorogenic, p-hydroxybenzoic, caffeic, ferulic, vanilic, rosmarinic and cinnamic acid, quercetin and naringenin. IC50 values ranged from 0.22-45.76 µg/ml for DPPH radical, OH radical, H_2O_2 and lipid peroxidation. The extract lowered the

187 University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, 21000 Novi Sad, Serbia. Corresponding author: branislava.teofilovic@mf.uns.ac.rs

intensity of lipid peroxidation and potentiated the activity of antioxidant enzymes, with statistically significant increase in catalase ($p<0.01$), glutathione reductase ($p<0.05$), glutathione transferase activities ($p<0.05$), except for glutathione peroxidase activity.

CONCLUSIONS:

The obtained results indicate that basil extract, produced by a simple, convenient, and widely accessible mode of extraction, easily done without any sophisticated equipment, exhibits several beneficial properties. In addition to high antioxidant in vitro activity, the present study demonstrated significant *in vivo* antioxidant potential of aqueous basil extract in a model of acetaminophen induced liver injury. Antioxidant effects were apparent though the increase in the activity of antioxidant enzymes and decreased lipid peroxidation.

T3-P-66 New androstane 1,3,4-thiadiazolines: synthesis and physicochemical analysis

Tijana Šestić, Marina Savić, Andrea Nikolić, Ivana Kuzminac, Jovana Ajduković[188]

KEYWORDS: aza-steroids; lactam; thiosemicarbazone; thiadiazoline; physicochemical properties.

INTRODUCTION:

Steroids are an essential class of natural compounds which regulate variety of metabolic processes and they are proven as good therapeutics for many diseases. In order to find new steroid derivatives with desirable biological activity, natural steroids are often modified by incorporation of heteroatoms or heterocyclic rings. 1,3,4-Thiadiazoline derivatives are the most studied among the different isomers, while they exhibit various biological activities due to the presence of =N-C-S- moiety.[189] On the other hand, incorporation of a nitrogen atom in the steroid A-ring can modify biological activity of parental molecules.[190] Aza-steroids are formed by replacement of one carbon atom in the steroid molecule by nitrogen, while aza-homosteroids are formed by incorporation of lactam moiety (-NH-CO-) into the steroid ring.

OBJECTIVES:

Considering the biological potential of steroid lactams and thiadiazolines, here we present synthesis of novel aza-steroids with 1,3,4-thiadiazoline ring (**2** and **3**) obtained by the cyclization reaction of thiosemicarbazone derivative **1** in the presence of acetanhydride or propionic anhydride. Furthemore, the physicochemical properties of the synthesized compounds were calculated using the web tool SwissADME, and compared with Lipinski, Veber, Egan, Ghose and Muegge criteria.[191]

METHOD / DESIGN:

All new compounds were characterized by IR and NMR spectroscopic techniques and *in silico* ADME profile was determined. The bioavailability radars allowed a first insight at the drug-likeness of the compounds, while the BOILED-Egg model provided information about gastrointestinal absorption and brain penetration.

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188 Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia. Corresponding author: ivana.kuzminac@dh.uns.ac.rs

189 S. Menta, S. Carradori, D. Secci, C. Faggi, L. Mannina, R. Cirilli, M. Pierini, J. Org. Chem. 80 (2015) 11932

190 J. J Ajduković, K. M. Penov Gaši, D. S. Jakimov, O. R. Klisurić, S. S. Jovanović-Šanta, M. N. Sakač, L. D. Aleksić, E. A. Djurendić, Bioorg.Med. Chem. 23 (2015) 1557

191 A. Daina, O. Michielin, V. Zoete, Sci. Rep.-UK 7 (2017) 42717

RESULTS:

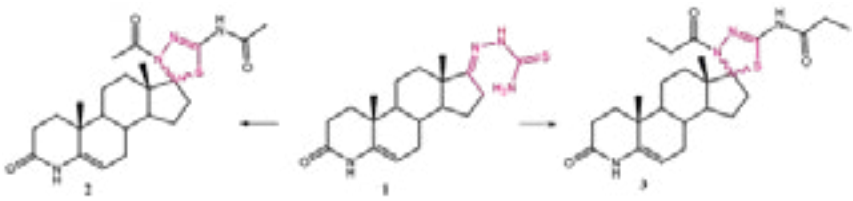

Table 1. In silico physicochemical properties of compounds 1, 2 and 3.

Compound	Formula	MW	HBD	HBA	LogP	nrotb	TPSA	MR	No.rings
1	$C_{19}H_{28}N_4OS$	360.52	3	2	2.52	2	111.60	107.90	4
2	$C_{23}H_{32}N_4O_3S$	444.59	2	4	2.71	3	116.17	132.68	5
3	$C_{25}H_{36}N_4O_3S$	472.65	2	4	3.39	5	116.17	142.29	5

MW: molecular weight expressed in Daltons; HBD: number of hydrogen bond donors; HBA: number of hydrogen bond acceptors; LogP: partition coefficient, average of five predictions (iLOGP, XLOGP3, WLOGP, MLOGP and Silicos-IT LogP); nrotb: number of rotatable bonds; TPSA: topological polar surface area in $Å^2$; MR: molar refractivity.

CONCLUSIONS:

In this work, the synthesis of new thiosemicarbazone and thiadiazoline aza-steroid derivatives was described. Analysis of *in silico* ADME physicochemical properties showed that all newly synthesized compounds possess drug-like properties according to Lipinski, Veber, Egan, Ghose and Muegge criteria. The bioavailability radars showed that all compounds are in the optimal range for lipophilicity, polarity, solubility, saturation and flexibility. The BOILED-Egg model indicated that these compounds are absorbable by the intestines but couldn't penetrate the brain. Obtained results indicate that all compounds are good candidates for further *in vitro* investigations.

T3-P-67 Transcriptional profiles of mitochondrial dynamics markers in human spermatozoa are associated with different types of spermiograms

Tamara Tomanic[192], Isidora Starovlah[192], Ana Jeremic[193], Milan Perovic[193], Tatjana Kostic[192], Silvana Andric[192]

KEYWORDS: male infertility; spermatozoa; mitochondrial dynamics markers; in vitro fertilization; real-time PCR.

INTRODUCTION:

Infertility has become one of the greatest health issues today, affecting millions of people worldwide, with significant contribution of male factor in many reported cases. Bearing in mind the increasing number of unexplained cases of infertile men in the peak of reproductive period and the lack of an accurate test for assessment of spermatozoa functionality, World Health Organization urges for the development of a new prognostic/diagnostic tool for detection of male infertility. Since mitochondria play important role in spermatozoa, regulating their homeostasis and functionality, it is reasonable to presume that they could be involved in these types of abnormalities and markers of their dynamics could be used as "mitochondrial-sperm-signature", to test the spermatozoa functionality. Regardless of that, little is known about mitochondrial dynamics markers in human spermatozoa.

192 Laboratory of Reproductive Endocrinology and Signaling, Laboratory for Chronobiology and Aging, Centre of Excellence for Reproductive Endocrinology and Signaling, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia. Corresponding author: silvana.andric@dbe.uns.c.rs

193 ART Department, Clinic for Gynecology and Obstetrics "Narodni Front", Belgrade, Serbia

OBJECTIVES:

The main objective of this research was to investigate the transcriptional profile of main mitochondrial dynamics markers in spermatozoa from the population of men diagnosed with normozoospermia, teratozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia.

METHOD / DESIGN:

Spermatozoa obtained from men participating in the National program of *in vitro* fertilization, diagnosed with normozoospermia, teratozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia, were separated from seminal plasma, capacitated and incubated with acrosome reaction inducer – progesterone. After RNA isolation and complementary DNA synthesis, samples were subjected to real-time PCR analysis. During quantification, all results were normalized to a normozoospermic group, used as a control and *GAPDH*, as a reference gene.

RESULTS:

The results showed significant increase in the level of *PPARGC1A* transcript in spermatozoa from teratozoospermic group comparing to normozoospermic. Conversely, the levels of *PPARGC1B* and *MFN1* transcripts significantly decreased. The levels of *NRF2, TFAM, MFN2, OPA1, FIS1 DRP1, PINK1* and *PRKN* remained unchanged. Although trends of stimulation in transcriptions were observed for *PPARGC1A, PPARGC1B* and *PRKN* in asthenoteratozoospermic and oligoasthenoteratozoospermic group, the levels of the changes were not significant. In the same samples, the levels of transcripts for other mitochondrial dynamics markers remained unchanged.

CONCLUSIONS:

Based on the obtained results it is evident that the markers of mitochondrial dynamics in human spermatozoa exerted different patterns depending on the type of spermiograms and the most remarkable changes were observed for the teratozoospermic group. However, it is important to point out that research was conducted on a small number of the samples (3-10 individuals per group), so the results should be considered preliminary.

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T4-IL-1 Novel approaches for extraction of proteins from alternative sources

Xianglu Zhu[1/2], Laura Healy[1/3], Brijesh K Tiwari[1]

KEYWORDS: Alternative proteins, cavitation, pre-treatment, processing

INTRODUCTION:

Alternative proteins from unconventional sources has shown significant increase in demand due to various factors namely negative perception associated with meat based proteins. Association of Green House Gases (GHGs) emission associated with Animal-based foods compared to plant-based foods (e.g. 1 kg of animal products requires about 2 to 15 kg of plant material). Moreover, global protein production faces unachievable demands due to population growth along with other socio-economic challenges unless alternative strategies are adopted.

OBJECTIVES:

The objective of this presentation is to outline various approaches employed for the extraction of proteins from a range of unconventional sources.

RESULTS:

There are many strategies available to respond to growing global protein demand, which span alternative sources of protein, reduction in protein losses and technological advancements. Novel unconventional sources of protein and improved protein recovery from food processing streams while employing novel biotransformation techniques will facilitate a bridging of the gap between protein supply and demand. Novel sources of protein require the development of new value chains, and attention to issues such as production costs, food safety, scalability and consumer acceptance. Innovative, green, sustainable bioprocessing technologies for recovery of proteins are gaining increased processors interest over conventional techniques. The obvious advantages of novel technologies include improved process efficiency, the use of clean solvents and allow the production of chemical residue free end product. A wide range of new conversion technologies have been employed to extract proteins from a range of matrices. New conversion technologies can be employed at various stages including pre-treatment, extraction or purification of proteins (*Fig 1.*). The key focus of the pre-treatment is to disrupt plant matrices and increase protein solublisation. New pretreatment methods are generally employed prior to extraction, and their combinations with conventional and green extraction technologies.

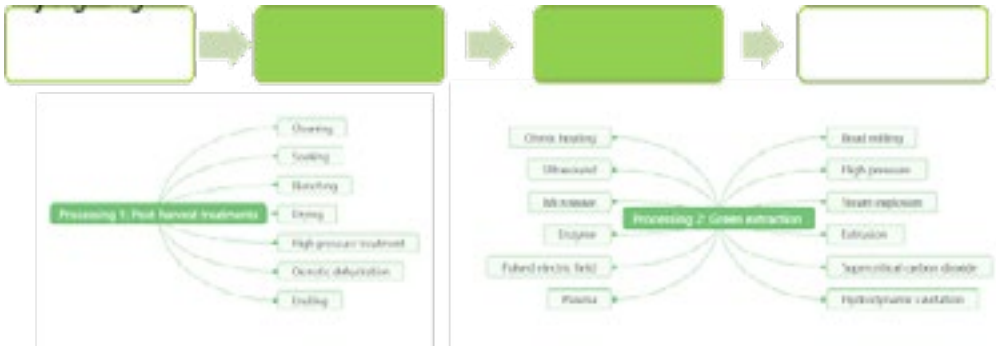

Figure 1. Novel approaches for extraction of proteins

1 *Teagasc Food Research Centre, Dublin, Ireland. Corresponding author: brijesh.tiwari@teagasc.ie*

2 *School of Biosystem and Food Engineering, University College Dublin, Ireland*

3 *School of Food and Environmental Health, Technological University Dublin, Ireland*

CONCLUSIONS:

Combinations of different technologies and extraction methods can enhance the extractability of proteins with higher purity and contribute towards improved process efficiency. It is expected that these new conversion technologies will contribute to develop a zero-waste sustainable approach. Although some of these technologies have been investigated extensively for the recovery of protein, the majority of them require further development prior to commercial adoption.

T4-IL-2 Digital services for farmers based on Sentinel-2 satelllite images and advanced machine learning

Oskar Marko, Marko Panić, Željana Grbović, Aleksandar Antić, Branislav Pejak, Vladimir Crnojević[4]

KEYWORDS: Image Processing; Machine Learning; AgTech; Precision Agriculture

INTRODUCTION:

The world's growing population is putting an immense pressure on agriculture to produce more with less. In the context of conflicting economic, environmental and societal demands, decision-making across the whole supply chain needs to be optimised. In order to make informed decisions, data coming from satellites, drones, sensors and other sources needs to be analysed. However, due to complexity and magnitude of data, advanced machine learning and data analytics algorithms need to be employed.

OBJECTIVES:

This paper tackles two critical tasks in precision agriculture – management zone delineation and yield prediction. Management zones are regions in the field that have large inter-region and small intra-region variability, meaning that their boundaries divide the field into homogeneous zones for which the agronomic operations should be separately adjusted. Secondly, yield prediction is essential for fertiliser optimisation and post-harvest logistics. Fertiliser type and amount are tied to the amount of nutrients extracted from the soil and in order to compensate for this, nutrients need to be replenished. The information about the yield is also key for optimising harvesting, logistics, storage and sales.

METHOD / DESIGN:

The choice of input data depends on the use-case, but generally, there is a trade-off between precision and scalability. Within the scope of image processing, drones may provide high-resolution data, but their use is limited by the need of physical presence of the human operator in regular intervals during the season. Sentinel-2 satellites on the other hand provide images at a 10 m resolution, but cover the whole globe every 5 days on average. For this reason, we chose them as the input data source. For management zone delineation, we calculated different spectral vegetation indices from satellite images and applied the k-means algorithm. The resulting maps were post-processed so that the resolution of the zones fits the width of the fertiliser/pesticide spreader. Yield prediction was set on a per pixel basis. We used the soya yield maps from combine harvesters acquired in the years 2018-2020 for model training (411 ha in total) and a number of machine learning models were implemented, such as: random forest, artificial neural networks, XGBoost and stochastic gradient descent.

RESULTS:

Proposed yield prediction algorithms were evaluated on the test set which included 14 out of 142 soya fields from the database. With the Pearson correlation coefficient of 0.74 and mean absolute error of 0.49 t/ha, stochastic gradient descent

4 *BioSense Institute. Oskar Marko: oskar.marko@biosense.rs*

achieved the best performance. As for management zone delineation, the tool cannot be validated on a similar basis, as there is no objective division of the field into zones. Rather than that, we left the algorithm parameters, such as choice of the spectral index (from a number of soil and plant-based indices), the number of zones and the width of the machine, to the user to decide on, according to his/her preference, experience and the desired output.

CONCLUSIONS:

The aforementioned machine learning models are essential tools for monitoring crop growth. The resulting maps provide precious information to the farmers, who can optimise their decisions based on them. In order to facilitate rapid transfer of technology from academia to industry, we implemented a management zone delineation module within AgroSense. With more than 20,000 users, and ¼ of all Serbian farmland managed through the system, this technology transfer signifies an important step in digital transformation of agriculture.

T4-IL-3 Prebiotic oligosaccharides: dietary strategies for improving gut health

Pissared Muangnil, Sakena K-da, and Fittree Hayeeawaema[5]

KEYWORDS: Dragon fruit oligosaccharides; Konjac oligo-glucomannan; *Gracilaria fisheri* oligosaccharides; gut motility; gut dysbiosis.

INTRODUCTION:

Prebiotic oligosaccharides are produced from many different sources which may alter gut microbiota composition and improve gut health. These novel prebiotics are showing their ability to deliver health benefits compared to well-known prebiotics.

OBJECTIVES:

This research aimed to show the effects of novel prebiotic oligosaccharides: dragon fruit oligosaccharides (DFO), konjac oligo-glucomannan (KOG), and *Gracilaria fisheri* oligosaccharides (GFO) on gut motility in normal, constipated, and colitis mice, respectively.

METHOD / DESIGN:

Normal mice received distilled water, 100, 500, and 1000 mg/kg DFO, 1000 mg/kg fructo-oligosaccharide (FOS), or 10^9 CFU *Bifidobacterium animalis* daily for 1-2 weeks. Constipated mice received distilled water, 100, 500, and 1000 mg/kg KOG, 100 mg/kg konjac glucomannan (KGM), 500 mg/kg lactulose, or 10^9 CFU *Bifidobacterium animalis* daily for 2 weeks. Colitis mice received distilled water, 100, 500, and 1000 mg/kg GFO, or 1000 mg/kg inulin daily for 2 weeks. Gut microbiota composition, defecation frequency, and gut transits were analyzed. Motility patterns, smooth muscle (SM) contractions, and morphological structures of the colons were assessed.

RESULTS:

DFO significantly increased fecal output, reduced gut transit time, and increased the amplitude and duration of colonic SM contractions when compared to the control group. Spatiotemporal maps of colonic wall motions showed that DFO increased

5 *Gut Biology and Microbiota Research Unit and Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Songkhla, Thailand. Corresponding author: pissared.k@psu.ac.th*

the number of colonic non-propagation contractions and fecal pellet velocity. Histological stains showed normal epithelia, crypts, goblet cells, and SM thickness in all groups. KOG ameliorated the effects of loperamide on defecation frequency, gut transit time, and contraction frequency of colonic SM. The motility patterns were changed from non-propagation to propagation contraction. KOG significantly inhibited the effects of loperamide on gut microbiota by increasing the numbers of Bifidobacterium spp. and decreasing the numbers of Clostridium spp. and Bacteroides spp. GFO attenuated histological change and shortening of the colon, reduced body weight loss, and lowered the disease activity index in acetic acid-induced colitis mice. GFO treatment prevented reductions in gut transit, propulsive motility, and SM contractility and also modulated Enterobacteria populations and short-chain fatty acids production in the gastrointestinal tract.

CONCLUSIONS:

The prebiotic effects of oligosaccharides derived from dragon fruit, konjac, and red seaweed (*Gracilaria fisheri*) could promote gut health and correct gastrointestinal motility disorders such as constipation and diarrhea symptoms of ulcerative colitis.

T4-IL-4 Natural deep eutectic solvents for green agri-food solutions

Alena Stupar[6], Nemanja Teslić[6], Aleksandra Cvetanović[7], Branimir Pavlić[7], Nadiia Khakimova[6/8], Živan Mrkonjić[7], Petar Vrgović[9], Anamarija Mandić[6], Milica Pojić[6], Aleksandra Mišan[6]

KEYWORDS: natural deep eutectic solvents, agri-food sector, plant phenolics, carotenoids, novel assisting extraction techniques.

INTRODUCTION:

The food industry faces the challenges of sustainable production demanding innovative solutions to exploit food waste and by-products as bioresources for our next generation of energy, chemicals, pharmaceuticals, cosmetics, foods and other high value added products. In line with that, the principles of "green chemistry" are gaining importance[10].

