

Josip Juraj Strossmayer University of Osijek Faculty of Food Technology Osijek Faculty of Agriculture in Osijek Faculty of Medicine in Osijek Department of Chemistry Department of Biology

# BOOK OF ABSTRACTS

1 Young Scientistor Day PhD Conference

# 14<sup>th</sup> June 2018

Aula of Josip Juraj Strossmayer University of Osijek



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# 1<sup>st</sup> YOUNG SCIENTIST DAY – PhD Conference

OSIJEK, 14th June 2018

AULA OF JOSIP JURAJ STROSSMAYER UNIVERSITY OF OSIJEK TRG SVETOG TROJSTVA 3, OSIJEK

# **BOOK OF ABSTRACTS**

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### 1<sup>st</sup> YOUNG SCIENTIST DAY – PhD Conference

### TABLE OF CONTENTS

SCIENTIFIC PROGRAMME	I
PRESENTATION ABSTRACTS	1
AUTHOR INDEX	24

### **SCIENTIFIC PROGRAMME**

8:30 - 9:00	Participant registration		
9:00 - 9:30	Opening ceremony		
9:30 - 11:20	Section I.		
Moderators: M. Molnar, Z. Antunović, T. Kovač			
9:30-9:45	<u>D. Iljkić,</u> M. Rastija, V. Kovačević		
	Genotype and agroecological effects on the microelements concentration and grain yield of maize Genotipski i agroekološki utjecaji na koncentracije mikroelemenata i prinos kukuruza		
9:45-10:0	0 <u>A. Vuković, I. Štolfa-Čamagajevac, R. Vuković, M. Matić, Z. Lončarić</u>		
	Influence of different selenium forms on the antioxidative status in wheat seedlings (Triticum		
10 00 10	Ujecaj razlicitih oblika selena na antioksidacijski status u klijancima psenice ( <i>Friticum destivum</i> L.)		
10:00-10	<b>15</b> <u>S. Guberac</u> , S. Petrovic, A. Rebekic, S. vila Winter wheat and climate change adaptability – finding functional markers in elite wheat		
	winter wheat and cumate change adaptability – finding functional markers in etile wheat germplasm		
	Ozima pšenica i prilagodljivost na klimatske promjene – potraga za funkcionalnim markerima u elitnoj germplazmi pšenice		
10:15-10:	30 <u>I. Varga</u> , M. Antunović, Z. Lončarić, M. Pospišil, A. Kristek		
	Sugar beet root yield and quality in relation to different planting densities Prinos i kvaliteta šećerne repe u različitoj gustoći sjetve		
10:30-10	45 <u>Ž. Klir</u> , J. M. Castro-Montoya, J. Novoselec, J. Molkentin, M. Domacinovic, B. Mioc, U. Dickhoefer, Z. Antunovic		
	Influence of pumpkin seed cake and extruded linseed on milk production and milk fatty acid profile in Alpine goats		
	Utjecaj pogače sjemenki bundeve i ekstrudiranog lana na proizvodnju i masnokiselinski profil mlijeka alpskih koza		
10:45-11:	00 <u>K. Gvozdanović</u> , V. Margeta, I. Djurkin Kušec, K. Salajpal, S. Džijan, G. Kušec		
	Genetic authentication of meat from autochthonous pig breed by the use of microsatellite markers		
	Genetska autentifikacija mesa autohtone pasmine svinja primjenom mikrosatelitskih markera		
11:00-11:	15 <u>N. Bilić-Dujmušić</u> , A. Matić (ex. Cosić), R. Vuković, A. Stupin, L. Rašić, G. Kralik, Z. Kralik, I.		
	Drenjančević		
	Positive effect of omega-3 enriched egg consumption on reducing oxidative stress level in young		
	neatiny women Destitiven utional konzumanija omoga 2 obogaćanih jaja na smanjanja razina altaidativnog straga kad		
	r oznivan ujecaj konzumacije omega-5 obogacenin jaja na smanjenje razina oksidativnog stresa kod mladih zdravih ženskih isnitanica		
	muum Zaruvin Zonskin ispiuniou		

#### **11:15 – 11:50** *Coffee break*

11:50 – 12:50 Sect	ion II
Mod	erators: I. Tomac, S. Šafranko, M. Molnar
11:50-12:05	M. Nujić, M. Habuda-Stanić
	Nitrate removal from aqueous solution by modified biomass
	Uklanjanje nitrata iz vodenih medija pomoću modificirane biomase
12:05-12:20	<u>G. Šelo</u> , A. Bucić-Kojić, M. Planinić, M. Tišma
	Biotransformation of grape pomace with Ceriporiopsis subvermispora
	Biotransformacije tropa grožđa s Ceriporiopsis subvermispora
12:20-12:35	<u>N. Pavlović,</u> M. Jakovljević, M. Miškulin, S. Jokić
	Green extraction techniques of bioactive components from cocoa shell
	Zelene tehnike ekstrakcije bioaktivnih komponenata iz kakao ljuske
12:35-12:50	M. Jakovljević, P. Kuś, I. Jerković, M. Molnar, S. Jokić
	GC-MS and LC-MS profiles of the supercritical fluid extracts of black poplar
	(Populus nigra L.) buds
	Određivanje sastava superkritičnih CO2 ekstrakata pupoljaka crne topole
	(Populus nigra L.) primjenom GC-MS i LC-MS tehnika
12.50 14.00 T	

#### 12:50 – 14:00 Lunch break

14:00 – 15:15 Sec	tion III	
Moderators: M. Nujić, I. Štofa Čamagajevac, I. Đerđ		
14:00-14:15	Ž. Lončarić, B.K.Hackenberger, D.K.Hackenberger	
	Application of matrix population models in biology	
	Primjena matričnih populacijskih modela u biologiji	
14:15-14:30	<u>A. Galir Balkić</u>	
	Zooplankton taxonomy, trophic structure and food web interactions	
	Taksonomija i trofička struktura zooplanktona te interakcije unutar hranidbene mreže	
14:30-14:45	<u>B. Vlaičević</u> , J. Vidaković	
	Structure and development of periphytic ciliate community in a Danube floodplain lake Struktura i razvoj zajednice trepetljikaša perifitona u jezeru poplavnog područja Dunava	
14:45-15:00	M. Varga, I. Štolfa Čamagajevac, J. Horvatić	
	Physiological response of Lemna gibba L. when exposed to ionic colloidal silver	
	Fiziološki odgovor vrste Lemna gibba L. na djelovanje ionskog koloidnog srebra	
15:00-15:15	I. Pivić-Kovačević, S. Matić, J. Barać, Ž. Salinger	
	Macular thickness changes in diabetic patients after uncomplicated phacoemulsification cataract	
	surgery	
	Promjene debljine makule u dijabetičara nakon nekomplicirane operacije mrene metodom ultrazvučne fakoemulzifikacije	
15.15 15.50 Coffa	a break	
15.13 – 13.30 Cojje		
15:50 – 18:00 Sectio		
Mode	rators: A. Stanković, K. Selthofer-Relatić, M. Jakovljević	
15:50-16:05	<u>M. Paurević</u> , R. Ribić, S. Tomić	
	Desmuramyl peptide derivatives – synthesis and adjuvant activity	
16.05-16.20	M Komar M Molnar	
10.05-10.20	Synthesis of heterocyclic compounds with biological activity in deep eutectic solvents	
	Sinteza heterocikličkih spojeva s biološkom aktivnošću u eutektičkim otapalima	
16:20-16:45	M. Jukić, T. Bernardi-Opačak, J. Matić, B. Žinić, Lj. Glavaš-Obrovac	
	Mechanism of action of newly synthetized N-9-sulfonylpurine derivatives	
	Mehanizam djelovanja novosintetiziranih N-9-sulfonilpurinskih derivata	
16:45-17:00	<u>P. Matic</u> , S. Ukic, L. Jakobek	
	Ravnotežna istraživanja adsorpcije flavan-3-ola na β-glukanu	
17:00-17:15	J. Bijelić, A. Stanković, B. Matasović, B. Marković, M. Bijelić, G. Štefanić, Ž. Skoko, Z. Jagličić, S.	
	Zellmer, T. Preller, G. Garnweitner, I. Djerdj	
	Modified aqueous sol-gel route towards quaternary metal oxides containing tungsten	
	Modificirana vodena sol-gel metoda za sintezu kvaternarnih metalnih oksida na bazi volframa	
17:15-17:30	<u>M. Hajduković</u> , M. Samardžić	
	Funkcionalizirane ugljikove nanocievčice kao novi senzorski materijal za određivanje kationskih	
	tenzida	
17:30-17:45	<u>I. Tomac</u> , M. Šeruga, L. Jakobek	
	Characterization of chlorogenic acids in different coffee varieties using electrochemical methods	
	Karakterizacija klorogenskih kiselina u različitim vrstama kave primjenom elektrokemijskih metoda	
17:45 - 18:00 Sho	rt break	
18:00 – 18:30 Con	18:00 – 18:30 Congress closing	
18:30 - 20:30 Garden party (Faculty of Food Technology, F. Kuhača 18, Osijek)		