Natural deep eutectic solvents (NADES) are a subgroup of eutectic solvents consisting only of natural, edible, nontoxic and biodegradable compounds. More precisely, sugars, fatty acids, organic salts, amino acids, terpenes, alcohols and other generally recognized as a safe (GRAS) compounds can be mixed in the adequate proportions to design multifunctional solvents with tailored properties for specific applications[11/12]. Their edible nature, high solubilisation capacity for poorly soluble natural compounds, ability to enhance stability of extracted compounds and to promote their biological activities, make them suitable for a wide area of food applications[10/11/12].

6 *University of Novi Sad, Institute of Food Technology, cara Lazara 1, 21000 Novi Sad, Serbia. Corresponding author: alena.tomsik@fins.uns.ac.rs*

7 *University of Novi Sad, Faculty of Technology, cara Lazara 1, 21000 Novi Sad, Serbia*

8 *Kaunas University of Technology, K. Donelaičio g. 73, 44249 Kaunas, Lithuania*

9 *University of Novi Sad, Faculty of Technical Sciences, Trg D. Obradovića 6, 21000 Novi Sad, Serbia*

10 *Mišan, A., Nađpal, J., Stupar, A., Pojić, M., Mandić, A., Verpoorte, R., & Choi, Y. H. (2020). The perspectives of natural deep eutectic solvents in agri-food sector. Critical reviews in food science and nutrition, 60(15), 2564-2592*

11 *Stupar, A., Šeregelj, V., Ribeiro, B. D., Pezo, L., Cvetanović, A., Mišan, A., & Marrucho, I. (2021). Recovery of β-carotene from pumpkin using switchable natural deep eutectic solvents. Ultrasonics Sonochemistry, 105638*

12 *Mišan, A., & Pojic, M. (2021). Applications of NADES in stabilizing food and protecting food compounds against oxidation. Eutectic Solvents and Stress in Plants, 97, 333*

OBJECTIVES:

The objective of the work was to test the application of NADES within the framework of "new food product development": 1) to identify sustainable plant sources rich in phenolic compounds and carotenoids; 2) to produce task specific hydrophilic and hydrophobic NADES; 3) to optimize the production of NADES extracts rich in bioactives; 4) to design a new functional beverage in the co-creation processes involving consumers and SMEs.

METHOD / DESIGN:

Interdisciplinary approach was applied, encompassing the employment of different engineering (novel extraction techniques), chemical (HPLC and spectrophotometric), mathematical (descriptive statistics, RSM and ANN modelling) and social science methods.

RESULTS:

Hydrophilic NADES composed of organic acids (citric, malic, lactic and tartaric acid) as hydrogen-bond donors and betaine or sugars (fructose and glucose) as hydrogen-bond acceptors could serve as efficient extraction agents for the phenolic compounds and alternative hydrolysis media which are edible and nontoxic for human health. On the other hand, hydrophobic NADES composed of medium chain fatty acids appear very efficient in extracting and protecting β-carotene from degradation. The selection of extraction technique and optimization of parameters have a crucial role in the process efficiency (2).

CONCLUSIONS:

Based on obtained results; NADES have a great potential in the food sector. However, a lot of fundamental research dealing with the nature of the interactions in NADES, along with their physicochemical properties needs to be done as they are relevant for industrial applications.

ACKNOWLEDGMENT: This work was funded by the Science Fund of the Republic of Serbia, PROMIS, Grant No. 6060592, DEStiny.

T4-IL-5 A 3D gelatin aerogel sorbent for the extraction of polycyclic aromatic hydrocarbons in tea drinks

Myo Myint Zaw[13/14], Sujittra Poorahong[15], Proespichaya Kanatharana[13/14], Panote Thavarungkul[13/14], Chongdee Thammakhet-Buranachai[13/14]*

KEYWORDS: gelatin aerogel; high performance liquid chromatography; polycyclic aromatic hydrocarbons; vortex assisted solid phase extraction.

INTRODUCTION:

Tea leaves could be contaminated by polycyclic aromatic hydrocarbons (PAHs), a well-known class of carcinogens commonly generated from incomplete combustion of gases during the drying process. Therefore, as a consumer safety precaution, it is important to determine the concentrations of PAHs in tea. The most potent PAHs, which are benzo(a)anthracene (BaA), benzo(b)fluoranthene (BaF) and benzo(a)pyrene (BaP) were selected as target PAHs in this study. However, the contamination

13 *Division of Physical Science. *Corresponding author: chongdee.t@psu.ac.th*

14 *Center of Excellence for Trace Analysis and Biosensor, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand*

15 *Functional Materials and Nanotechnology Center of Excellence, Faculty of Science, Walailak University, Nakhon Si Thammarat 80160, Thailand*

level is very low, therefore an extraction before the analysis was required. Gelatin aerogel sorbent was prepared and used as a 3D-porous sorbent for the extraction of PAHs in tea samples. The aerogel was rich in carbonyl (C=O) groups that can adsorb PAHs through carbonyl-π or π-π interactions. After extraction, the extractant was analyzed by a high-performance liquid chromatography with diode array detector (HPLC-DAD).

OBJECTIVES:

To develop a simple, affordable and effective extraction method for the analysis of PAHs in tea samples

METHOD / DESIGN:

15% gelatin was added in water before a crosslinking agent (25% glutaraldehyde) was added. Then the mixture was transferred into a template and kept at -20°C for 16 h. The gelatin cryogels were removed from the template and lyophilized at -60°C for 12 h to obtain the gelatin aerogel and used for the extraction of PAHs by the vortex-assisted solid phase extraction.

RESULTS:

Under the optimum conditions, the method provided a good linearity in the concentration range of 0.005-0.2 µg L^{-1} with low limits of detection for all three PAHs. Good reproducibility and reusability were achieved over 40 extraction cycles. BaA was detected in six different tea samples at concentrations ranging from 1.02±0.02 to 5.0±0.2 µg L^{-1}. BbF was found in four tea samples at a concentration rage of 0.32±0.03 to 2.50±0.02 µg L^{-1}. Recoveries in the range of 89.4±1.3% to 100.0±7.0% for BaA, 82.3±0.9% to 100.1±5.0% for BbF and 83.4±1.5% to 100.2±4.3% for BaP, respectively.

CONCLUSIONS:

The 3D-gelatin aerogel sorbent exhibited excellent adsorption and desorption towards target analytes. The sorbent is more cost effective than commercial ones. The PAHs analysis with the developed gelatin aerogel sorbent showed good accuracy, reproducibility and reusability with acceptable RSDs and recoveries. Finally, the developed method was successfully applied for the determination of the polycyclic aromatic hydrocarbons in tea samples.

T4-IL-6 Novel sensing technologies in food supply chains

Aneesh Chauhan[16]

KEYWORDS: food supply chains, sensing technologies

INTRODUCTION:

Typical food supply chains begin at farms, proceeded by logistics, processing and retail, finally reaching the end-user for consumption. These chains are under constant pressure to provide increasingly more food, with better quality and in a sustainable manner (animal welfare, optimal land and water use), while reducing food losses (caused by diseases, pests, spoilage, quality loss, consumer habits etc.) and delivering a safe product, leading to a healthy consumer. Data-driven technological advances, supported by sensing innovations, are playing a key role in addressing these challenges.

Novel sensing technologies can lead to new insights at scale which can help tackle multiple challenges across the food supply chain. In particular, there is a necessity to measure non-destructively, non-invasively and on a smaller scale than is currently common: from batch level to product level; from population segment to an individual. For this, we need to consider a number of technological hurdles and advances in the areas of non-destructive sensing. The goal of the talk is to demonstrate

16 *Wageningen University and Research Wageningen, 6708 PB, The Netherlands*

through practical examples how novel sensing methods can be used to measure and (semi)-automatically make better decisions based on the measured product properties.
To demonstrate the possibilities we will look at the developments and investigations over multiple examples across the supply chain, with examples from production to post-harvest processes. In particular we will look at how old and new non-destructive and non-invasive optical sensing approaches are being explored at Wageningen University and Research.

T4-IL-7 Potential of plasma applications in a circular food systems approach

Oliver Schlüter[17/18], Julia Durek[17], Shikha Ojha[17]

KEYWORDS: Plasma sources, food quality, decontamination, up-scaling, safety aspects

INTRODUCTION:
Cold plasma technology is leading major breakthroughs in addressing a plethora of issues in the agriculture and food sectors, ranging from mitigating produce losses due to pathogens and pests to enhancing the yield and safety of food. Being an ionized gas, cold atmospheric pressure plasma (CAPP) constitutes of a complex mixture of active agents, such as UV photons, charged particles, radicals and other reactive nitrogen, oxygen and hydrogen species[19].
The complexity of these molecule-molecule interactions underlines the importance of a tailor-made process design. In order to achieve the goal, which might be a gentle microbial inactivation in most cases, plasma sources need to be adapted to the specific purpose of application. This will be one of the major tasks in the field of CAPP and food before large-scale processes can be implemented in industry[20].

OBJECTIVES:
The aim of the presentation is to discuss the important development of criteria to assess safety and quality attributes related to plasma treatment of foods, which requires not only a detailed, standardized characterization of the process parameters and the method, but also elucidation and characterization of potential changes to substances in the treated foods. CAPP induced physical/chemical/biochemical/microbiological changes in different food matrices will be discussed along the value-added chain.

METHOD / DESIGN:
The introduction of a new decontamination technology in industry requires understanding of the mechanisms of microbial inactivation and the associated interactions between process and product. Therefore, experimental studies will be presented with the following objectives: i) to investigate the underlying inactivation mechanisms in plasma treatment of microbial contaminants; ii) to clarify how the different process- and product-related parameters influence the inactivation process; and

17 Quality and Safety of Food and Feed, Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Max-Eyth-Allee 100, 14469 Potsdam, Germany. Corresponding author: oschlueter@atb-potsdam.de; oliver.schluter@unibo.it

18 Department of Agricultural and Food Sciences, University of Bologna, Piazza Goidanich 60, 47521 Cesena, Italy

19 Darmanin, M.; Fröhling, A.; Bußler, S.; Durek, J.; Neugart, S.; Schreiner, M.;Blundell, R.; Gatt, R.; Schlüter, O.; Valdramidis, V.P. (2021). Plasma applications for the treatment of bean sprouts: safety, quality and nutritional assessments under aqueous and gaseous set-ups. Scientific Reports. 11:19681 (https://doi.org/10.1038/s41598-021-97823-1)

20 Misra, N.; Schlüter, O. (2019). Editorial Note, Securing the food production chain through cold plasma technologies. Innovative Food Science and Emerging Technologies. 53, May, 1-2.(https://doi.org/10.1016/j.ifset.2019.04.001)

iii) to evaluate the treatment effects on relevant quality parameters of selected food materials.

RESULTS:

The individual aspects follow a comprehensive overall approach, from the targeted inactivation of microorganisms on temperature-sensitive food systems to the customized influence of quality attributes along the entire food value chain. The multi-scale approach takes into account the influence of cold atmospheric pressure plasma on molecules, macromolecules, single cells, complex plant food systems and food processing equipment[21].

CONCLUSIONS:

Future perspectives for the transfer of plasma applications to industrial food production will be discussed and examples for possible implementation concepts of the plasma technology in a circular food systems approach will be derived.

21 Ojha, S.; Fröhling, A.; Durek, J.; Ehlbeck, J.; Tiwari, B.K.; Schlüter, O.; Bußler, S. (2020). Principles and Application of Cold Plasma in Food Processing. In: Knoerzer, K., Muthukumarappan, K. (Eds.), Innovative Food Processing Technologies: A Comprehensive Review, vol. 1. Elsevier, pp. 519–540. https://doi.org/B978-0-08-100596-5.23033-3

T4-P-1 Drug-like properties of phytocannabinoids

Maja Milanović, Nataša Milošević, Milana Rajačić, Nataša Milić[22]

KEYWORDS: endocannabinoids; phytocannabinoids; drug-like properties; lipophilicity; oral bioavailability

INTRODUCTION:

Phytocannabinoids could have therapeutic potential in a variety of pathological conditions regulated by endocannabinoid system. Hence, *in silico* analysis serves as powerful tool for the optimization of new therapeutic agents that target endocannabinoid system related diseases.

OBJECTIVES:

In silico evaluation of drug-like properties of different cannabinoids through the determination of their physicochemical and pharmacokinetic parameters and receptor binding affinity.

METHOD / DESIGN:

Molecular descriptors such as molecular weight (Mw), number of rotatable bonds, hydrogen bond acceptors and hydrogen bond donors (NHBA and NHBD, respectively), together with polar surface area (PSA), lipophilicity (MlogP) and solubility (logS) were calculated online for 80 compounds (endocannabinoids, phytocannabinoids from *Cannabis sativa* and phytocannabinoids from other natural resources) using Molinspiration and SwissADME programs. Based on the two-dimensional structures of analysed compounds, the human intestinal absorption and the permeability through Caco-2 cells were predicted by admetSAR tool. The enzyme inhibitory potential and the binding affinity to G-protein coupled receptors and nuclear receptors were calculated by Molinspiration program.

RESULTS:

The rules derived by Lipinski and Veber suggest that a compound would have a good oral bioavailability if Mw < 500 g/mol, Mlog P < 4.15, NHBD < 5, NHBA < 10, PSA < 140 Á^2 and the number of rotatable bonds is below 10. The 46% of analysed compounds met the empirical rules requirements for drug-likeness. However, most of the analysed compounds had molar mass less than 500 g/mol (92%), MlogP < 4.15 (71%) and PSA < 140Á^2 (95%), which indicated good oral bioavailability. The number of hydrogen bond acceptors and donors corresponded to Lipinski`s rule for 94% of analysed compounds while 82% of them had less than 10 rotatable bonds according to Veber`s rule. All compounds had the potential to penetrate through blood-brain barrier and expressed appropriate human intestinal absorption, Caco-2 cells permeability as well as the receptor binding affinity to G-protein coupled receptors and nuclear receptors.

CONCLUSIONS:

A more than half of the analysed compounds were in agreement with the requirements of Lipinski`s and Veber`s rules and would theoretically have appropriate oral bioavailability. In terms of drug-likeness, the analysed compounds could contribute to the discovery and optimization of suggesting drug candidates for the treatment of various pathological conditions.

22 *University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia. Corresponding author: maja.milanovic@mf.uns.ac.rs*

T4-P-2 Evaluation of spirulina antioxidative potential in hyperlipidaemia

Nataša Milić, Maja Milanović[23], Ivan Milovanović[24], Jelica Simeunović[25], Dajana Blagojević[25], Aleksandra Mišan[26], Danijela Kojić[25], Anamarija Mandić[26]

KEYWORDS: antioxidative activity; functional food; hepatoprotective effects; hyperlipidaemia; spirulina

INTRODUCTION:

Nowadays, hyperlipidaemia is a worldwide public health problem with emerging incidence and prevalence. Dietary approach and exercise are one of the most recommended lifestyle changes for managing hyperlipidaemia. Spirulina is a valuable source of vitamins, minerals, carotenoids and essential fatty acids and amino acids. In some experimental models *Spirulina plantensis* demonstrated certain antioxidative, anti-inflammatory and antidiabetic potential. Therefore, it can be hypothesized that spirulina supplementation can ameliorate in vitro enzyme activity and decrease oxidative stress in hyperlipidaemia.

OBJECTIVES:

The objective of this research was to analyse the effects of spirulina supplementation against hyperlipidaemia induced oxidative stress through the evaluation of antioxidant biomarkers.

METHOD / DESIGN:

Male Wistar rats (approved by the Institutional Bioethics committee No. III-2011-01) were randomly divided into five groups based on the applied diet (I-normal diet; II-normal diet with *Spirulina plantensis*; III-lipogenic diet; IV-lipogenic diet with concomitant spirulina supplementation and V-lipogenic diet with spirulina treatment). The activity of superoxide dismutase (SOD), catalase (CAT), xanthine oxidase (XOD), glutathione S-transferase (GST), glutathione peroxidase (GPx) was measured in hemolysate. Additionally, lipid peroxidation and total antioxidant activity were determined using commercial kits.

RESULTS:

The activity of analysed oxidative stress biomarkers was noticed in all studied groups. Atherogenic diet induced lipid peroxidation and decreased GST, GPx as well as TAC levels due to the increased oxidative stress. The significant changes in SOD, CAT and TAC values were observed between group III (lipogenic diet) and V, when this microalga was added to the diet after hyperlipidaemia occurred. The obtained differences are also reflected by separation on principal component analysis plots (*Figure 1*).

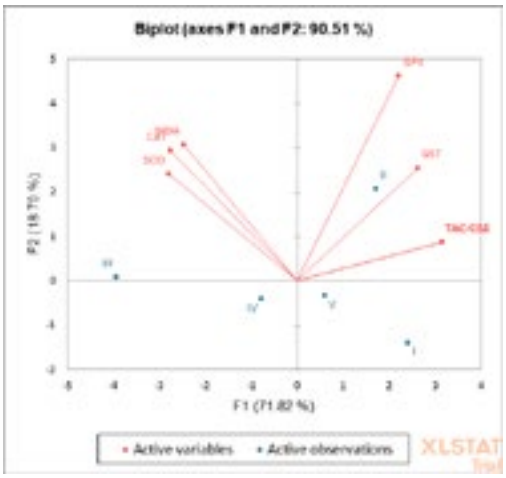

Figure 1. Principal Component Analysis (PCA) of the impact of different feeding regime on antioxidative biomarkers in rats

CONCLUSIONS:

Based on the obtained results, spirulina supplementation can be considered

23 *University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia. Corresponding author: maja.milanovic@mf.uns.ac.rs*

24 *State Laboratory, Backweston Laboratory Campus, Co. Kildare, Ireland*

25 *University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia*

26 *University of Novi Sad, Institute of Food Technology, Novi Sad, Serbia*

in managing hyperlipidaemia. Spirulina stabilized the amounts of reactive oxygen species in hyperlipidaemic rats through the amelioration of antioxidative biomarkers.

Acknowledgement: This research was financially supported by the Ministry of Education, Science and Technological Development Republic of Serbia (Project No. TR31029).

T4-P-3-ORAL Ultrasound assisted depolymerization of sulfated polysaccharide (fucoidan) from seaweed

Viruja Ummat[27/28], Saravana Periaswamy Sivagnanam[27], Colm O'Donnell[28], Gillian E Conway[29], Shane M Heffernan[29], James Curtin[30], Brijesh Kumar Tiwari[27]

KEYWORDS: Seaweed; fucoidan; extraction; ultrasound; depolymerization

INTRODUCTION:

Seaweeds present a wide range of high value biomolecules, amongst which fucoidan, a sulfated polysaccharide, has gained a lot interest of researchers and pharmaceuticals, owing to its anti-oxidant, anti-coagulant, anti-inflammatory and anti-tumor properties. Ultrasound, being a green extraction technology also has emerged as one of the most efficient methods for enhancing the extraction yield from various sources and has shown the potential of being an efficient depolymerization method. Advantages of ultrasound technique in the depolymerization process can result in uniform molecular weight distribution. Depolymerization of a polysaccharide can help in reducing the viscosity, structural heterogeneity and permeability, so that biomolecules can easily penetrate through the cell membranes. In addition, depolymerization has been shown to significantly improve the anticancer properties of biomolecules by reducing their molecular weight, while retaining the structure of the polysaccharide and involves short treatment time.

OBJECTIVES:

The objectives of the study were 1) to extract fucoidan from seaweed *Fucus vesiculosus* using a green solvent and compare with the most commonly used extraction solvent i.e. hydrochloric acid 2) Determine the effect of ultrasound assisted depolymerization of the fucoidan extracts and investigate the cytotoxic effect of the different molecular weight fractions.

METHOD / DESIGN:

Fucoidan extraction was carried out using the conventional extraction method involving use of hydrochloric acid/ a green extraction solvent and compared with a commercially available fucoidan sample. Following which, different ultrasonic amplitude and green solvents were used for depolymerization of the fucoidan. The samples obtained were then analyzed for changes in molecular weight and cytotoxic impact evaluated in cancer cells *in vitro*.

RESULTS:

The results showed that the green solvent along with the ultrasound treatment with a short treatment time, was efficient in reducing the molecular weight of the sample. Furthermore, a significant reduction of cell viability was observed in a selec-

27 *Teagasc Ashtown Food Research Centre, Teagasc, Ashtown, Dublin 15, Ireland. Corresponding author: Viruja.ummat@ucdconnect.ie*

28 *UCD School of Biosystems and Food Engineering, University College Dublin, Belfield, Dublin 4, Ireland*

29 *In vitro Toxicology Group, Institute of Life Science, College of Medicine, Swansea University, Swansea, Wales, UK*

30 *School of Food Science & Environmental Health, College of Sciences & Health, Technological University Dublin-City Campus, 7 Dublin, Ireland*

tion of samples demonstrating anticancer potential

CONCLUSIONS:

In relation to obtaining fucoidan from seaweed *Fucus vesiculosus*, green extraction solvent showed comparable results with the commonly used harsh extraction solvent, i.e. hydrochloric acid. Ultrasound was found to be an efficient depolymerization method and therefore, can be used for carrying out depolymerization of crude fucoidan from seaweed *Fucus vesiculosus*.

T4-P-4 Nutritional and technological properties of cookies prepared from minor cereals and fruit and vegetable by-products

Miona Belović, Aleksandra Torbica, Lidija Peić Tukuljac, Boško Marić, Jovana Kojić, Jelena Krulj, Jelena Perović[31], Vesna Vujasinović, Goran Radivojević[32]

KEYWORDS: minor cereals, cookies, fruit and vegetable by-products, dietary fiber

INTRODUCTION:

Cookies are one of the cereal products typical for the area of Vojvodina, where they are usually consumed with coffee or tea. However, they are usually produced from wheat flour, leaving the nutritional potential of minor cereals growing in the area of Vojvodina unused. On the other hand, fruit and vegetable processing industry in Vojvodina generate large amounts of by-products that could be used as natural flavorings and colorings. The combination of these raw materials could be used to produce cookies with improved nutritional properties and interesting sensory properties, which would be appealing to consumers, especially those concerned with the consumption of natural ingredients.

OBJECTIVES:

The objective of this research was to develop optimal formulations of cookies prepared from triticale, rye, spelt and barley flour with the addition of apple pomace, beetroot pomace, pumpkin pulp and pumpkin oilseed cake. Then, technological and nutritional properties of these cookies were assessed.

METHOD / DESIGN:

Wholegrain rye, spelt and barley flours bought in the market, and refined triticale flour obtained by milling grains were used as the basic ingredients for the manufacturing of cookies. Apple, beetroot and pumpkin pomace were dried and powdered, while powdered pumpkin oilseed cake was obtained from the producer of pumpkin seed oil. Cookies were prepared using the previously optimized formulation as a basis, with flours substituted with powdered fruit and vegetable by-products. The following combinations of basic ingredients were used: triticale flour substituted with 10% of apple pomace; spelt flour substituted with 5% of beetroot pomace; rye flour substituted with 10% of pumpkin pomace; and barley flour substituted with 10% of pumpkin oilseed cake. Cookies prepared from refined wheat flour were used as a control sample.

Regarding the nutritional properties, moisture, ash, protein, total carbohydrates, sugar, fatty acids composition, soluble and insoluble dietary fiber and energy value were determined and calculated using standard methods. Of technological quality parameters, spread ratio was calculated from dimensions; color of upper and lower surface of cookies was measured using a Chrome Meter CR-400 (Konica Minolta, Japan), and hardness and fracturability of cookies were determined by using a TA.XT Plus texture analyser (Stable Micro Systems, UK).

31 *University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21102 Novi Sad, Serbia. Corresponding author: miona.belovic@fins.uns.ac.rs*

32 *University of Novi Sad, Faculty of Science, Trg Dositeja Obradovića 3, 21102 Novi Sad, Serbia*

RESULTS:

Nutritional properties of all manufactured cookies were similar. Their proximate composition and energy value were in the range common for this type of confectionary products. Cookies prepared from rye and pumpkin pomace had the highest dietary fiber content (8.90 g/100 g), followed by spelt and beetroot pomace cookies (7.09 g /100 g), barley and pumpkin oilseed cake (6.69), while triticale and apple pomace cookies had the lower dietary fiber content (4.50 g/100 g). According to Regulation EC No 1924 (2006) on nutrition and health claims made on foods, cookies prepared from wholegrain flours (spelt, rye and barley) can bear the label "high fiber", while cookie prepared from refined flour (triticale) can be labelled as a "source of fiber" since total fiber content in cookies was higher than 6 g/100 g and 3 g/100 g, respectively.

Spread ratio of triticale and spelt cookies was similar to those of wheat cookies, while rye and barley cookies had much higher values. However, this difference was not important from the processing point of view, since all doughs were easy to manipulate. Spelt and barley cookies were less hard than other cookies, and their fracturability was similar. Differences in color originated from the use of different raw materials.

CONCLUSIONS:

All developed cookies had technological properties similar to control sample, indicating that they can be easily manufactured in coffee shops and restaurants. All cookies can contribute to the increase of daily intake of dietary fiber. Using fruit and vegetable by-products as natural colorings can support the sustainability of food industry. Nutritional properties and attractive appearance of cookies can make them recognizable products from Vojvodina, improving the gastro-tourist offer of this region.

T4-P-5-ORAL RSM and ANN optimization of polyphenols recovery from *Thymus Serpyllum* herbal dust using microwave-assisted extraction

Živan Mrkonjić[33], Dušan Rakić[33*], Aleksandar Takači[33], Nemanja Teslić[34], Zoran Zeković[33], Ivana Lazarević[33], Branimir Pavlić[33*]

KEYWORDS: Wild thyme; Antioxidant activity; Multi-response optimization; Artificial neural network (ANN); Industrial waste

INTRODUCTION:

Nowadays is valorization of industrial waste put into focus, by being underlined with the main principles of green extraction[35]. Commonly, the green extraction principles are easily followed by using more effective energy and solvents, which are recognized as safe. Furthermore, designing the production line with minimal number of processing steps and obtaining a safe and non-denatured and biodegradable extract without concomitants as a final product, lead to the fact that green extraction processes of natural products present desirable approach for isolation of antioxidants[35/36].

OBJECTIVES:

The main objective of our study was to valorize *T. serpyllum* herbal dust by microwave-assisted extraction (MAE) and to evaluate extraction parameters (ethanol concentration, extraction time, liquid-solid ratio and irradiation power) affecting total extraction yield (Y), total phenols yield (TP) and antioxidant properties of obtained extracts. Response surface methodology (RSM) and ANN approaches were applied to optimize antioxidants recovery from *T. serpyllum* herbal dust and comparative

33 *University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia. *Corresponding authors: drakic@tf.uns.ac.rs; bpavlic@uns.ac.rs*

34 *University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*

35 *Chemat, F., Vian, M. A., & Cravotto, G. (2012). Green extraction of natural products: concept and principles. International Journal of Molecular Sciences, 13(7), 8615-8627*

36 *Rombaut, N., Tixier, A. S., Bily, A., & Chemat, F. (2014). Green extraction processes of natural products as tools for biorefinery. Biofuels, Bioproducts and Biorefining, 8(4), 530-544*

analysis in terms of influence analysis, model fitting and optimization accuracy was done.

METHOD / DESIGN:

With the aim of investigating impact of the MAE parameters on target responses and optimizing extraction process, the face-centered central composite experimental design (CCD) with RSM and ANNs were used. The impact of ethanol concentration (45, 60 and 75%), extraction time (5, 12.5 and 20 min), liquid-solid ratio (10, 20 and 30 mL/g) and irradiation power (400, 600 and 800 W) were used as input parameters in both cases. As responses were selected Y, TP, as well as antioxidant activity parameters obtained by DPPH and ABTS assays.

RESULTS:

Optimized MAE conditions obtained by RSM were ethanol concentration of 52%, 20 min extraction time, 24 mL/g liquid-solid ratio and irradiation power of 400 W. On the other hand, optimized MAE conditions obtained by ANN were ethanol concentration of 45%, 5 min extraction time, 30 mL/g liquid-solid ratio and irradiation power of 400 W. Based on values of R^2 of RSM obtained for Y, TP, DPPH and ABTS (0.9242, 0.8487, 0.9216 and 0.6661, respectively), and R^2 of ANN where its lowest value, taking into account all four responses, was 0.9507, it could be concluded that there is a good fit between experimentally observed and predicted values.

CONCLUSIONS:

Results suggested that both RSM and ANN approaches could be successfully used for optimization MAE process of polyphenols recovery from *T. serpyllum* herbal dust. It could be also concluded that MAE is an efficient technique for the extraction of biologically active compounds from *T. serpyllum* herbal dust, which represents the high-valuable source of natural antioxidants with great potential for further use in various forms within different branches of industry.

ACKNOWLEDGMENT:

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T4-P-6 Effect of climatic variables and sowing date on winter rapeseed (*Brassica napus L.*) development and yield

Ana Marjanović-Jeromela[37], Sreten Terzić[37], Mirjana Jankulovska[38], Miroslav Zorić[37], Ankica Kondić-Špika[37], Nevena Nagl[37]

KEYWORDS: climate change; cultivar x environment interaction; growth stage; winter rapeseed; seed yield.

INTRODUCTION:

Climate change differentially affects crops, since the effects are caused by combination of changes in growing conditions and the timing of phenological phases. Winter rapeseed is vulnerable to local climatic conditions because of its lengthy growth period and overwintering ability. The information on the cultivar x environment interaction provides valuable data to plant breeders and agronomists for the identification of superior cultivars in specific environments, and defines site-specific best management practices. A further step in the cultivar (C) and year (Y) interaction analysis (C x Y) for rapeseed, would be

37 *Institute of Field and Vegetable Crops, Novi Sad, Serbia. Corresponding author: ana.jeromelal@ifvcns.ns.ac.rs*

38 *Department of Genetics and Plant Breeding, Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University, Skopje, North Macedonia*

to investigate the effect of specific climatic variables throughout developmental stages. Such data could be used to dissect the year effect and determine which variables are the most significant for an optimal plant development at each growth stage. The effect of climatic variables on the winter rapeseed developmental stages and yield in Southeast Europe has not yet been analysed simultaneously, although their interaction is important to breeders and growers.

OBJECTIVES:

The aim of the study was to understand year-related interactions and the effect of climatic variables in different growth stages on seed yield and oil content.

METHOD / DESIGN:

Sources of variability for the seed yield and oil content of four rapeseed cultivars were evaluated, during the four growing seasons, under the influence of three sowing dates. Six climatic factors: the temperature (minimum on 5 cm above ground; minimum; maximum; and mean), total precipitation, and relative air humidity, were observed during the germination, overwintering, budding, flowering and ripening.

RESULTS:

A Wald F test showed a highly significant effect of C × Y for both, the seed yield and oil content. The treatment × year (T × Y) interaction was significant for the oil content. A set of individual factorial regression models was developed in order to test the hypothesis about the effect of climatic variables on C x Y and T x Y interactions. Out of thirty available climatic variables, nineteen had a highly significant effect on the C x Y interaction for the oil content and six variables had a significant effect. The largest proportion of the explained interaction variance was obtained for precipitation at the budding stage (60.3%), the maximum temperature at overwintering (60.2%), and the relative air humidity at flowering (59.0%). As a consequence of the decreased level of significance of the T x Y interaction for the oil content, only three climatic variables were found to be important. A highly significant effect was observed only for precipitation at overwintering (81.4%), whereas the effect of the relative air humidity at the budding stage (76.4%) and precipitation at the germination stage (61.1%) accounted for a significant proportion of the T x Y interaction.

CONCLUSIONS:

The study successfully dissected the effect of year-related climatic variables on the agronomical traits in winter rapeseed.

T4-P-7 Within-field correlation between satellite-derived vegetation indices and grain yield of wheat

Dragana Blagojević, Stefanija Stojković, Sanja Brdar, Vladimir Crnojević[39]

KEYWORDS: vegetation indices; yield; correlation; agriculture; satellite.

INTRODUCTION:

In recent times, satellite-derived vegetation indices were widely used in the field of agriculture especially in the assessment of crop damage and crop progress as well as in clarification of spatial variability of yield. These types of analyses play an important role in the estimation of the health condition of each crop during its growth and provide an opportunity for timely decision making.

39 BioSense Institute, Research and Development Institute for Information Technologies in Biosystems, University of Novi Sad, Dr. Zorana Đinđića 1, 21000 Novi Sad, Serbia. Corresponding author: dragana.blagojevic@biosense.rs

OBJECTIVES:

This research aimed to inspect the correlation coefficients, during the crop growth stages, between vegetation indices (VIs) derived from Sentinel-2 imagery and grain winter wheat yield derived from yield monitoring and select the most promising indices for monitoring crop growth and yield estimation.

METHOD / DESIGN:

The satellite images in 10m resolution were selected based on crop growth stages, from the end of tillering phase (beginning of March 2019) until the full ripening (end of June 2019). For the analysis, the BBCH-scale for cereals was used. Yield observations were performed at harvest on five fields in one season and twelve VIs were calculated across 10 growth stages. To designate their correlation and dependence, a statistical comparison of the VIs and yield was made. The Pearson's and Spearman's correlation coefficients were calculated, and their statistical significance was tested using *p*-value (at p=0.01, p=0.05).

RESULTS:

According to the crop growth stages, the highest correlation coefficients were detected from the early boot stage (BBCH 41) until the middle of development of the fruiting stage (BBCH 73 – early milk). In that period the correlation coefficients varied from 0.39 to 0.84 depending on the field. Based on the location, the highest correlation coefficient values for all 12 indices were recorded for the parcel named C-6 (April 15), and the lowest values for the parcel named C-10 (June 29). Most of the indices showed statistically significant dependence (at the p<0.01 and p<0.05 significant levels) on the yield in the first five growth stages except the chlorophyll vegetation index (CVI) for the parcel named C-11 (p=0.21, p=0.39).

CONCLUSIONS:

To conclude, the last growth stage named ripening showed the lowest values both for correlation coefficient and statistical significance which means that VIs also had low values because the reflectance is weak in this growth stage and wheat is about to be harvested. In the first five stages, VIs showed significantly high spectral reflectance values since in this period the leaf is full of chlorophyll pigments. Analyzing the correlation coefficient in different stages of wheat growth, we look at the current state of crops and have the opportunity to take appropriate measures in time to increase yields or save inputs at specific locations.

T4-P-8-ORAL Incorporation of bioactive compounds from oregano plant to greek whey cheeses

Stamatia Christaki, Thomas Moschakis, Ioannis Mourtzinos[40]

KEYWORDS: essential oil; extract; oregano; whey cheeses; nanoemulsion

INTRODUCTION:

Whey cheeses are susceptible to microbial spoilage due to their characteristics (high moisture content, high pH, low salt content) with a limited shelf-life, properties that limit their commercialization. Nowadays, natural ingredients from plants are employed from the food industry as additives in different products for the replacement of harmful synthetic additives. Essential oils and extracts from aromatic plants exhibit potent antimicrobial and antioxidant activities and their use as alternative food additives has increased over the years.

40 Department of Food Science and Technology, School of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, 541 24, Thessaloniki, Greece. Corresponding author: mourtzinos@agro.auth.gr (Ioannis Mourtzinos)

OBJECTIVES:

Bioactive compounds from oregano plant (*Origanum vulgare*) have been incorporated into Greek whey cheeses. The role of these compounds as preservatives and antioxidants in these products has been studied and the extension of the shelf-life has been evaluated.

METHOD / DESIGN:

Novel oil-in-water nanoemulsions as delivery systems have been formulated containing both essential oil (lipid phase) and extracts (aqueous phase) from oregano plant using ultrasonication. The particle size distribution and the microstructure of nanoemulsions were analyzed using the laser diffraction method and confocal laser scanning microscopy (CLSM), respectively. Additionally, microbiological analysis has been performed to nanoemulsions (*in vitro*) and their particle size stability was assessed over one-month-period. Nanoemulsions were incorporated as coatings in Greek whey cheeses, i.e., Mizithra and Anthotyros. Antimicrobial activity against *Penicillium expansum* and extension of shelf-life has been evaluated.

RESULTS:

The nanoemulsions presented antimicrobial activity against the mold *P. expansum in vitro* and potent antioxidant activity. Nanoemulsions with both oregano essential oil and extract presented higher antioxidant activity *in vitro*, compared to the other formulations. Particle diameter in all nanoemulsions was <100nm and remained stable after one month. Furthermore, nanoemulsions presented antimicrobial activity against *P. expansum in situ*.

CONCLUSIONS:

Novel oil-in-water nanoemulsions combining both the essential oil and extracts from aromatic plants can boost their action as effective preservatives and antioxidants in different food products.

T4-P-9 Mealworm as a valuable source of essential fatty acids in animal feed production

Danka Dragojlović[41], Olivera Đuragić[41], Matija Milković[42], Slađana Rakita[41], Viktor Stojkov[41], Nedeljka Spasevski[41], Strahinja Vidosavljević[41]

KEYWORDS: insects; animal feed; *Tenebrio molitor*; fatty acid; lipid content

INTRODUCTION:

To meet the need for meat intake due to the growth of the human population, the demand and research on alternative protein sources is of the great importance. In the last eight years, the price of protein sources for animal feed production has doubled and already represents 60-70% of the total cost. Insects present valuable source of proteins, fat and minerals therefore one of the possible solution could be application of insect meal in feed production. Although those insects are already recognized as novel protein food, it is important to emphasize that most of edible insects are rich in fat content also. The average fat content ranges from 13 to over 33% depending on the rearing conditions. Moreover some insect species are rich in essential fatty acid and further research on the influence of rearing condition on insect meal fatty acid profile are very desirable.

41 *Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia. Corresponding author: danka.dragojlovic@fins.uns.ac.rs*

42 *University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia*

OBJECTIVES:

The main goal of this research was to enrich the fatty acid profile of *Tenebrio molitor* by changing rearing condition. Different rearing conditions in terms of feed have been chosen to enrich essential fatty acids.