**PRESENTATION ABSTRACTS** 

# Genotype and agroecological effects on the microelement concentration and grain yield of maize

Genotipski i agroekološki utjecaji na koncentracije mikroelemenata i prinos kukuruza

<u>D. Iljkić<sup>\*</sup></u>, M. Rastija, V. Kovačević

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This study examined the effects of hybrids, year and location (environment) on the grain yield of the concentration of iron maize, (Fe), manganese (Mn), zinc (Zn) and copper (Cu) in leaf and grain dry matter. Furthermore, protein, oil and starch content in maize grain were also examined. A field experiment was conducted in a randomized complete block with four replications at two locations, during a two-year period with 10 commercial maize hybrids of different FAO groups originating from the Agricultural Institute Osijek. The location Osijek has eutric brown soil type, very low pH reaction and a higher concentration of copper and manganese available to plants, while soil in

Podgorač belongs to a pseudogley type, has very acid pH reaction, and a greather concentration of iron and zink available to plants. Grain yield of maize was significantly influenced by hybrid (H), location (L) and years (G) and their interaction. The average grain yield of maize was 8.61 t ha<sup>-1</sup> with variations from 8.20 t ha<sup>-1</sup> to 8.92 t ha<sup>-1</sup>. Generally, at location Osijek, average yield of a 2-year experiment was higher when compared to the location Podgorač. Nutrient concentrations in leaf and grain dry matter were significantly different for all main factors (G, L, H), while the location was not statistically justified only for the Fe grain concentration, and location was not statistically justified for Cu grain concentration. There were significant variations in leaf and grain trace elements concentration. Protein, oil and starch content in maize grain was significantly affected by hybrid, location and years with the exception of the impact of the oil content and the impact of location on the content of starch in maize grain. In our other experiments we deal with the residual impact of liming on the yield and quality parametres of field crops.

Keywords: maize hybrids, agroecological conditions, yield, trace elements, seed quality

# Influence of different selenium forms on the antioxidative status in wheat seedlings (*Triticum aestivum* L.)

Utjecaj različitih oblika selena na antioksidacijski status u klijancima pšenice (*Triticum aestivum* L.)

<u>A. Vuković<sup>1,\*</sup></u>, I. Štolfa-Čamagajevac<sup>1</sup>, R. Vuković<sup>1</sup>, M. Matić, Z. Lončarić<sup>2</sup>

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Selenium (Se) is an important microelement with a dual effect; at lower concentrations it is an essential element for animals, humans and some lower plants, but at higher concentrations, it can become toxic. The aim of this study was to investigate the influence of different concentrations of two selenium forms, selenate and selenite, on the oxidative and antioxidative status of wheat shoots and roots. Wheat grains were exposed to five different concentrations  $(0.1 \text{ mg L}^{-1}, 1 \text{ mg L}^{-1}, 5 \text{ mg L}^{-1}, 10 \text{ mg L}^{-1}, 100$ 

mg L<sup>-1</sup>) of Se in a form of selenite and selenate forseven days. As biomarkers of the oxidative status in wheat, lipid peroxidation (LPO) levels and total H<sub>2</sub>O<sub>2</sub> content were determined. The antioxidative status was determined by total reduced glutathione levels (tGSH), as well as by catalase (CAT) and ascorbate peroxidase (APX) activities. Both Se forms did not cause any significant changes in LPO levels and H<sub>2</sub>O<sub>2</sub> content in shoots, while in roots selenite and selenite induced oxidative stress. tGSH levels in roots and shoot were reduced after the exposure to various concentrations of selenite and selenate relative to their controls. The results showed that both forms of Se decreased CAT activity in roots, while in shoots CAT activity did not change. Furthermore, only the highest concentrations of selenite and selenite increased the APX activity in shoots and roots. In conclusion, the plant response to Se depends on its chemical form, applied concentration and the analyzed part of the plant. The obtained results can serve as a basis for further studies of molecular and biochemical effects of Se on plants.