METHOD / DESIGN:

Larvae of *Tenebrio molitor* were reared in plastic boxes. Every box contained wheat bran as a base diet and every third day larvae were fed with carrots (diet 1), or cabbage (diet 2) or mix of carrot, cabbage and flax seed (diet 3). Afterwards in order to produce mealworm, larvae were collected and inactivated in boiling water, dried and milled. Insect oil was obtained by extraction with 2:1 chloroform-methanol mixture. Fatty acid methyl esters (FAMEs) were then determined using an Agilent GC equipped with a flame ionization detector (FID) (Agilent, 7890 Series, USA) and SP-2560 fused silica capillary column.

RESULTS:

The obtained results showed that mealworm had high content of lipids. Lipid content ranged from 25 to 33% based on dry matter. The highest lipid content was found in the mealworm which diet included mix of carrot, cabbage and flaxseed. Among saturated fatty acid (SFA) the most dominant was palmitic acid in all samples. Oleic acid was predominant monounsaturated acid (MUFA), while linoleic was predominant polyunsaturated fatty acid (PUFA, n-6 fatty acid). Furthermore, the concentration of n-3 increased tenfold in mealworm which diet included flaxseed and it ranged from 10,5 to 14%.

CONCLUSIONS:

Fatty acid profile of mealworm can be enhanced wit the adequte insect feed selection, while the most beneficial fatty acid profile was achieved with feed which was based on mix of carrot, cabbage and flax seed. Considering the content of essential n-3 and n-6 fatty acids it could be concluded that inclusion of mealworm into animal diet is very desirable and has a huge potential in animal feed industry.

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T4-P-10-ORAL Cherry seed oil: supercritical fluid extraction of lipophilic bioactive compounds

Ivana Dimić[43], Nastasija Cvetković[43], Bojana Milinković[43], Jovana Pantelić[43], Nemanja Teslić[44], Zoran Zeković[43], Branimir Pavlić[43*]

KEYWORDS: cherry seed oil; supercritical fluid extraction, Box-Behnken design, fatty acid profile, tocopherol content

INTRODUCTION:

Along with the growth of the world population, food consumption has been increased which leads to generation of the great amount of food waste to be valorized. Cherry seeds have been recognized as a by-product after fruit processing for production of juices, jams, alcoholic drinks and processed fruit products and may be used as a resource to recover oil rich in polyunsaturated fatty acids, tocopherols and other active compounds. Recent research has been aimed towards the development of green techniques to increase bioactive compounds yield, reduce production costs and simultaneously minimize hazardous effects on the environment. One of the prominent techniques is supercritical fluid extraction (SFE) which is per-

43 *University of Novi Sad, Faculty of Technology, Blvd. cara Lazara 1, 21000 Novi Sad, Serbia. *Corresponding author: bpavlic@uns.ac.rs*

44 *University of Novi Sad, Institute of Food Technology, Blvd. cara Lazara 1, 21000 Novi Sad, Serbia*

formed at mild extraction conditions and uses CO2 which is cheap, available in high purity, non-toxic and environmentally friendly. Additionally, SFE process parameters can be easily adjusted to reach the maximal concentration of target compounds from the plant material.

OBJECTIVES:

The main objective of this study is to apply Box-Behnken design to optimize the SFE process in terms of the total extract yield (Y), tocopherol content and suitable fatty acid profile for the healthy human diet. Evaluation of the process parameters (pressure, temperature and flow rate) could be used to examine which parameters have a positive influence on the process and provide significant practical information necessary for possible industrial application.

METHOD / DESIGN:

The optimization of the process was performed by applying Box-Behnken design with fifteen regular and six additional runs to determine the influence of independent variables on total extraction yield: pressure (200 – 350 bar), temperature (40 – 70°C) and CO_2 flow rate (0.2 – 0.4 kg/h). Fatty acid methyl esters were prepared from the extracted lipids using a method based on 14% boron trifluoride–methanol solution. Tocopherol content was determined by high-pressure liquid chromatography (HPLC).

CONCLUSIONS:

Cherry seed oil is rich in different bioactive compounds and has shown its potential to be used in the pharmaceutical, cosmetic and food industry. Among the polyunsaturated fatty acids, the most abundant was linoleic acid, while oleic acid was the predominant fatty acid belonging to monounsaturated fatty acids. Pressure of 275 bar was most favorable to achieve the highest yield of the tocopherols, while increase of temperature and CO_2 flow rate has decreased tocopherol content. Supercritical fluid extraction represents an alternative to conventional extraction methods and the process parameters can be optimized to recover cherry seed oil with the highest concentration of bioactive compounds.

T4-P-11 Comparison of peduncle vascular tissue of wild *Perennial helianthus* species

Jelena Jocković[45], Lana Zorić[45], Sreten Terzić[46], Milan Jocković[46], Dragana Miladinović[46], Jadranka Luković[45]

KEYWORDS: sunflower; peduncle; vascular system; condutivity.

INTRODUCTION:

A common problem in sunflower cultivation is poor achene filling. The sunflower fruit growth is a result of the influx of water, minerals and assimilates through xylem and phloem of peduncle into receptacle. Variations in penducle vascular system characteristics among wild perennial *Helianthus* species could indicate their different conductivity capacity which is reflected in differences in seed yield potential.

OBJECTIVES:

Taking into account the importance of the functional vascular network of peduncle, its close correlation with sunflower seed yield potential, and the importance of wild species in breeding programmes, we made a comparative analysis of peduncle vascular tissues of 19 wild perennial *Helianthus* species.

45 *University of Novi Sad, Faculty of Sciences, Trg D. Obradovica 2, Novi Sad, Serbia. Corresponding author: jelena.lazarevic@dbe.uns.ac.rs*

46 *Institute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, Serbia*

METHOD / DESIGN:

Plant material was grown at the experimental field of Rimski Šančevi, Novi Sad. For anatomical observation, five plants of each species were randomly selected and cross sections were obtained from the middle part of peduncle, using cryotechnic procedure. Measurements of vascular tissues' features were performed using light microscopy.

RESULTS:

Our study showed that the number of vascular bundles was not always in a positive correlation with the lenght of peduncle, size of the peduncle cross section and the size of the vascular bundles. Multivariate Discriminant Analysis showed that species *H. eggertii*, *H. resinosus*, *H. hirsutus*, and *H. mollis* had remarkably higher cross-section area of vascular bundles as well as size of lumen and number of vessels than other analysed species. In the species *H. tuberosus*, we noticed large vascular bundles with well developed xylem composed of a slightly smaller number of vessels of the wider lumen. In addition to the more developed xylem, species *H. eggertii, H. resinosus, H. hirsutus*, and *H. tuberosus* had the most developed phloem and sclerenchyma tissues. Also, Principal Componentes Analysis showed the most frequent presence of vessels with the widest lumen (300-500 µm2 and 500-1000 μm^2) in these species, although in a small percentage. On the other hand, species *H. salicifolius, H. glaucophyllus, H. laevigatus* and *H. divaricatus* were positioned in the negative zone of the graph with significantly narrower lumen of vessels.

CONCLUSIONS:

Understanding the peduncle vascular characteristics is of a fundamental importance for improvement of cultivated sunflower seed yield. Wild species with larger vascular bundles, a higher number of vessels and its wider lumen could indicate a higher conductivity capacity of the penducle.

T4-P-12 From byproducts to bioproducts

Ana Kalušević[47],
Steva Lević, Saša Despotović, Mile Veljović, Viktor Nedović[48]

KEYWORDS: bioactives; vinification byproducts; spray drying; freeze drying; food application

INTRODUCTION:

Food lifecycle creates enormous amounts of processing byproducts and waste that can be used for production of valuable bioactives and potential food additives. Researchers and experts from food industry are very interested in developing of innovative functional foods and bioproducts in accordance with circular economy and low or zero waste concepts. For instance, food with encapsulates containing bioactives from grape processing byproducts, as active component could be one of directions since these bioactives still posses health-promoting effects, colour, flavour and can be stabilized by various encapsulation technology.

OBJECTIVES:

The main objective of this research was the application of spray dried and freeze dried extracts obtained from byproduct of grape processing into the final food products. Previously, the effects of applied carriers and techniques on the efficiency and physicochemical properties of microencapsulates were examined.

47 *Academy of Applied Studies Belgrade, Belgrade, Serbia. Corresponding author: ana.kalusevic@vhs.edu.rs*

48 *Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*

METHOD / DESIGN:

The raw material for the extraction of bioactives was the grapeskin of red grape variety as byproduct of vinification. Microencapsulates were obtained using different carriers such as maltodextrin, gum Arabic and skimmed milk powder, and spray and freeze drying as encapsulation techniques widely used in the food sector. Oat meals and yoghurts enriched with microencapsulates were the final products which characteristics were tested by sensorial panel.

RESULTS:

All tested microencapsulates showed extremely low water activity (0.2-0.3), and very high solubility (around 90% m/m). Microencapsulation yields varied from around 65 to 93%. Total phenol contents ranged from 5.8 to 11.6 mg GAE/g and was the highest in microencapsulates produced by freeze drying with gum Arabic. The results of the assessment of sensorial characteristics showed very high average sensory scores, over 7 and 8. In comparison to the standard products the colour change was the most noticeable, due to the anthocynins content in the added microencapsulates. Sensorial analysis indicated that the highest potential for the application in food products have shown microencapsulates based on maltodextrin.

CONCLUSIONS:

These results have shown that spray dried and freeze dried microencapsulates of grapeskin as byproducts of agri-food processing could be used as a source of natural pigments and bioactives with improved stability. Microencapsulates obtained in this research can be applied as multipurpose additives in dairy, confectionery, bakery products as well as beverages and soft drinks.

Namely, except their bioactive potentials, these microencapsulates could be a substitute for artificial colorants present in the numerous food products nowadays.

T4-P-13 Machine learning chemometric model for Raman spectroscopy based honey quality assessment

Stefan M. Kolašinac, Ilinka Pećinar, Zora P. Dajić Stevanović[49]

KEYWORDS: honey; adulteration; raman spectroscopy; support vector machine

INTRODUCTION:

According to Codex Alimentarius (2001), "Honey is the natural sweet substance, produced by honeybees from the nectar of plants or from secretions of living parts of plants, or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature". Honey is mostly made up of sugars, as well as enzymes, amino acids, organic acids, vitamins, aromatic compounds, minerals and carotenoids. It contains a lot of flavonoids and phenolic acids, which have a lot of biological effects and functions such as natural antioxidants, anti-inflammatory and antimicrobial properties. Its composition is particularly variable, depending on its botanical and geographical origins Because of its exclusive flavor and high dietary value, natural honey is more expensive than other sweeteners. This is the reason why honey is a target of adulteration. The problem is that counterfeiting honey is relatively easy, but detection is difficult. Further, the authenticity of honey is a global important problem for commercial producers and consumers. Accordingly, a fast and non-destructive method of detecting counterfeits is needed.

49 *University of Belgrade, Faculty of Agriculture, Nemanjina 6, Belgrade, Serbia. Corresponding author: stefan.kolasinac@agrif.bg.ac.rs*

OBJECTIVES:

The aim of this paper is to verify the possibility of Raman spectroscopy and Support Vector Machine (SVM) for classification of two different honeys and their fake duplicates. For this purpose, meadow and acacia honeys were selected.

METHOD / DESIGN:

Spectra of homemade and counterfeits honey were recorded using XploRA Raman spectrometer (Horiba Jobin Yvon). Raman scattering was excited by laser at a wavelength of 785 nm equipped with a 600 lines/mm grating; spectra were recorded by applying exposure time 10 s and accumulated from 10 times scans, using 100% filter. Spectral resolution was 3 cm−1 and autocalibration was done each time before recording of spectra by 520.47 cm^{-1} line of silicon. In order to assess a possible sample inhomogeneity, thirty Raman spectra in the region from 200-3400 cm^{-1} were recorded for each sample. All spectra were baseline-corrected, normalized and smoothed. After that PCA (Principal component analysis) was conducted and obtained PCs (first two PCs) served as a features for support vector machine (SVM) classification method. Data were divided into training model (70 %) and training data (30 %). Pre-processing was done by Unscrambler X 10.4 software (CAMO software, Norway). In order to determine the best shape of the hyperplane and decision boundary, several kernel function were used: linear, radial basis and polynomial function. The SVM was conducted by Python and Scikit-learn package.

RESULTS:

Support vector machine showed high accuracy in classification of different honey samples. Accordingly, the best discrimination power showed SVM with polynomial function (100%), followed by radial basis (96.67%) and linear (81.82%).

CONCLUSIONS:

Results showed that SVM algorithm can be used as a tool for detection of fraudulent honey products.

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T4-P-14 Mistletoe (*Viscum Album L.*) as a source of valuable antioxidants

Stefan M. Kolašinac[50], Aleksandar Ž. Kostić[50], Danijela Maksimović[51], Sanja Mijatović[51], Nikola Tanić[51], Nebojša Arsenijević[52], Zora P. Dajić Stevanović[50]

KEYWORDS: *Viscum album sp.*; phytochemicals; antioxidants

INTRODUCTION:

Viscum album L. (Loranthaceae Juss.) is semi-parasitic evergreen shrub distributed in Europe, northwestern part of Africa and Anatolia. The species is hosted by different woody gymnosperm and angiosperm species and it is known that host preference usually reflects in differences in morphological traits and possibly in phytochemical composition. Therefore, there are different subspecies, out of three subspecies (*V. album* subsp. abietis (Wiesb.) Janchen, subsp. *creticum* N. Böhling & al. and

50 University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia. Corresponding author: stefan.kolasinac@agrif.bg.ac.rs

51 University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia

52 University of Kragujevac, Faculty of Medical Sciences, Svetozara Markovića 69, 34000 Kragujevac, Serbia

subsp. *austriacum* (Wiesb.) Vollm.) occur most frequently on conifers while subsp. *album* is confined to deciduous host trees and shrubs. In addition to religious and mystical uses, the mistletoe is used in the folk medicine for circulatory and respiratory disorders and as an anticancer remedy.

OBJECTIVES:

The main object of this research was to examine and compare biological materials obtained from two different subspecies of *V. album* (*V. album* subsp. *abietis* labeled as S1 and *V. album* subsp. *album* labeled as S2 sample) as potential source of the total phenolics, total flavonoids and total dihydroxycinnamic acid derivatives. For that purpose the ethanolic extracts were prepared and were analyzed for their antioxidant properties.

METHOD / DESIGN:

Extraction of plant material (shoot) was performed using 70% ethanol. The content of selected phytochemicals was determined by application of the standard spectrophotometric methods: Folin-Ciocalteu (total phenolics, TPC), aluminum chloride (total flavonoids, TFC) and Arnow's (total dihydroxycinnamic acid derivatives, HCA) and expressed as mg/g equivalents of gallic acid (GAE), quercetin (QE) and chlorogenic acid (CGAE) calculated on fresh weight (F.W.) of sample respectively. For determination of antioxidant properties the following *in vitro* assays were used: total antioxidant capacity (TAC) determined via phosphomollybdenum test, ferric reducing power (FRP) and cupric reducing antioxidant activity (CUPRAC) as well as DPPH radical quenching ability. All results for antioxidant analyses were expressed as mg/g of ascorbic acid equivalents (AAE) per gram of F.W. To investigate differences between two *V. album* subspecies the Student's t-test with a significance level of $p \leq 0.05$ was performed. All measurements were carried out in triplicate.

RESULTS:

It was observed that results for TPC (5.41 mg/g GAE for S1; 7.69 mg/g GAE for S2), HCA (11.98 mg/g GAE for S1; 14.33 mg/g CGAE for S2), TAC (78.86 mg/g AAE for S1; 237.61 mg/g AAE for S2), FRP (16.45 mg/g AAE for S1; 21.64 mg/g AAE for S2) and DPPH" (13.14 mg/g AAE for S1; 14.86 mg/g AAE for S2) were significantly different ($p \leq 0.05$). In all cases *V. album* subsp. album exhibited better bioactivity compared to *V. album* subsp. abietis. However, *V. album* subsp. abietis exhibited higher TFC value (8.27 mg/g QE F.W.) but it was not significantly different The CUPRAC values didn't differ much in two studied subspecies.

CONCLUSIONS:

Obtained results indicate that both mistletoe subspecies are good source of bioactive compounds, in particular, the different phenolic acids. Both subspecies are the excellent antioxidant source. The further research will be focused on deeper phytochemical characterization of different *Viscum* populations and the bioactivity of target extracts and their individual components.

ACNOWLEDGMENT:

This work was supported by bilateral cooperation between Republic of China and Republic of Serbia named "Biological effects of extracts and molecules isolated from plants from the Balkans".

T4-P-15 Application of mutation breeding in creation of climate resilient cereal crops

Ankica Kondić-Špika, Milan Mirosavljević, Bojan Jocković, Ljiljana Brbaklić, Dragana Trkulja, Sanja Mikić, Svetlana Glogovac, Dragana Miladinović[53]

KEYWORDS: induced mutations; gamma irradiation; optimal doses; wheat; barley

INTRODUCTION:

In a global climate change scenario crop varieties with increased tolerance to drought, heat and other abiotic stresses are needed. So far, at the Institute of Field and Vegetable Crops many different studies for testing the existing varieties to these stresses were performed, but there was no breeding program for active development of tolerant varieties. Using mutation breeding we will try to develop wheat and barley varieties with incorporated drought and heat tolerance in order to be more adaptive to the changing climate.

OBJECTIVES:

The objectives of this study were to determine the optimal doses of gamma irradiation for mutation induction in two wheat and one barley varieties, and to apply the identified doses in order to produce mutant populations.

METHOD / DESIGN:

Radio-sensitivity test for determination of optimal irradiation doses was carried out according to the FAO/IAEA Manual on Mutation Breeding. Dried seeds of two winter wheat (NS40S and Simonida) and one barley varieties (Rudnik) were exposed to 75, 150, 300, 450 and 600 Gy gamma irradiation. The treated seeds and non-treated control were sown at equal depths in a tray filled with soil in rows (20 seeds each). Per assay three replicates were performed, one tray per replicate. After fourteen days of growing in a greenhouse, the germination and seedling height was measured to determine the Growth Reduction Value 50 or GR50.

RESULTS:

The results have shown that treated wheat and barley varieties had different reactions to applied doses of gamma irradiation. Germination of both wheat varieties was very good at all applied doses (over 90%), and there was no significant difference in the germination rate among doses or varieties. However, barley seeds were more susceptible to gamma irradiation, where doses of 300, 450 and 600 Gy reduced germination rate for 14.2, 33.2 and 42.1%, respectively. The seedlings' growth was more affected by irradiation treatment then germination process in both wheat and barley varieties. The dose of 300 Gy was lethal for Rudnik and NS-40S, while Simonida expressed higher tolerance regarding this dose. Accordingly, the dose of 210 Gywas identified as GR50 for varieties Rudnik and NS-40S, while 310 Gy was determined for Simonida. These doses were used for the treatment of 2000 seeds of each variety and mutation populations were produced. Further, mutation populations of these cereal crops will be used in a breeding programs for creating the varieties with increase resilience to climate change.

CONCLUSIONS:

Gamma irradiation had negative effect on seed germination and growth in wheat and barley varieties, but the varieties had different reactions to applied doses. The GR50 values were identified for each variety and used for production of mutation populations. The obtained populations will be used in wheat and barley breeding programs for improved tolerance to climate change.

53 Institute of Field and Vegetable Crops. Corresponding author: ankica.spika@ifvcns.ns.ac.rs

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T4-P-16 Molecular diversity of autumn garlic genotypes using SSR markers

Svetlana Glogovac, Jelica Gvozdanović-Varga, Miroslav Zorić, Nevena Nagl, Dragana Trkulja, Ljiljana Brbaklić, Ankica Kondić-Špika[54]

KEYWORDS: classification; germplasm collection; microsatellites

INTRODUCTION:

Garlic (*Allium sativum L.*) is one of the most important *Allium* species in terms of worldwide production and various usages in human nutrition, medicine, pharmacy and cosmetics. Basic method of garlic propagation is vegetative and creation of new varieties is mainly achieved by clonal selection. The characterization and preservation of samples in germplasm collections is of crucial importance in plant breeding, as well as availability of information about number and characteristics of samples in gene banks. Since pheotypic traits can vary significantly under the influence of environmental factors the characterization is more reliable by using DNA markers. Effective characterization of samples in collections and identification of duplicates is important from the economic aspect, ie. space saving and maintenance costs. The garlic collection of the Institute of Field and Vegetable Crops in Novi Sad (IFVCNS) includes 63 samples of autumn and 67 samples of spring garlic. These genotypes represent a valuable genetic pool for the selection of clones with appropriate characteristics, highly adapted for the production in the agro-climatic conditions of Serbia. Molecular characterization will provide more complete insight into diversity of samples, identification of potential duplicates and enable breeders more efficient selection and development of new cultivars.

OBJECTIVES:

The objectives of this study were to assess diversity of autumn garlic collection based on SSR data and to identify potential duplicates in the collection.

METHOD / DESIGN:

From the IFVCNS autumn garlic collection 52 samples originating from 11 countries, were selected for analysis. DNA extraction from young leaves was performed according to the Somma (2004) protocol[55]. For molecular evaluation 30 SSRs markers were selected, while 10 SSRs were determined to be polymorphic. Separation of amplified PCR products was performed on metaphor agorase gel (3% and 3,5%) by horizontal electrophoresis. The visualization of the product was done under UV light on a Wilber Bio-Print device. All data analyzes were performed within the R software environment, version 4.0.5 (R Core Team, 2020).

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54 Institute of Field and Vegetable Crops. Corresponding author: ankica.spika@ifvcns.ns.ac.rs

55 Somma, M. (2004): Extraction and purification of DNA. In M. Querci, M. Jermini, G. Van den Eede (ed.), The analysis of food samples for the presence of genetically modified organisms (Special Publication No. I.03.114) (Session 4, 13–17). European Commission DG-JRC.

RESULTS:

A total of 36 alleles were revealed by 10 polymorphic SSR loci. The number of alleles per locus ranged from 2 to 7, with an average number of 3.6. PIC values were 0.073-0.610 and the most informative markers were As 5944 and As 11065. The genetic distance between the analyzed genotypes ranged from 0 to 0.80 with an average value of 0.30. Out of a total of 52 samples in our study, 23.1% of the samples had an identical genotype for 10 examined SSR loci, with at least 1 genotype, while 76.9% were at a certain genetic distance with all analyzed samples. Molecular analysis provided distinguishing of most of the analyzed genotypes by PCoA and classification into 4 groups. No regularity in the grouping of genotypes according to origin was observed.

CONCLUSIONS:

The obtained results enabled more complete insight into diversity of collection and easier identification of potential genotypes for selection. Since this is the first research using DNA markers of the IFVCNS garlic collection, and considering the size and complexity of its genome, the obtained molecular results can be considered preliminary. Although the presence of duplicates in the collection was revealed based on 10 SSR loci these results represent guidelines for further research using more DNA markers.

ACKNOWLEDGEMENT:

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number: 451-03-68/2020-14/200032.

T4-P-17 Phytosterol composition of selected nuts and seeds from the Serbian market

Ivana Sofrenić[56], Jovana Ljujić[56], Katarina Simić[57], Jelena Kukić-Marković[58], Nevena Ivanović[59], Margarita Dodevska[60]

KEYWORDS: phytosterols; nuts; seeds; GC/MS

INTRODUCTION:

Edible nuts and seeds are nutrient-rich food, and also valuable source of various bioactive compounds. Among them are phytosterols, plant triterpenes with proven antioxidant, anti-inflammatory and antibacterial properties. Due to their similar structure with cholesterol, these plant sterols, when digested, compete with cholesterol for small intestine absorption leading to lowering of the cholesterol level in blood.

OBJECTIVES:

The aim of this work is to investigate phytosterol composition of selected nuts and seeds used in nutrition from the Serbian market.

56 *University of Belgrade – Faculty of Chemistry, Belgrade, Serbia. Corresponding author: jelena.kukic@pharmacy.bg.ac.rs*

57 *University of Belgrade – Institute of Chemistry, Technology and Metallurgy, Belgrade, Serbia*

58 *Department of Pharmacognosy, University of Belgrade – Faculty of Pharmacy, Belgrade, Serbia*

59 *Department of Bromatology, University of Belgrade – Faculty of Pharmacy, Belgrade, Serbia*

60 *Institute of Public Health of Serbia „Dr Milan Jovanović Batut", Center for Hygiene and Human Ecology, Belgrade, Serbia*

METHOD / DESIGN:

Gas chromatography with flame ionization detector (GC-FID) and gas chromatography with mass spectrometry (GC-MS) analysis of the unsaponifiable fractions was performed on an Agilent 7890A GC equipped with 5975C (inert XL EI/CI) MSD and a FID detector connected by a capillary flow technology two way splitter with make-up (250 °C). A HP-5MS capillary column (Agilent, 30 m × 0.25 mm, 0.25 µm film thickness) was used. The identification of the compounds was based on the comparison of their retention indices (RI), Rt, and mass spectra from NIST/NBS 05, Wiley libraries 8th edition and NIST Chemistry WebBook.29.

RESULTS:

Phytosterols were analyzed as volatile derivatives obtained by the silanization of residual unsaponifiable fractions. Cholesterol standard was used for quantification. Among them, the most abundant was β-sitosterol, followed by stigmasterol, isofucosterol and campesterol (*Figure 1*).

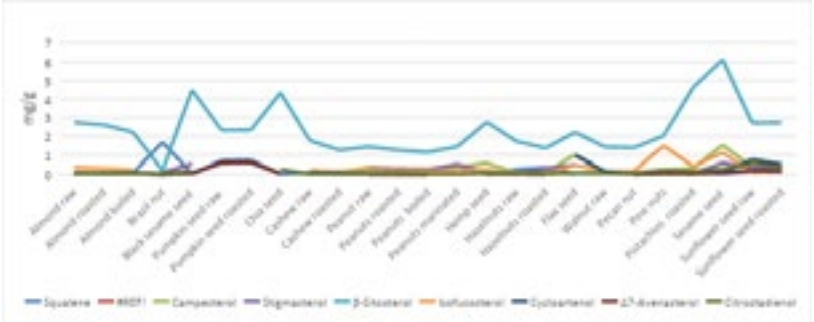

Figure 1. Phytosterols composition (mg/g oil).

CONCLUSIONS:

Our results indicate that the seeds (sesame, black sesame, chia seed) contain higher amount of phytosterols in comparison to the nuts. These results are in correlation with literature data. The product quality on Serbian market is appropriate according to phytosterols content.

T4-P-18-ORAL Screening of hydrophobic deep eutectic solvents for the extraction of tomato carotenoids

Anastasia Kyriakoudi, Alexandros Tsiouras, Ioannis Mourtzinos[61]

KEYWORDS: tomato; carotenoids; lycopene; hydrophobic deep eutectic solvents; extraction

INTRODUCTION: Tomatoes, the edible part of the plant *Solanum lycopersicum*, are one of the most widely consumed fruits worldwide. They can be consumed either fresh or as a variety of processed forms[62]. Tomatoes constitute a rich source of different valuable phytochemicals, among which, carotenoids prevail. Their *de novo* biosynthesis is observed only in higher plants and some fungi and bacteria, whereas animals and humans cannot synthesize them, so they must include them in their diet. Carotenoids constitute one of the most widespread groups of pigments in nature with colors ranging from pale yellow to deep red. Moreover, they are involved in various health-promoting biological activities (e.g. provitamin A action,

61 *Laboratory of Food Chemistry and Biochemistry, Department of Food Science and Technology, School of Agriculture, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece. Corresponding author: Anastasia Kyriakoudi; ankyria@chem.auth.gr*

62 *Costa, J.M. and Heuvelink, E. Introduction: The tomato crop and industry. In Tomatoes. 2005, pp. 1-19. Ed. E. Heuvelink, Cromwell Press, Trowbridge*

protection against certain types of cancer, cardiovascular and eye diseases etc.). The major carotenoid in tomato is lycopene constituting ~90% of total carotenoids. Lower amounts of other carotenoids, i.e. β-carotene, lutein, phytoene, phytofluene are also present[63]. Till recently, the extraction of these carotenoids was carried out using conventional organic solvents (e.g. hexane, acetone, ethyl acetate etc.)[64]. Lately, the interest of the scientific community has focused on the use of alternative, green, solvents such as deep eutectic solvents (DES). The latter ones are composed of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) forming a network of hydrogen bonds resulting in a lower melting point than that of the individual components. DES present certain advantages such as biodegradability, biocompatibility, easy preparation process, low cost. Apart from hydrophilic DES, the preparation of hydrophobic ones (HDES) has been recently reported[65]. To the best of our knowledge, till now, extremely limited is the use of HDES for the recovery of tomato carotenoids[66].

OBJECTIVES: In the present study, different HDES based on terpenes and fatty acids were prepared, characterized and examined for their effectiveness as extraction solvents of tomato carotenoids.

METHOD / DESIGN: HDES were prepared at different molar ratios by mixing menthol or thymol with lauric acid and decanoic acid as well as lauric acid with decanoic acid, by magnetic stirring (10 min) at 50ºC until transparent homogeneous liquids were obtained. The prepared HDES were physicochemically characterized and used for the extraction of carotenoids from a freeze-dried tomato sample with the aid of magnetic stirring at room temperature. Quantitative data were obtained by RP-HPLC-DAD using appropriate calibration curves. All manipulations were performed away from direct light to minimize photodecomposition of carotenoids throughout the analytical procedure.
RESULTS: To the best of our knowledge, the results obtained in the present work constitute the first report on the use of HDES for the extraction of carotenoids from the edible part of tomato fruits. The proposed solvent system, based on a combination of fatty acids, that serve as hydrogen bond donors and hydrogen bond acceptors simultaneously, was found to exhibit comparable extraction capacity with conventional organic solvents.

CONCLUSIONS: In this study, a simple and effective process for the extraction of lycopene and *β*-carotene from tomato is presented. Taking into account that the solvents used are considered non-toxic and biodegradable, the obtained carotenoid rich extracts are expected to be of use in industrial applications.

Acknowledgments: This research has been financed by Greek national funds through 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).

63 *Perveen, R., Suleria, H.A.R., Anjum, F.M., Butt, M.S., Pasha, I., Ahmad, S. Tomato (Solanum lycopersicum) carotenoids and lycopenes chemistry; Metabolism, absorption, nutrition, and allied health claims—A comprehensive review, Crit. Rev.Food Sci. Nutr. 2015, 55, 919-929*

64 *Deng, Y., Zhao, S., Yang, X., Hou, F., Fan, L., Wang, W., Xu, E., Cheng, H., Guo, M., Liu, D. Evaluation of extraction technologies of lycopene: Hindrance of extraction, effects on isomerization and comparative analysis - A review. Trends Food Sci. Technol. 2021, 115, 285-296*

65 *Zainal-Abidin, M.H., Hayyan, M., Wong, W.F. Hydrophobic deep eutectic solvents: Current progress and future directions. J. Ind. Eng. Chem. 2021, 97, 142-162*

66 *Silva, Y.P.A., Ferreira, T.A.P.C., Jiao, G., Brooks, M.S. Sustainable approach for lycopene extraction from tomato processing by-product using hydrophobic eutectic solvents. J Food Sci Technol., 2019, 56, 1649-1654.*

T4-P-19-ORAL Natural carotenoids and pectin from the juice by-product of microwave-heated persimmon fruits (Cv. Jiro)

Sofia Lalou[67], Stella A. Ordoudi[67], Fani Th. Mantzouridou[67/68]

KEYWORDS: Persimmon; juice solid residue; microwave heating; carotenoids; pectin

INTRODUCTION:

Microwave (MW) heating has been suggested as a post-process treatment of fruit juice residues to facilitate the recovery of valuable ingredients. However, it is still not well comprehended how the quality of these residues might be affected by integrating MW-heating in the pre-treatment step of juice processing. In the case of persimmon fruits, the orange-red residue from the conventional juicing processes has been reported to be a good source of β-carotene and β-cryptoxanthin that exhibit provitamin A activity as well as of pectin. This is of particular importance for the potential valorization of the Persimmon Juice Residue (PJR) in new added-value products as (a) natural carotenoids constitute a market segment of increasing growth rate and numerous applications for different end-use industries (food/beverage, animal feed, personal care/cosmetics and pharmaceuticals) and (b) pectin, usually isolated from citrus or apple pulps, is widely used as a thickener, water binder and stabilizer in foods. Thus, novel industrial strategies for effective persimmon juice processing that will result in PJR with potential health benefits are considered worthy of further investigation.

OBJECTIVES:

The objective of the present study was to assess the solid PJR as a potential source of pro-vitamin A carotenoids and pectin.

METHOD / DESIGN:

Persimmon pulp (PP) was treated under three different MW-heating conditions (0.7, 4.2, 8.4 kJ/g for 30, 60, 120 s respectively) prior to enzymatic maceration. The generated PJRs were evaluated for (a) total and individual carotenoid content via RP-HPLC-DAD and (b) pectin content and its degree of methylesterification (MED) via FT-IR spectroscopy. The results were compared with those for PJR of the non-MW-heated PP (PJR-C) and commercial citrus pectin.

RESULTS:

The total content and profile of carotenoids in the PJRs were markedly affected after MW-heating of the PP. At the most intense conditions tested (8.4 kJ/g for 120 s), the respective PJR (PJR-M120) was richer in total carotenoids by almost 40%, compared to the PJR-C. Although intensification of MW-heating conditions was found to favor the release of several types of free xanthophylls from their bound forms, provitamin-A active carotenoids like β-carotene and β-cryptoxanthin along with its fatty acid mono and diesters were still the dominant ones. PJR-M120 contained 997.4 ± 7.5 μg of (all-E)-β-carotene and 4687.7 μg of total β-cryptoxanthin per 100 g (on fresh basis) and retained ~278 μg Retinol Activity Equivalents. All the PJRs had a pectin content of 9.0 to 11.0 % w/w, showing a promising potential as a commercial source compared to citrus peel and apple pomace (with 25-35 % and 10-15% w/w respectively). Evaluation of the FT-IR spectra of PJRs indicated that MW-heating of the PP did not have alter the structural features of the extracted pectins, providing evidence for relatively low MED values of this product (51.3% in PJR-M60 to 49.4 in PJR-M120) and potential use as food additive.

67 Laboratory of Food Chemistry and Technology, School of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece. Co-correspondence: fmantz@chem.auth.gr (F.Th.M): Tel: +302310997774; steord@chem.auth.gr Tel: +302310997847

68 Natural Products Research Center of Excellence (NatPro-AUTH), Center for Interdisciplinary Research and Innovation (CIRI-AUTH), 57001 Thessaloniki, Greece

CONCLUSIONS:

Overall, our findings suggest that the persimmon juice residue resulting from MW-heated pulp is enriched in provitamin-A active carotenoids and in low-methoxy pectin supporting also the view that non-conventional treatments in juice processing could assist the local industry to implement waste valorization strategies via the production of high-value added products.

ACKNOWLEDGMENTS: Foundation of State Scholarships (I.K.Y., Athens, Greece) is acknowledged by SL for a post-doctoral fellowship.

T4-P-20-ORAL Recovery of bioactive compounds using green extraction solvents

Anastasia Loukri, Ioannis Mourtzinos[69]

KEYWORDS: coffee pulp; deep eutectic solvents; cyclodextrins; caffeine; phenolics

INTRODUCTION:

Large amounts of coffee by-products are generated, during the consumption of the famous brew. Coffee pulp, a main by-product of coffee production line, contains valuable compounds such as caffeine and chlorogenic acid, that possess anti-radical activity. Novel solvent systems have been proposed as alternatives to conventional organic solvents, such as aqueous solutions of cyclodextrins and deep eutectic solvents.

OBJECTIVES:

The purpose of the study was the optimization of the extraction of caffeine and antioxidant compounds from coffee pulp using non-conventional solvents.

METHOD / DESIGN:

A response surface analysis was conducted for the evaluation of the efficiency of the aqueous solutions of cyclodextrins (CD) and deep eutectic solvents (DES). The concentration of the solvent (C), the temperature of the extraction (T) and the liquid to solid ratio (L/S) were the independent variables. As depended variables, the antiradical activity (A_{AR}) and caffeine extraction yield (C_{Caf}) were used. Also, the non-conventional systems were compared with other solvents. Moreover, an HPLC analysis was carried out for the determination of the main bioactive compounds, that are presented.

RESULTS:

The different nature of extraction media influenced the recovery of caffeine and antioxidantive compounds. The increase of water content in DES enhances the recovery of both examined responses. However, in cyclodextrin assisted extraction, the concentration of cyclodextrin had a different efficiency compared to the nature of extracted compounds. It was observed that the deep eutectic solvent was more efficient in comparison with the other solvents.

CONCLUSIONS:

The extraction with deep eutectic solvent is a promising green method for obtaining valuable extracts, that are rich in bio-active compounds, that can be used as natural food additives.

69 Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.

Corresponding author: loukriap@agro.auth.gr

T4-P-21 Fortification of commercially available gluten free baking mix - enhancing protein content of cookies

Nikola Maravić, Jelena Tomić, Dubravka Škrobot, Tamara Dapčević-Hadnađev, Miroslav Hadnađev[70]

KEYWORDS: chickpea; *Cucurbita*; proteins; gluten-free; cookies

INTRODUCTION:

In the population that follows gluten free diet, a lot of complicate immune reactions can occur. The intestinal mucosa can be damaged which can cause inability to absorb nutrients. Furthermore, that can lead to development of a lot of diseases such as anaemia, osteoporosis, arthritis, autoimmune and malignant diseases. Gluten free diet is characterized by lower content of vitamins, fibres, minerals, proteins, and imbalanced nutrient content. Addition of chickpea and pumpkin seed oil press-cake flour to commercial gluten free baking mix could have positive impact on nutritive content of obtained final product. That assumption is based on fact that chickpea has high protein content (23-27%), especially lysine, high fibre content and low glycaemic index. On the other hand, pumpkin seed oil press-cake flour contains up to 50% of proteins with favourable amino acid content and is source of fibre and minerals (P, K, Mg, Mn i Ca). Lower content of lysine in pumpkin seed press-cake is compensated with addition of chickpea flour.