Keywords: selenate, selenite, wheat, oxidative stress, antioxidative enzymes

#### Winter wheat and climate change adaptability – finding functional markers in elite wheat germplasm Ozima pšenica i prilagodljivost na klimatske promjene – potraga za

funkcionalnim markerima u elitnoj germplazmi pšenice

<u>S. Guberac</u>\*, S. Petrović, A. Rebekić, S. Vila

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Wheat is a dominant crop in temperate climate and its success depends on its adaptability and high yield, as well as on specific quality traits. Large natural variation of this species is enabled by its hexaploid genome structure. Adaptation of wheat genotypes to diverse environmental conditions is under the influence of three genetic mechanisms, Rht, Ppd and Vrn genes. The aim of this study was to detect presence of functional markers for plant height, response to photoperiod and vernalisation requirement among 70 elite cultivars. The selected cultivars, wheat originating from Croatia, Serbia, Italy Austria, Germany, France, Hungary and Russia, were evaluated for plant height and flowering date in a field trial located in the Eastern part of Croatia.

DNA extraction, at three leaves phase, was carried out by the CTAB method. The average plant height was 88.92 cm, it ranged from 62 to 159.32 cm, with CV of 19.23 %. Days to heading ranged from 142 to 119,with CV of 4.06 %. Our results indicated that Rht-B1b, Rht8 (192bp) and Ppd-D1a alleles dominated with frequencies of 62 %, 68 %, and 72 % respectively, followed by Rht-D1a, Rht-D1b and Ppd-D1b alleles. Seventeen cultivars with Rht-B1a and Rht-D1a had plant height  $\geq$  98 cm, while 25 cultivars carrying dwarf alleles had plant height from 62 to 96 cm. Among Croatian cultivars, Vrn-A1a allele was detected in only one cultivar, Vrn-A1c in three cultivars, while vrn-A1 dominated with 92 %. Prevalence of vrn-B1 and vrn-D1 was recorded with 95 % and 100 %.

Keywords: wheat, functional marker, adaptability

*Acknowledgments:* Published in the Book of Abstracts (p. 155) of the 20th EUCARPIA General Congress, 29 Aug – 1 Sep 2016, Zurich, Switzerland. Research is a part of the Research Installation Project PHENOWHEAT (No.2000) financed by the Croatian Science Foundation.

#### Sugar beet root yield and quality in relation to different planting densities Prinos i kvaliteta šećerne repe u različitoj gustoći sjetve

I. Varga<sup>1</sup>, M. Antunović<sup>1</sup>, Z. Lončarić<sup>1</sup>, M. Pospišil<sup>2</sup>, A. Kristek<sup>1</sup>

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During 2014 and 2015 growing seasons, the sesonal dynamics of sugar beet leaf growth,root yield and quality were analyzed. The hybrid Serenade, KWS, was sown on March 18th 2014 and March 25th 2015 at inter-row spacing of 50 cm and four different intra-row spacings: 13, 15, 17 and 19 cm (60 000, 80 000, 100 000 and 140 000 plants ha<sup>-1</sup>). In 2014, the mean air temperature in vegetation was not very different when compared to the long-term mean (LTM), but the total rainfall was 24% higher. In 2015, there was 14% less rainfall from March to October when compared to the LTM. The average sugar beet root yield in 2014 was 98.9 t ha<sup>-1</sup> and sucrose content 13.15 %, whereas in 2015 the root yield was smaller, on average 70.9 t ha<sup>-1</sup> with the average sucrose content of 15.50 %. In relation to

plant density, in 2014 the highest root and white sugar yield was foundat 140 000 plants ha<sup>-1</sup>, whereas the lowest yield was at 60 000 plants ha<sup>-1</sup>. Different spring nitrogen fertilization did not have significant influence on the root and white sugar yield, but significant differences were found in the root quality. In 2015, the highest root yield was at 100 000 plants ha<sup>-1</sup> and the lowest at 60 000 plants ha<sup>-1</sup>. Different plant density significantly influenced brei impurities in 2014, whereas in 2015, significant influence was not found.