OBJECTIVES:

The objective of this study was to enrich commercially available gluten free baking mix in terms of protein content and nutritive profile with chickpea flour and pumpkin seed oil press-cake.

METHOD / DESIGN:

In formulation of cookies commercial gluten free mix, pumpkin seed oil press-cake flour, chickpea flour, vegetable fat, soya lecithin, powdered sugar, salt, baking powder and water were used. The constant level of the gluten free mix (30%) was fortified with different ratio of chickpea flour and pumpkin seed oil press-cake flour. Formulation for control sample production contained 70% of chickpea flour, while in formulations for PC20:50 and PC35:35 samples, 20% and 35% of chickpea flour was replaced with pumpkin seed oil press-cake flour, respectively. Proximate chemical composition, fatty acid profile, mineral content, nutritive profile, texture profile and sensory evaluation were examined. The obtained cookies were compared to commercial.

RESULTS:

As expected, protein content of PC20:50 and PC35:35 was higher than control sample where both of created cookies can be considered as source of proteins. The PUFA/SFA ratio in fatty acid profile is around 0.7 in all samples which suggest that choice of raw materials contributed to favourable fatty acid composition. Also, substitution with pumpkin seed press-cake significantly increased Ca and Mg content in cookies. Addition of pumpkin seed oil press-cake in formulation positively contributed to overall likeability and colour, taste and aroma likeability of obtained cookies compared to control and commercial cookies. Nutritive composition of cookies was significantly improved by addition of pumpkin seed oil press-cake in formulation and the new formulation have similar properties to commercial final products.

70 *University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia. Corresponding author: nikola.maravic@fins.uns.ac.rs*

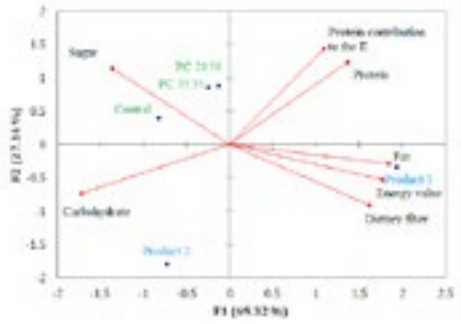

Figure 1. Graphical representation of the position of created cracker samples in PC space in relation to coomercially available gluten-free crackers

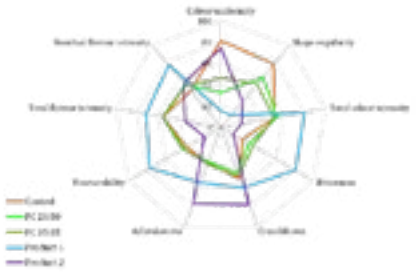

Figure 2. Sensory profile of gluten-free cookies

CONCLUSIONS:

It could be concluded that addition of pumpkin seed oil press-cake in commercial gluten free mix contributed to enhancing protein content. Also, nutritive content of obtained products had been significantly improved.

T4-P-22 Technological and nutritional quality of high protein gluten-free pasta

Dubravka Škrobot, Nikola Maravić, Jelena Tomić, Mladenka Pestorić, Olivera Šimurina[71]

KEYWORDS: gluten-free; pasta; protein; rice; pea

INTRODUCTION:

Nowadays following gluten-free diet became not just a need for people who suffer celiac disease, but the way of modern life, which lead to development of wide spectrum of gluten-free products. Palatability, likeability, low costs, nutritional quality, and simplicity of preparation are some of the reasons why pasta is one of the worldwide most consumed products. These facts indicate that development of gluten-free pasta represent one of the promising products for production. Although pasta is a good source of carbohydrate with a low glycaemic response, it is not considered as a nutritionally balanced product due to its low protein biological value and low fibre content. Also, traditional gluten free flours lack proteins and antioxidants. In that terms, fortification of gluten free pasta is necessary.

OBJECTIVES:

The aim of this study was to examine the suitability of protein rich pea flour as functional ingredient which will forficate the rice pasta.

METHOD / DESIGN:

Three variations of pasta samples were formulated. The first variation was made from 100% rice (Rp), the second one was rice based with 5% of pea flour and the third one was pea based with 5% rice. Proximate composition, physical, textural and colour properties, and cooking quality of samples were examined. Pasta colour was measured by using Konica Minolta colorimeter (CR-400, Konica, Minolta, Tokyo, Japan), while textural properties of uncooked (hardness, flexibility and toughness) pasta samples were measured by using texture analyser TA.XT Plus (Stable Micro System, U. K.)

71 *University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia. Corresponding author: nikola.maravic@fins.uns.ac.rs*

RESULTS:

The sample P95/R5 had more than two times higher content of proteins compared to R95/P5 pasta and Rp pasta. Comparing textural properties of uncooked pasta, results showed higher hardness of pasta based on rice flour (Rp). Pea based pasta had significantly ($p<0.05$) more red and yellow nuance, although during cooking red tone was washed into cooking water. Cooking loss for P95/R5 and R95/P5 pasta samples was at the same level and significantly lower compared to Rp pasta.

Figure 1. ARABIC 1. Textural properties (hardness, flexibility and toughness) of uncooked pasta

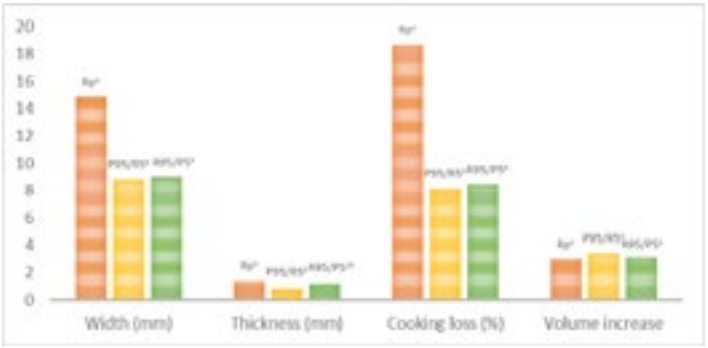

Figure 2. ARABIC 2. Physical properties (width, tickness) and cooking quality (cooking loss, volume increase) of pasta

CONCLUSIONS:

The results indicate that pea flour may be used for production of protein enriched pasta without altering quality properties. Parameters such as protein content and cooking loss were significantly improved with addition of pea flour.

T4-P-23-ORAL Oil crops breeding at IFVCNS – new tools for tackling changing environment and market demands

Dragana Miladinović, Ana Marjanović Jeromela, Sandra Cvejić, Siniša Jocić, Aleksandra Radanović, Milan Jocković, Boško Dedić, Sonja Gvozdenac, Nada Hladni, Ankica Kondić-Špika[72]

KEYWORDS: oil crops; breeding; molecular tools; environment

INTRODUCTION:

Oil crops breeding at Institute of Field and Vegetable Crops (IFVCNS) has a successful 50-year long tradition that resulted in collection of 7000 sunflower inbred lines, as well as collection of wild sunflowers and substantial collections of genetic resources of rapeseed, pumpkins and 24 minor oil crops. Creation of new oil crop varieties using classical breeding methods is a long-term process, sometimes not efficient enough to meet demands of changing environment and market demands of 21st century.

OBJECTIVES:

The introduction of modern techniques, such as high-throughput phenotyping, marker-assisted and genomic selection, into IFVCNS breeding programs, for more efficient incorporation of desired traits into commercial varieties.

METHOD / DESIGN:

The most common application of molecular tools in oil crops breeding at IFVCNS is marker-assisted backcross breeding for

72 *Institute of Field and Vegetable Crops, National Institute of Republic of Serbia, Novi Sad, Serbia. Corresponding author: dragana.miladinovic@ifvcns.ns.ac.rs*

gene introgression, as well as mapping of agronomically important traits. Climate change and its consequences placed focus also on selection of oil crops genotypes tolerant to abiotic stresses. Since abiotic stress related traits are mostly quantitative, genomic selection along with epigenomic selections are gaining importance in IFVCNS breeding activities.

RESULTS:

Marker-assisted backcross breeding is routinely used for introduction of resistance traits into sunflower germplasm, with the emphasis on downy mildew, where markers for identification of P_{l6}, P_{l15} and P_{larg} genes are available. Marker-assisted selection is also used in production of rapeseed hybrids, as well as high-oleic sunflower genotypes. Genomic selection is already applied in soybean breeding, with the initial efforts made for introduction of epigenetics into sunflower breeding with the aim of developing epiQTLs. Tolerance to abiotic stresses is also addressed through phenotyping efforts, focused predominantly on root traits and drought resistance, with the first results obtained in sunflower and rapeseed. Tolerance to abiotic stresses is also addressed through phenotyping efforts, focused predominantly on root traits and drought resistance, with the first results obtained in sunflower and rapeseed.

CONCLUSIONS:

There is still room for improvement, especially in data collection and integration. Further efforts should be made in better combining of phenotypic and molecular data and their integration into the breeding process through envirotyping and identification of traits and markers of real practical value for the breeders.

ACKNOWLEDGEMENTS: This work is part of the project supported by Ministry of Education, Science and Technological Development of Republic of Serbia, grant number 451-03-9/2021-14/200032, and COST Actions CA 19125, CA 18111 and CA 16212.

T4-P-24 Specificity of heavy metal accumulation in vegetable species and health risk assessment in relation to cultivation site

Nataša Nikolić, Slobodanka Pajević, Danijela Arsenov, Milan Borišev, Milan Župunski[73]

KEYWORDS: heavy metals; vegetables; human health risk; metal pollution index

INTRODUCTION:

Enrichment of the environment by heavy metals (HM) due to various human activities occurs at the global scale. Besides the essential mineral nutrients, edible food crops simultaneously absorb and accumulate metals whose role in plant metabolism has not been discovered yet (e.g. cadmium, lead, chromium). Concentration of HM in edible cultivated plants above the recommended values (i.e. maximum permitted concentration) poses significant human health risks due to dietary exposure to high levels of the pollutants (Ogunkunle et al., 2017), considering the tendency of plant-based food increase in regular diet of the modern man. Surveys dedicated to food quality assessment especially in small, economically week, developing countries are inestimable, because the excessive accumulation of HM might also disturb levels of essential nutrients, resulting in malnutrition diseases of the inhabitants. Concentration of heavy metals in edible parts of cultivated plants depends on plant species, soil characteristics, and the metal in question (Murray et al., 2009).

73 Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 2, Novi Sad, Serbia.

Corresponding author: natasa.nikolic@dbe.uns.ac.rs

OBJECTIVES:

Analysis of the chemical composition of vegetables cultivated at different localities performed in the present work aimed at determination of Cd, Ni, Cr, and essential elements (K, Na, Ca, P) concentration, human health risk related to chronic consumption of potentially contaminated vegetables, and summarizing variation among species by principal component analysis.

METHOD / DESIGN:

Randomly selected root samples of parsley (*Petroselinum crispum* (Mill.) Fuss), celeriac (*Apium graveolens L.*), carrot (*Daucus carota* subsp. *Sativus*) and parsnip (*Pastinaca sativa L.*) were produced by individual producers at small farms located in various districts (Srem, Banat, Bačka) of the Vojvodina Province. Leaves of parsley and celeriac were also analyzed, due to their common use in human nutrition. Concentration of K, Ca and Na was determined by flame photometry, and of P spectrophotometrically. Concentration of Cd, Ni and Cr was measured by Inductively Coupled Plasma Mass Spectrometry (ICP/MS, Agilent Technologies 7700) according to EPA method 6020B (SW-846). All measurements were performed in triplicate. Obtained results were processed by analysis of variance using Statistica V 13.5.0.17, a post-hoc Fisher's test and (at p <0.05) and Principal Component Analysis (PCA).

RESULTS:

Concentration of HM in edible plant parts varied among plant species, cultivation locality and plant organ (leaf/root). Carrot showed the lowest potential for Cd, Ni and Cr accumulation while the highest potential was shown for parsley leaves, with respect to average values. Considerable variability among cultivation sites was recorded for root to shoot HM translocation in parsley and celeriac. The hazard quotient (HQ) values revealed highest values for Cd, followed by Ni and Cr. Positive Pearson correlation between Cr and Ni has been observed in many cases suggesting the possibility of simultaneous occurrence of high Cr and Ni concentrations in studied vegetables. Concentration of Ca, P, K, and Ca varied considerably between the cultivation sites, and concentrations of K were higher than of Ca, P and Na in all analysed species. PCA analysis indicated differences in plants ability to accumulate certain nutrients and pollutants, while samples diversification was closely related to growing sites properties.

CONCLUSIONS:

Results obtained in the present work suggest the absence of health risk due to consumption of studied vegetables, with respect to HQ of Cr and Ni, while HQ of Cd calculated for adults and/or children exceeded the threshold value of 1 in several cases.

ACKNOWLEDGMENT: Research was conducted and funded within the project entitled: "Biologically active components and medical potential of functional food grown in Vojvodina Province, Serbia" no. 114-451-2149/2016-03, financed by the Provincial Secretariat for Science and Technological Development, Autonomous Province of Vojvodina, Serbia.

T4-P-25-ORAL A deep learning-based prediction model for soybean yield

Petra Djuza, Gordan Mimić, Oskar Marko, Sanja Brdar[74]

KEYWORDS: crop yield prediction; food security; AgriTech; deep learning; convolutional neural networks

INTRODUCTION:

The threat of global food insecurity is accelerating rapidly and has become a critical concern for global leaders pursuing the Sustainable Development Goal of Zero Hunger, which involves food security and sustainable agriculture. The development of accurate crop yield prediction models is thus essential for ensuring that agricultural product demands will be met in the future. While recent years have seen an exponential rise in the use of machine learning techniques for crop yield predictions, few studies have focused on its implementation with both time-series and remote-sensing data, captured throughout the growing season.

OBJECTIVES:

The objective of the paper is to propose a state-of-the-art deep learning approach to crop yield prediction for soybean based specifically on artificial neural networks (ANNs) and convolutional neural networks (CNNs) using time-series meteorological data, soil data and the Enhanced Vegetation Index (EVI). The performance of the proposed model is to be compared to the random forest model, a standard model used for crop yield prediction. Furthermore, the influence of the input parameters on the yield are to be analyzed so as to determine their significance in yield prediction models.

METHOD / DESIGN:

The methodology involved extracting meteorological time-series data, soil data, and the Enhanced Vegetation Index (EVI) for soybean farms located throughout Vojvodina from 2006 to 2020. The dataset representing seven soil parameters at various soil depths was inputted into a deep ANN, whereas the time-series datasets representing seven meteorological variables and the EVI values respectfully were entered into two separate CNNs, each with a different architecture. The use of CNNs was due to their promising performance when time dependencies are present in the dataset. The three neural networks were thereafter concatenated and put through three fully connected layers. The resulting yield prediction was thereafter evaluated using the coefficient of correlation (R^2) and the root mean square error (RMSE).

RESULTS:

The results show that the proposed model produces yield predictions that deviate, on average, 4% from the true ground value. Moreover, the proposed CNN-ANN model outperforms the random forest implementation as it produces an R^2 score of 63%, 10% higher than that of the random forest, and an RMSE value of 0.11 tons per hectare as opposed to the random forest's RMSE value of 0.74 tons per hectare. Graphical comparisons between the two methodologies are provided. The analysis of the input data confirms the significance of vapour pressure deficit, precipitation, surface solar radiation, and soil moisture on the yield and further implies the insignificance of including minimum temperature values in the model. In terms of soil features, evidence is presented to suggest that high nitrogen levels at the 0-5cm depth could be detrimental to the yield while soil compositions with higher clay percentages could aid yield production. There also appears to be a strong positive correlation between yield and the percentage change in EVI values throughout the growing season.

74 *The BioSense Research Institute. Corresponding author: petra.djuza@biosense.rs*

CONCLUSIONS:

The proposed CNN-ANN model produces relatively accurate yield predictions for soybean farms located in Vojvodina, Serbia and should be the preferred method to random forests. However, further research is required to determine the model's performance when more geographically diverse regions are considered and when additional farm management practices are available for inclusion in the model.

T4-P-26 Contents of chlorophyll, epidermal flavonols and anthocyanins in field grown buckwheat cultivars during different developmental stages

Gordana Racić[75], Emilija Svirčev[76], Biljana Bajić[77], Danijela Žunić[78], Vladimir Sabadoš[78], Dejana Panković[75], Neda-Mimica Dukić[76]

KEYWORDS: Dualex; pigments; *Fagopyrum esculentum*;

INTRODUCTION:

Common buckwheat (*Fagopyrum esculentum* Moench) is a fast-growing pseudo cereal rich in flavonoids which are partially responsible for its biological activity. Due to increasing demands for food supply rich in biologically beneficial compounds, use of non-destructive methods for a fast pre-screening of plants is of high interest in sustainable agriculture. These methods allow screening of plants for the relative contents of secondary metabolites, photosynthetic pigments, presence of biotic or abiotic stress.

OBJECTIVES:

The purpose of this study was to investigate contents of chlorophyll, epidermal flavonols and anthocyanins in field grown buckwheat (*Fagopyrum esculentum* Moench) cultivars from different origin during different developmental stages using non-destructive measurement with Dualex® Scientific.

METHOD / DESIGN:

Experiment was conducted at experimental open field at Nenadic (Sombor). Fourteen different cultivars (Oberon, B. Petrovac exp 1., Darja 1, Populacija B.T., Novosadska plus, Češka, Bamby, Novosadska, B. Petrovac exp 2., B. Petrovac exp 3., K-11, Bily, Ajda and Darja 2) were sown in 3 m long rows with 25 cm of inter-row spacing and 15 cm spacing between plants in the row. The standard growing technique was applied. Indices of chlorophyll (Chl), epidermal flavonols (Flav) and ther ratio, NBI as well as antocyanines (Anth) were measured in vivo non-destructively with Dualex sensor (Force-A, Orsay, France). Measurements were done on 28 uniform, fully developed and sunexposed leaves of the buckwheat plants, each foutheenth days starting from sowing in soil.

RESULTS:

Relative index of Chl, Flav and ther ratio, NBI as well as antocyanin (Anth) content measuerd with Dualex sensor showed similar trend for each cultivar during plant growth and development. In the all examined cultivars, Chl content did not change significantly among cultivars nor during different developmental stages. Highest values for Chl were measured for cultivars

75 Faculty of Ecological Agriculture, Educons University. Corresponding author: gordana.racic@educons.edu.rs

76 Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad

77 ABioTechLab,Sremska Kamenica

78 Agricultural Extension Service, Sombor

Bamby, Ajda and Darja 2. Content of Flav ranged from 1.7046 to 2.0955 for all examined cultivars, whereas its content was highest in the 2nd, 3rd and 4th week of measurment. Anth content was the highest in the first and in the last week of measurment forming the U shaped curve, with minimal values in 5th and 6th week of measurement i.e. about 14th week after sowing of plants.

CONCLUSIONS:

Pre-screening of biologically active compounds and pigments with sensors can be used for a prediction of their content in plants during their development. Our results might help in choosing optimal time of the harvest for buckwheat plants due to its uneven maturation, as well as for flavonoid content estimation facilitating selection of cultivars with high flavonoid content.

ACKNOWLEDGEMENT: This work was funded by Ministry of Education, Science and Technological Development, Serbia, Grant No. 451-03-9/2021-14/200125.

T4-P-27 Occurrence of Alternaria toxins in maize harvested in Serbia during 2012–2017

Elizabet Janić Hajnal[79], Radmila Radović[79], Jovana Kos[79],
Alexandra Malachová[80], Rudolf Krska[80], Michael Sulyok[80], Bojana Radić[79]

KEYWORDS: *Alternaria* toxins; maize; LC-MS/MS; weather conditions; Serbia

INTRODUCTION:

Due to significant amounts of nutrients, vitamins, and minerals, and several health benefits on human and animal organisms maize is one of the most widely used staple foods and animal feeds in the world. During cultivation, maize is exposed to numerous abiotic and biotic stress factors which can cause maize contamination with a large number of different fungal secondary metabolites. *Alternaria* presents one of the most common fungal genera with the ability to produce a wide spectrum of toxic secondary metabolites. Due to the frequent and high presence of *Alternaria* species and their toxins in food and feed, there is a need for their continuous monitoring, identification, and analysis.

OBJECTIVES:

The objective of the work was to determine the presence of different *Alternaria* toxins (tenuazonic acid, alternariol, alternariol methyl ether, tentoxin, and infectopyron) in maize samples collected during six years (2012-2017) from the main maize-producing regions (Bačka, Banat, and Srem) in Northern Serbia. For this purpose, the sophisticated analytical technique liquid chromatography-tandem mass spectrometric (LC-MS/MS) was employed for the identification and quantitation of target *Alternaria* toxins.

METHOD / DESIGN:

During six years investigated period, maize samples were collected and managed according to standard agricultural procedures and good professional practice. Sample preparations, instrumental parameters, and LC-MS/MS analysis of maize

79 University of Novi Sad, Institute of Food Technology in Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Republic of Serbia.

Corresponding author: radmila.radovic@fins.uns.ac.rs

80 University of Natural Resources and Life Sciences Vienna (BOKU), Department IFA-Tulln, Konrad Lorenz Str. 20, 3430 Tulln

samples were performed according to the method published by Sulyok et al. (2020)[81] without any modifications, as well as with the same chemicals, reagents, and equipment.

RESULTS:

Among all analyzed maize samples from 2012, 2013, 2014, 2015, 2016, and 2017 production years, 67, 18, 29, 27, 34, and 25% were contaminated with tenuazonic acid; 6, 6, 23, 14, 8 and 9% with alternariol; 47, 18, 63, 33, 14 and 7% with alternariol monomethyl ether, respectively. On the other hand, in 2012, 2013, 2014, and 2017 maize growing season, 10, 6, 6 and 2% of maize samples were contaminated with tentoxin, respectively. Infectopyron was quantified in 6 and 3% of maize samples from the 2012 and 2017 production years, respectively. The most commonly detected *Alternaria* toxin in maize samples from all investigated years was tenuazonic acid with the highest frequency 67% in 2012 and with the highest mean concentration 599±1.29 µg/kg in 2016.

CONCLUSIONS:

Based on the findings obtained in this study, it could be concluded, that the weather conditions (especially air temperature and amount of precipitation) in the investigated maize growing seasons, had a huge influence on the contamination frequency and determined concentration of *Alternaria* toxins in maize.

ACKNOWLEDGMENT:

This paper is a result of the research conducted within Project of Multilateral Scientific and Technological Cooperation Projects in the Danube Region (DS2016-0059); European Union's Horizon 2020 research and innovation program under grant agreement no. 692195 (MultiCoop); and Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-9/2021-14/200222). The authors are thankful to the agricultural advisory services from Bačka, Banat, and Srem, which provided the maize samples and agronomic data for the presented investigation.

T4-P-28 Presence of trichothecenes in maize produced in Northern Serbia

Jovana Kos, Bojana Radić, Elizabet Janić Hajnal[82],
Alexandra Malachová, Rudolf Krska, Michael Sulyok[83]

KEYWORDS: deoxynivalenol, trichothecenes; maize; LC-MS/MS; weather conditions

INTRODUCTION:

Infection of crops and stored cereals with fungi can lead to the production of secondary toxic metabolites commonly known as mycotoxins, which can result in great economic losses and negative impacts on human and animal health. Trichothecenes are the largest group of mycotoxins produced by *Fusarium* species and frequently occur in cereals such as maize, wheat, barley, oats and rye.

81 *Sulyok, M., Stadler, D., Steiner, D., Krska, R. (2020). Validation of an LC-MS/MS-based dilute-and-shoot approach for the quantification of > 500 mycotoxins and other secondary metabolites in food crops: challenges and solutions. Analytical and Bioanalytical Chemistry, 412(11), 2607-2620.*

82 *Institute of Food Technology in Novi Sad, University of Novi Sad, Novi Sad, Republic of Serbia. Corresponding author: bojana.radic@fins.uns.ac.rs*

83 *Department for Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna (BOKU), Tulln, Austria*

OBJECTIVES:

The main objective of the present study was to determine the presence of trichothecenes: deoxynivalenol (DON), nivalenol (NIV), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), T-2 toxin, HT-2 toxin, monoacetoxyscirpenol (MAS), diacetoxyscirpenol (DAS), neosolaniol, and conjugated forms of trichothecenes such as deoxynivalenol-3-glucoside (DON-3G) and HT-2-glucoside in maize samples collected in Northern Serbia during a period of six years. The second objective of this study was to examine the influence of weather conditions on the levels of detected mycotoxins.

METHOD / DESIGN:

The concentrations of trichothecenes in maize samples were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS)[84].

RESULTS:

The obtained data indicate significant differences in the occurrence of examined trichothecenes in maize samples collected over the period of six years. Rainy conditions during the maize growing season in the year 2014 were favorable for the synthesis of DON (100%), DON-3G (100%) and 15-ADON (98%). However, high air temperatures as well as lack of precipitation recorded during the maize growing seasons in 2012, 2013, 2015, 2016 and 2017 resulted in their lower frequency as well as lower concentrations. DON was detected in 63%, 35%, 63%, 76% and 6% of the samples from years 2012, 2013, 2015, 2016 and 2017, respectively. DON-3G was detected in 71% of the samples from 2016, while the frequencies of DON-3G and 15-ADON were lower in the other years (≤18%). Also, the detected concentrations of DON, DON-3G and 15-ADON in maize samples from the year 2014 were higher as well as significantly different compared to the detected concentrations of these toxins in maize samples from other investigated years. According to European and Serbian Regulations, the maximum level of DON for unprocessed maize intended for human consumption is 1750 µg/kg, which is exceeded in 2% and 84% of the examined samples from 2012 and 2014, respectively. In terms of maize intended for animal nutrition, 6% of samples from 2014 had DON concentrations higher than the maximum level (8000 µg/kg).

Furthermore, NIV was detected in 37% of samples from 2014, and T-2 toxin in 37% and 35% of samples from 2013 and 2014, respectively. The frequency of other detected mycotoxins (HT-2 toxin, MAS, DAS, neosolaniol and HT-2 glucoside) in maize samples from Serbia, in all six investigated years, was low, while all samples were negative for 3-ADON.

CONCLUSIONS:

Based on all the above, it can be noticed that DON is a frequent contaminant of maize from Northern Serbia, but it should be noted that its concentration largely depends on the amount of precipitation during the maize growing season. Therefore, the contamination of maize samples with DON should be continuously monitored due to its potential negative effects on human and animal health.

ACKNOWLEDGEMENT:

This paper is a result of the research conducted within Project of Multilateral Scientific and Technological Cooperation Projects in the Danube Region (DS2016-0059); European Union's Horizon 2020 research and innovation program under grant agreement no. 692195 (MultiCoop); and Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-9/2021-14/ 200222).

84 Sulyok, M., Stadler, D., Steiner, D., Krska, R. (2020). Validation of an LC-MS/MS-based dilute-and-shoot approach for the quantification of > 500 mycotoxins and other secondary metabolites in food crops: challenges and solutions. Analytical and Bioanalytical Chemistry, 412(11), 2607-2620

T4-P-29 Influence of carrier agents on the chemical composition and physical properties of blueberry (*Vaccinium Myrtillus L.*) powder produced by spray drying

Živilė Tarasevičienė, Miglė Daunaravičiūtė, Aurelija Paulauskienė[85]

KEYWORDS: blueberry juice; powder; encapsulation; quality.

INTRODUCTION:

Phenolic compounds, especially anthocyanins, are the main bioactive compounds with functional benefits in blueberries. During the processing and storage, significant amount of these compounds decreases. One of the pronounced methods for the increasement of the shelf life of the bioactive compounds, protecting them from environmental conditions and avoiding oxidation, is encapsulation.

OBJECTIVES:

The aim of the research was to evaluate the influence of maltodextrin and inulin on the quality of blueberry juice powder.

METHOD / DESIGN:

Corn maltodextrin (14 – 17 dextrose equivalent) and chicory inulin were used for the experiment. The blueberry juice was mixed with 10, 20 and 30 % of maltodextrin, also with 10, 20 and 30 % of maltodextrin and inulin at the ratio 1:1. The same additives were added to the blueberry juice dissolved in distilled water at the ratio 1:2 (the additive:distilled water). The blueberry juice with additives was spray dried by LabPlant SD-06 (Keison products, United Kingdom). Juice drying parameters: supplied liquid flow - 436.5 ml h^{-1} or 7.28 ml min^{-1} (± 10%); air flow-87.6 m^3val^{-1}. Inlet and outlet air temperatures were 140 °C / 70 °C. A constant pressure of 0.8 bar was maintained, a 2 mm nozzle was used. The equation of Saavedra-Leos et al., 2019 was used to calculate the blueberry juice powdered yield, and the spectrophotometric method was used to determine the content of total phenols, anthocyanins and antioxidant activity.

RESULTS:

Higher concentration of the additive and its dissolution in water increased the yield of blueberry juice powder. The highest yield was determined with 30% dissolved maltodextrin additive (4,40 %). In blueberry juice powder with 20% of maltodextrin and inulin the highest content of phenolic compounds was present (938,99 mg GAE 100 g^{-1}). The highest anthocyanin content and its retention was found in the powder with 10% of maltodextrin (328,03 mg 100 g^{-1} and 82,67 %). Dissolution of the additive in water before spray drying resulted in significantly lower amount of anthocyanin in the powder. The highest antioxidant activity had a blueberry juice powder with 20% of maltodextrin (91,44%) and 20% of maltodextrin and inulin additive (91,79%). Dissolution of the additives in water significantly contributed to the lower antioxidant activity of the powder.

CONCLUSIONS:

The results obtained in this study demonstrate that additives dissolution before spray drying gave a higher powder yield, but a lower amount of biologically active substances and antioxidant activity of powder. The mixture of inuline and maltodextrine resulted in higher content of phenols, anthocianins and antioxidant activity of powder. Prebiotic dietary fibers,such as inulin, demonstrate the potential use as alternative carrier agents with additional nutritional value.

85 Vytautas Magnus University Agriculture Academy, Faculty of Agronomy, Studentų str. 11, LT-53361 Akademija, Kauno r. Lithuania.

Corresponding author: zivile.taraseviciene@vdu.lt

T4-P-30 Volatiles other than ethanol in unrecorded and recorded fruit spirits – health risk assessment

Branislava Srđenović Čonić[86/87]*, Ljilja Torović[86], Nebojša Kladar[86/87], Biljana Božin[86/87], Nebojša Salaj[86], Kusonić Dejan[86], Jan Suđi[86/88]

KEYWORDS: Volatiles, spirits, HHS-GC-FID, margin of exposure

INTRODUCTION:

Per capita alcohol consumption in the WHO European Region is the highest in the world, which results in proportionally higher levels of burden of disease attributable to alcohol use compared to other regions. Mortality attributed to alcohol consumption remains a problem in countries located at Southeast Europe and consumption of unrecorded homemade spirits is stated to be one of the leading causes. Some alcoholic beverages contain volatile components; other than ethanol. Methanol has been described to be the most common cause for surrogate toxicity, while acetaldehyde may contribute to the carcinogenicity and higher alcohols have also been speculated as a cause for unrecorded alcohol toxicity (liver cirrhosis) in Eastern Europe. They are not subject to safety control, thus raising the question about health risks related to the presence of carcinogenic and non-carcinogenic volatiles.

OBJECTIVES:

Out of 153 fruit spirit samples collected during 2020 in Vojvodina (Serbia), 26 with tax stamp were marked as recorded, whereas 127 produced in private homes or small-scale distilleries and obtained mainly directly from the producers were marked as unrecorded. All samples were analyzed by HSS-GC-FID for the presence of acetaldehyde, ethyl acetate, methanol and higher alcohols (n-propanol, n-butanol, isobutanol, isoamyl alcohol, n-amyl alcohol) and resulting concentrations were compared with toxicological thresholds proposed by the Alcohol Measures for Public Health Research Alliance project (AMPHORA). The margin of exposure approach was used to assess the health risk of unrecorded and recorded spirits.

METHOD / DESIGN:

The margin of exposure approach (MOE-ratio of toxicological threshold values and estimated daily exposure) was used for the risk assessment, where NOAEL (no observed adverse effect level) was used as the toxicological threshold value, except for methanol where lower one-sided confidence limit of benchmark dose (BMDL) was used. Estimated daily exposure for each substance of interest (mg/kg bw/day) was calculated by multiplying measured concentration of substance (mg/L of pure alcohol) and daily alcohol consumption (L of pure alcohol per day) divided with body weight (kg).

For the daily alcohol consumption in Serbia four scenarios were employed – average consumption on the population level, regular drinkers only, chronic heavy drinkers version A (share of recorded and unrecorded alcohol consumption) and chronic heavy drinkers version B (exclusive consumption of recorded or unrecorded spirits)

RESULTS:

For the unrecorded spirits, AMFORA limit was exceeded for acetaldehyde, ethyl acetate, methanol and higher alcohols in

86 University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, 21000 Novi Sad, Serbia.

Corresponding author: BRANISLAVA.SRDJENOVIC-CONIC@mf.uns.ac.rs

87 University of Novi Sad, Faculty of Medicine, Center for Medical and Pharmaceutical Investigations and Quality Control, Hajduk Veljkova 3, 21000 Novi Sad, Serbia

88 Institute of Occupational Health Novi Sad, Futoska 121, 21000 Novi Sad, Serbia

5%, 66%, 21% and 1% of the samples, respectively, while for the recorded spirits limits were exceeded only in case of ethyl acetate (4% of the samples) and higher alcohols (1%). Statistically significant differences in content of volatiles between unrecorded and recorded spirits were noticed in case of ethyl acetate, isobutanol and n-amyl alcohol.
The MOE values for acetaldehyde dropped below 1000 even at average consumption level for 31% of unrecorded and 35% of recorded spirit samples for both sexes, rising up to 98% for both men and women considering consumption of solely unrecorded or 88% in case of exclusive consumption of recorded spirits. Similar situation was noticed for methanol where in average consumption scenario MOE values dropped below 100 in 73% of unrecorded and 58% of recorded samples, rising up to 85% for recorded and even 97% for unrecorded spirits in chronic heavy drinkers scenario B. Higher alcohols exerted health risk only in chronic heavy drinkers scenario version B (around 70% for both types of spirits), while only 4% of recorded spirits posed a risk in heavy drinkers scenario version A.

CONCLUSIONS:
The obtained results suggest that, although slightly higher content of some volatiles was observed in unrecorded spirits, there was no substantial difference in consequent health risk. Control measures should be included in order to maintain the quality of both recorded and unrecorded spirits and minimize the potential adverse health effects.

T4-P-31 Antihyperglycemic potential of hemp extracts (*Cannabis Sativa*, Cannabaceae)

Nebojša Kladar*, Marija Perić, Katarina Bijelić, Blagoje Prpa, Dejan Kusonić, Biljana Božin, Branislava Srđenović Čonić[89]

KEYWORDS: Hemp; *Cannabis sativa*; α-amylase; α-glucosidase; antioxidant potential

INTRODUCTION:
Cannabis sativa L., Cannabaceae is the only representative of *Cannabis L.* genus. The exploitation of *C. sativa* by mankind has a long history. It is important to highlight the classification of *Cannabis* species based on their primary purpose of utilization. Namely, the species containing more than 0.2, or 0.3% (depending on national regulations) of Δ^9-tetrahydrocannabinol (Δ9-THC) are considered psychoactive and are in most of the countries illegal to possess and use. On the other hand, species containing lower amounts of Δ^9-THC and higher amounts of cannabidiol (CBD) are legal for cultivation and are better known as industrial hemp, or simply hemp. They show demonstrated history of usage for production of fiber, as well as different food products, because of the exceptional nutritional value of hemp fruits, commonly marked as "seeds". Besides the previously mentioned terpenophenolic compounds, *Cannabis* species contain other classes of secondary metabolites which have the potential to exhibit beneficial biological effects.

OBJECTIVES:
The aim of the conducted study was to evaluate the antihyperglycemic and antioxidant potential of water and ethanolic hemp extracts, followed by preliminary and detailed chemical characterization of the obtained extracts.

METHOD / DESIGN:
The plant material included five samples of commercially available hemp teas which were further extracted in a form of infusion and ethanolic macerate (70% v/v, 24h). The solvents were evaporated and dry extract yield was quantified. The

89 *University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Hajduk Veljkova 3, Serbia.*

**Corresponding author: nebojsa.kladar@mf.uns.ac.rs*

obtained dry extracts were preliminary chemically characterized in term of total phenolics (expressed as mg of gallic acid equivalents (GAE) per g of dry extract (d. e.)) and flavonoids content (expressed as mg of quercetin equivalents (QE) per g of d. e.), as well as by high performance liquid chromatography (HPLC-DAD) for the quantities of gallic, caffeic, *trans*-cinnamic, *p*-coumaric, chlorogenic, rosmarinic and ferulic acid, quercetin, rutin and quercitrin. The potential of the prepared extracts to inhibit α-amylase and α-glucosidase was evaluated *in vitro* at two concentration levels, as well as the potential to scavenge 2,2-diphenyl-2-pycrylhydrazyl (DPPH), nitroso (NO) and hydroxyl (OH) radicals. Furthermore, the ability of extracts to inhibit lipid peroxidation process and to reduce ferric ions (FRAP-test) was also tested. The antioxidant potential of the examined extracts was expressed as concentration required for neutralization of 50% of free radicals (RSC_{50}) or inhibition of 50% of oxidation process (IC_{50}), except in the case of FRAP test in which the extracts were characterized as ascorbic acid equivalents (AAE) per g of d. e.