Keywords: sugar beet, plant population, yield, quality

#### Influence of pumpkin seed cake and extruded linseed on milk production and milk fatty acid profile in Alpine goats

#### Utjecaj pogače sjemenki bundeve i ekstrudiranog lana na proizvodnju i masnokiselinski profil mlijeka alpskih koza

#### <u>Z. Klir<sup>1,\*</sup></u>, J. M. Castro-Montoya<sup>2</sup>, J. Novoselec<sup>1</sup>, J. Molkentin<sup>3</sup>, M. Domacinovic<sup>1</sup>, B. Mioc<sup>4</sup>, U. Dickhoefer<sup>2</sup>, Z. Antunovic<sup>1</sup>

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Pumpkin seed cake (PSC) is a by-product with a potential to be used for ruminants' feeding, with a higher crude protein concentration than soybean meal whose effects on goats' milk yield and composition have not been studied. Similarly, extruded linseed (ELS) is one of the most common by-products available and already adopted for the enrichment of dairy products with beneficial fatty acids. Thus, the aim of this study was to investigateif PSC inclusion in the diets of dairy goats influences yield, composition and fatty acids profile of their milk as compared with a conventional diet containing soybean meal and a diet where soybean meal has already been replaced with ELS as a protein source. Goats were fed with mixtures containing soybean meal (control),

ELS or PSC in the trial lasting for 75 days. The addition of ELS or PSC did not influence milk yield and milk composition, compared with the control. The supplementation of ELS resulted in higher proportions of branched-chain fatty acids (BCFA) and a total n-3 fatty acids, compared with the control and PSC. Total n-3 fatty acids were accompanied by increased  $\alpha$ -linolenic acid (ALA, C18:3n-3; 0.56 g/100 g fatty acids) and eicosapentaenoic acid (EPA, C20:5n-3; 0.12 g/100 g fatty acids) proportions in milk of the ELS group. The PSC diet decreased total n-6 fatty acids compared with the control. Oleic acid (c9-C18:1), CLA (c9,t11-18:2) and t11-C18:1 did not differ between treatments, although stearic acid (C18:0) increased in ELS diets compared with the control. Partially substituted soybean meal with ELS may increase beneficial n-3 fatty acids, BCFA and C18:0. Pumpkin seed cake completely substituted soybean meal in the diet of dairy goats without any decrease in the milk production or sharp changes in fatty acid profile that may have a commercial or a human health relevancy.

Keywords: fatty acid profile, extruded linseed, pumpkin seed cake, goat milk

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#### Genetska autentifikacija mesa autohtone pasmine svinja primjenom mikrosatelitskih markera

#### Genetic authentication of meat from autochthonous pig breed by the use of microsatellite markers

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The Regulation EC No 178/2002, also "General Food Law", known as describes the traceability as "the ability to trace and follow a food, feed, food producing animal or ingredients, through all stages of production and distribution". All European Union member countries have developed some of the traceability systems depending on the manner in which they obtain information. The aim of this study was to evaluate a set of 26 microsatellite markers for the genetic identification of

the pig breed Crna Slavonska, and subsequently to minimize the number of loci needed for the successful authentication of meat originating from this breed. Based on the match probability value, a set of five microsatellite markers was created which demonstrated high sensitivity in the identification of Crna Slavonska breed. When the number of microsatellite markers was pooled, the match probability value within Crna slavonska pig breed increased. The reduction of microsatellite markers decreases the cost of the genetic analysis used for the breed identification and the time needed for the product authentication. A practical consequence of the present study is the introduction of a complementarity system for the conventional traceability of pork based on product labelling such as PDO/PGI. This system could be applied when more confident identification is needed, for example in legal issues of product liability, such as adulteration by other, undeclared meat. In order to establish a method for breed identification in sausages and similar products that may originate from higher number of animals in the batch, further investigations are suggested.

Keywords: autochthonous pigs, pork traceability, microsatellite markers, match probability

*Acknowledgments:* This work has been fully supported by the Croatian Science Foundation under project number 3396.

## Positive effect of omega-3 enriched egg consumption on reducing oxidative stress level in young healthy women

#### Pozitivan utjecaj konzumacije omega-3 obogaćenih jaja na smanjenje razina oksidativnog stresa kod mladih zdravih ženskih ispitanica

N. Bilić-Dujmušić<sup>1</sup>, A. Matić (ex. Ćosić)<sup>1</sup>, R. Vuković<sup>2</sup>, A. Stupin<sup>1</sup>, L. Rašić<sup>1</sup>, G. Kralik<sup>3</sup>, Z. Kralik<sup>3</sup>, I. Drenjančević<sup>1\*</sup>