RESULTS:

The recorded amount of total phenolics was similar in ethanolic (35,45-62,11 mg GAE/g d.e.) and water extracts (25,85-72,47 mg GAE/ g d. e.). Total flavonoids ranged 7,58-33,48 mg QE/g d.e. and 8,41-17,69 mg QE/ g d.e. in ethanolic and water extracts, respectively. The caffeic acid was the only compound found in both types of the extracts, whereas its amount was significantly higher in water extracts (0.054-0.166 mg/g d. e.). Water extracts showed better potential to inhibit α-amylase at tested concentrations (80 and 240µg/mL), whereas at higher concentration level the inhibition ranged 57.69-93.36%. On the other hand, ethanolic extracts were superior inhibitors of α-glucosidase, while the recorded inhibition ranged 79.24-97.44% at concentration level of 133 µg/mL. Ethanol extracts were better scavengers of DPPH (RSC_{50} = 36.4-45.80 µg/mL) and hydroxyl (RSC_{50} = 219.18-346.28 µg/mL) radicals, as well as better inhibitors of lipid peroxidation process (IC_{50} = 651.25-890.00 µg/mL). On the other hand, both types of the extracts did not manage to neutralize 50% of generated NO radicals in the tested concentration range. The antioxidant potential recorded in FRAP test was similar for both types of the extracts (25.68-77.35 mg AAE/g d. e. and 29.36-58.43 mg AAE/g d. e for water and ethanolic extracts, respectively).

CONCLUSIONS:

The obtained results show promising antihyperglycemic and antioxidant potential of hemp water and ethanolic extracts. However, of particular importance is the recorded anti-α-glucosidase activity of hemp ethanolic extracts, especially when compared with antihyperglycemic potential of acarbose obtained under the same experimental condition (IC_{50}=45.87 µg/mL). This highlights the importance of conducting future preclinical *in vivo* studies in order to better evaluate the possible beneficial effects of hemp ethanolic extracts in treatment of diabetes type 2.

T4-P-32 Fatty acid composition of hemp-based food products

Ljilja Torović[90/91], Nebojša Kladar[90/92]

KEYWORDS: food; hemp; fat; fatty acid; GC-MS

INTRODUCTION:

Rising popularity and market presence of hemp-based food products has emphasized the need for detailed characterization of their nutritional composition. In the food industry the most exploited raw materials are hemp fruits, which are commonly

90 *University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia. Corresponding author: ljilja.torovic@mf.uns.ac.rs*

91 *Institute of Public Health of Vojvodina, Novi Sad, Serbia*

92 *University of Novi Sad, Faculty of Medicine, Center for Medical and Pharmaceutical Investigations and Quality Control, Novi Sad, Serbia*

named "seeds". Hemp seeds after cold pressing result in hemp seed oil, leaving a cake which after grinding contains hemp protein. All of these products – seeds, oils, and proteins, are sources of fatty acids.

OBJECTIVES:

The objective of this research was to study and compare composition of fatty acids (FA) and characteristic groups of fatty acids (saturated – SFA, monounsaturated - MUFA, polyunsaturated – PUFA, omega 3 – ω3FA, omega 6 – ω6FA, omega 9 – ω9FA) in different hemp-based food products available to the consumers in European countries and to estimate intake of nutritionally valuable fatty acids.

METHOD / DESIGN:

Hemp-based food products were obtained from European countries during 2018–2021. Thirty-five products were classified as cold pressed hemp seed oils (19 samples), hemp seeds (5 unpeeled and 4 peeled) and hemp proteins (7 samples). In case of seeds and proteins, sample preparation included extraction of fats using Soxhlet apparatus prior to esterification of fatty acids to methyl esters, which enabled their GC-MS profiling. The intake of fatty acids was calculated based on the recommended usage data available on product's labels and expressed per average portion (oil 15 g, seed 20 g, protein 20 g).

RESULTS:

Mean fat content of hemp seeds was 34.7 and 51.2% in unpeeled and peeled samples, respectively, while in proteins was 11.3%. Composition of most abundant individual fatty acids and characteristic groups of fatty acids in cold pressed hemp seed oil and seed and protein fat portion, presented in Figure 1 (A) and (B), respectively, shows great similarity, as expected, with dominance of ω6 PUFA. Content of characteristic groups of fatty acids in hemp seeds and proteins as whole products is presented in Figure 1 (C). A portion of hemp-based food product boasts an abundance of linoleic (18:2 cis-9,12; ω6) and alpha-linolenic acid (18:3 cis-9,12,15; ω3): cold pressed oil 8.4 and 2.6 g, seeds 3.9 and 1.2 g (unpeeled) and 5.8 and 1.5 g (peeled), proteins 1.3 and 0.3 g, respectively.

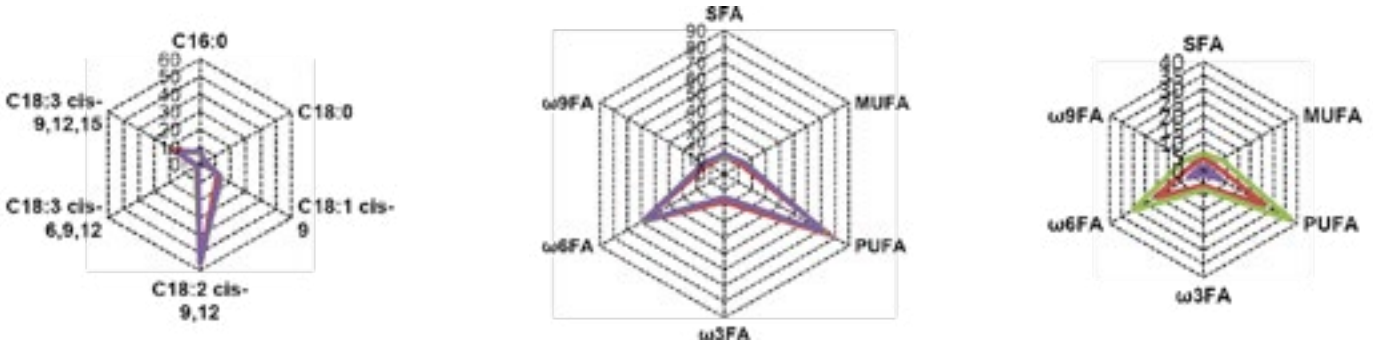

*Figure 1. Composition of most abundant individual fatty acids (A) and characteristic groups of fatty acids in cold pressed hemp seed **oil** and seed (**unpeeled** and **peeled**) and **protein** fat portion (B); Fatty acids composition of hemp seeds (**unpeeled** and **peeled**) and **proteins** (C)*

CONCLUSIONS:

The reported results present an input to the database of fatty acid composition of hemp-based food products. In depth characterization should be regarded as a valuable foundation in valorisation of these products in nutritional recommendations. Essential fatty acids from ω6 (linoleic) and ω3 (alpha-linolenic) groups, in a desirable ratio of ~4, provide substantial nutritional value to the consumers of hemp-based foods. The necessary prerequisite is safety of these products, above all in terms of contamination with cannabinoids, a class of terpenophenolic compounds with psychoactive potential.

T4-P-33 Fatty acid composition of a variety of plant oils used as food

Ljilja Torović[93/94]

KEYWORDS: food; plant oil; fatty acid; GC-MS

INTRODUCTION:

The most exploited raw plant materials for oil production in the food industry are sunflower seeds and olives, but a variety of oils produced from other plants has been offered on the market, usually by small and medium enterprises, most often obtained using cold pressing in order to preserve sensitive oil components. The main common characteristic of all of these oils is that they are sources of fatty acids.

OBJECTIVES:

The objective of this study was to screen various plant oils available to the consumers in Serbia, in terms of their identity, by investigation of fatty acid (FA) composition. Furthermore, the content of characteristic groups of fatty acids (saturated – SFA, monounsaturated - MUFA, polyunsaturated – PUFA, omega 3 – ω3FA, omega 6 – ω6FA, omega 9 – ω9FA) was evaluated. Oils' fatty acid composition also provides an insight into oils' potential to contribute to the dietary intake of nutritionally beneficial fatty acids.

METHOD / DESIGN:

A collection of 36 plant oils obtained during 2020–2021 provided a good representation of sunflower and olive oils available to the Serbian consumers (12 and 11 brands, respectively), and additionally included 13 samples of different less used plant oils, all categorized as food, with one sample of black cumin oil labeled as food supplement. GC-MS profiling of fatty acids (37 FA in total) was enabled by their esterification to methyl esters.

RESULTS:

Composition of most abundant individual fatty acids and characteristic groups of fatty acids in plant oils, presented in *Figure 1* (A) and (B), respectively, showed expected variability, depending on the oil source.

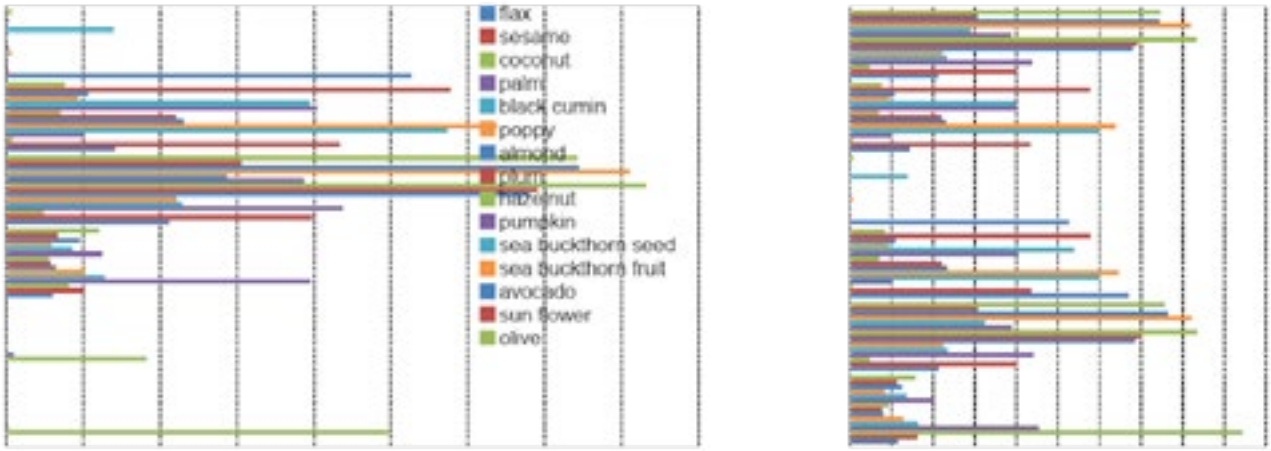

Figure 1. Composition of most abundant individual fatty acids (A) and characteristic groups of fatty acids in plant oils (B)

93 University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia. Corresponding author: ljilja.torovic@mf.uns.ac.rs

94 Institute of Public Health of Vojvodina, Novi Sad, Serbia

According to the content of fatty acids, all investigated samples were in accordance with relevant regulations (flax seed, sesame, coconut, palm, pumpkin and all samples of sunflower and olive oils) or literature data, thus contributing to the confirmation of their identity. The majority of the oils showed dominant content of oleic (C18:1 cis-9; ω9) or linoleic fatty acid (C18:2 cis-9,12; ω6). Exceptions were coconut oil with high content of middle-chain SFA, and flax seed oil, rich in alpha-linolenic acid (C18:3 cis-9,12,15; ω3), found also in lower quantity in sea buckthorn seed oil.

CONCLUSIONS:

Fatty acid composition is a valuable indicator of the origin of various plant oils. It is encouraging that market screening indicates correct labeling of oil sources. Only flaxseed oil is a rich source of ω3 alpha-linolenic acid, whereas ω6 fatty acids are much more abundant in numerous plant oils. To enable nutritional utilization of mono- and polyunsaturated fatty acids, oils should not be subjected to factors affecting their sensitive structure.

T4-P-34-ORAL Light quality and biostimulant application: a sustainable approach to improve antioxidant properties and photosynthesis in soybean (*Glycine max L.* Merril) sprouts

Ermenegilda Vitale[95], Violeta Velikova[96], Tsonko Tsonev[97], Giulia Costanzo[95], Carmen Arena[95/98]

KEYWORDS: soilless cultivation; modulation of light spectrum; amino acid based biostimulant; sprout nutritional content; PSII photochemical efficiency

INTRODUCTION:

The increasing market demand for functional foods, such as soybean seeds and sprouts, has generated significant interest among researchers and consumers in order to accelerate the development of new practices for improving food nutritional value and reducing wastes. The high food production leads to an overexploitation of the natural resources, especially of the soil, which additionally is exacerbated by the ongoing climate change.

In this context, new indoor cultivation strategies are compulsory to reduce the environmental impacts, maximizing crop yield and food quality. Currently, the modulation of the light spectrum through the light-emitting diodes technology (LEDs) and the application of biostimulants are considered eco-friendly and innovative strategies to control plant morphology, physiology and metabolism. The selection of proper light quality regimes and the use of biostimulants may successfully replace the common chemical fertilizers defining new 'natural' fertilization protocols. The merging of these two promising approaches provides new insights in the framework of sustainable cultivation practices.

OBJECTIVES:

This work explores the possibility to produce better functional food with improved photosynthetic traits implementing a new eco-friendly approach in soilless cultivation. The combined effect of amino-acid based biostimulant and specific light quality regimes was tested in soybean (*Glycine max* L. Merril) seeds.

METHOD / DESIGN:

95 *Department of Biology, University of Naples Federico II, Napoli, Italy. Corresponding author: ermenegilda.vitale@unina.it*

96 *Institute of Plant Physiology and Genetics, Bulgarian Academy of Science, Sofia, Bulgaria*

97 *Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria*

98 *Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, Portici (NA), Italy*

The soybean seeds were soaked for 4 hours in different solutions with increasing concentrations of the amino acid-based biostimulant 'Kaishi' (B) (i.e., K-0.01%, K-0,05%, K-0,5%, H_2O used as control).
At 4 days after sowing (DAS), the sprouts were moved under four different light quality regimes (LQ) (dark, D; white fluorescent, FL; full-spectrum, FS and red-blue, RB) to evaluate if the interaction between biostimulant and light quality (B x LQ) may improve the sprout nutritional value. Variations in antioxidant compounds, protein and carbohydrate content were evaluated in sprouts at 8 DAS by biochemical assays.
Part of the sprouts was transplanted in pots filled with water and grown under W, FS, and RB light regimes until 24 DAS. Then, seedlings were screened for morphological parameters, leaf functional and anatomical attributes, and photosynthetic activity to assess the possible valuable effect of the interaction B x LQ on their early developmental stage.

RESULTS:
The seed pre-treatment with the biostimulant, irrespective of the applied concentration, determined a significant rise of sprouts' antioxidant compounds, sugars and proteins compared to the untreated control. The positive effect of the biostimulant was enhanced in sprouts grown under FS and RB compared to D and W light regimes. Higher levels of bioactive co pounds were found in sprouts treated with K-0,05% x FS and K-0,05% x RB.
At the seedling stage, the specific leaf area (SLA) and the PSII photochemical efficiency increased after the treatment with K-0.05%. Such positive effect was enhanced by the interaction B x LQ which contributed to modify the leaf pigment content and the leaf anatomy, favoring the photosynthetic activity, mainly under FS and RB light regimes compared to W.

CONCLUSIONS:
Our results suggest that the seed-pretreatment with the amino-acid based biostimulant Kaishi in combination with plant growth development under specific light quality regimes is an effective and sustainable means to produce high-quality soybean sprouts in terms of antioxidants and seedlings with improved key physiological traits.

T4-P-35 Liquid herbal dietary supplements preserved with benzoates as possible source of benzene

Sladjana Vojvodić[99/100], Dejan Kusonić[99], Branislava Srdjenović Čonić[99/101], Ljilja Torović[99/102]

KEYWORDS: dietary supplements; benzoates; benzene; HSS-GC-MS

INTRODUCTION:
Liquid dietary supplements often contain preservatives, among others, benzoates, used for prolongation of product's shelf-life. Ascorbic acid, naturally present in plants or added as vitamin C or a food additive (antioxidant), can react with benzoates under certain conditions (e.g. heat, UV-light) to form benzene. Benzene is a known human carcinogen, and thus its ingestion in the diet could pose risk for consumers' health. Low levels of benzene have even been found in a variety of foods without added benzoates. In such cases, benzene is considered naturally occurring substance. Higher levels of benzene were found in some, but not all, foods preserved with benzoates.

99 University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia. Corresponding author: sladja.vojvodic@uns.ac.rs

100 University Business Academy in Novi Sad, Faculty of Pharmacy, Novi Sad, Serbia

101 University of Novi Sad, Faculty of Medicine, Center for Medical and Pharmaceutical Investigations and Quality Control, Novi Sad, Serbia

102 Institute of Public Health of Vojvodina, Novi Sad, Serbia

OBJECTIVES:

Study objective was to investigate benzene occurrence in liquid dietary supplements preserved with benzoates and consequent exposure of consumers.

METHOD / DESIGN:

Thirty-four herbal dietary supplements, purchased in pharmacies in Novi Sad in 2021, were liquids with benzoates (sodium benzoate) on their ingredients list. Additional 18 samples of liquid herbal dietary supplements preserved with sorbates (without benzoates) were acquired for the purpose of benzene analysis quality control (similar matrix containing herbal extracts, expected blanks). The method of analysis was HPLC-UV for preservatives, whereas benzene analysis was carried out using HSS-GC-MS. Benzene exposure assessment was based on experimentally obtained concentration data and products' usage instructions given on the labels. Health risk was assessed based on comparison of estimated exposure with Oral Reference Dose for benzene of 0.004 mg/kg bw/day (RfD, U.S. EPA).

RESULTS:

Analysis of preservatives confirmed their presence as presented on the products' labels. Sodium benzoate concentrations ranged from 663 to 8037 mg/L. Benzene was detected in 14 out of 34 samples (41.2%), in concentration ranging from 1.2 to 54.2 µg/L, and in samples containing sodium benzoate in concentration range 663-2123 mg/L. No correlation was established between concentrations of sodium benzoate and benzene. Benzene was not detected in any of the samples preserved using sorbates (confirmed blanks). Measured levels of benzene were greater than maximum allowed concentration of benzene in drinking water in the Republic of Serbia, set at 1 µg/L. It was interesting to note that one of six producers of 14 samples with measurable amounts of benzene was associated to even 5 samples (36%), mostly with higher range benzene content.

Regarding exposure assessment, it has to be highlighted that out of 23 samples intended for adults 8 were positive for benzene, 12 out of 27 for adolescents, 11 out of 25 for children (7-10 year), 10 out of 25 for preschool children, and one out of 8 for toddlers. Compared with RfD of benzene, the one benzene containing sample intended for toddlers was responsible for low level exposure (0.39% of RfD), exposure of preschool children ranged from 0.003-0.70%, children 0.005-0.65%, adolescents 0.006-0.84%, and adults 0.036-0.57%. These products are used over a limited period of time, usually to support respiratory or immune system, and therefore exposure to benzene caused by their consumption is also limited. However, considering the fact that co-exposure to benzene and ethanol can increase benzene toxicity in humans, it is important to emphasize that many of investigated products contain ethanol used for preparation of plant extracts which were their active principles.

CONCLUSIONS:

Considering that children constitute a group that is consuming majority of investigated liquid herbal supplements, it is of the utmost importance to prevent benzene presence. Indeed, product reformulation could eliminate in situ benzene generation and thus mitigate health risk.

Index

A

B

C

D

Đ

E

K

L

M

N

O

P

R

S

T

V

W

X

Y

Z

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