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<u>AIM</u>: We have previously demonstrated that omega-3 enriched eggs reduced the levels of oxidative stress in young healthy men (Rašić et al. ESH 2015). The present study was aimed to determine the effect of omega-3 enriched egg consumption on the systemic oxidative stress level and blood antioxidant enzymes' activity in young healthy women. <u>MATERIALS AND</u> <u>METHODS</u>: 14 healthy women (age: 20-26) were included in the protocol. Half of them consumed omega-3 enriched chicken eggs (OE), and other half consumed regular chicken eggs (RE) (both groups: 2 eggs per day/21 days). Omega-3 content: OE: 403,10 mg/day; RE: 74,63 mg/day. Ferric reducing ability of

plasma (FRAP), Thiobarbituric Acid Reactive Substances (TBARS), antioxidative enzymes' glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity were determined from serum samples, before and at the end of the protocol. To test differences among groups, Student t-test was used, and intra-group differences were tested by a paired t-test; p<0.05 was considered significant. <u>RESULTS</u>: No significant changes of TBARS, FRAP and enzymes' activity were detected in RE group after the protocol. In OE group, TBARS was significantly decreased, and FRAP was not affected by the omega-3 egg consumption. GPx activity significantly increased, while SOD activity was unaffected by a dietary protocol in OE group. Initial values of TBARS, FRAP and antioxidative enzymes' activity. However, GPx activity was significantly increased in OE after dietary protocol, compared to RE group. <u>CONCLUSION</u>: These results suggest the positive effect of omega-3 fatty acids on reducing systemic oxidative stress in young healthy women due to the increased blood antioxidative activity.

#### Keywords: oxidative stress, antioxidative activity, omega-3 eggs

*Acknowledgments:* The study protocol and procedures conformed to the standards of the Declaration of Helsinki and were approved by the Ethical Committee of the Faculty of Medicine, University of Osijek.

#### Nitrate removal from aqueous solution by modified biomass Uklanje nitrata iz vodenih medija pomoću modificirane biomase

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Modified hazelnut shells, brewers' spent grain and grape seeds, all lignocellulosic food industry by-products, were evaluated as lowcost sorbents for nitrate removal from aqueous solutions in batch and column studies. Characterisation of the materials was performed in terms of elemental analysis (C, H, O, N), FTIR and SEM. The effects of contact time (2 – 1440 min), initial nitrate concentration (10 – 300 mg L<sup>-1</sup>), sorbent concentration (1 – 10 g L<sup>-1</sup>) and pH (2 – 10) were investigated. Removal efficiencies over 90 % were achieved. Maximum adsorption capacities ranged from 22 to 25 mg g<sup>-1</sup>, which is in accordance with the literature. It was shown that the adsorption data

could be fitted in the linearized Freundlich and Langmuir model. Additionally, the sorption was very rapid and was well described by the pseudo-second order model, while intraparticle diffusion model suggests at least two steps during the adsorption process. Column experiments showed that these materials maintained nitrate removal ability during minimum three adsorption and desorption cycles. The column could be effectively regenerated with 0.1 M NaCl. The future aim is to study the adsorption process of other pollutants on different biomass.

Keywords: nitrate, biomass, sorption, column study

#### Biotransformation of grape pomace with *Ceriporiopsis subvermispora* Biotransformacije tropa grožđa s *Ceriporiopsis subvermispora*

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Grape pomace is a lignocellulosic-type of waste originating from winemaking process. It primarily consists of the skin, pulp, seeds and stems, and presents a source of polyphenolic compounds that are entrapped within the lignocellulosic structure. Due to the complex enzymatic system, filamentous fungi have a great potential for biotransformation of lignocellulose into value-added products during solid-state fermentation (SSF).

The aim of this study was to investigate the influence of a biological treatment of grape pomace with white-rot fungus *Ceriporiopsis* subvermispora on the production enhancement of a variety of polyphenolic compounds . SSF

was performed forfifteen days. Nineteen different phenolic compounds, such as hydroxybenzoic and hydroxycinnamic acides derivates, flavan-3-ols, flavonols, procyanidins dimers and stilbene were analysed before, during and after the biological treatment by the gradient UHPLC method. The enhanced level of phenolic acids was observed after first two days of a biological treatment. The initial content of gallic acid (GA), syringic acid (SA), vanillic acid (VA) and *p*-coumaric acid (*p*-CA) prior to biological treatment was 199.9  $\mu$ g g<sub>db</sub><sup>-1</sup>, 79.0  $\mu$ g g<sub>db</sub><sup>-1</sup>, 34.4  $\mu$ g g<sub>db</sub><sup>-1</sup> and 4.5  $\mu$ g g<sub>db</sub><sup>-1</sup>, respectively. After the biological treatment, the content of GA, SA, VA and *p*-CA was higher, up to 1.37-, 1.73-, 1.54-, and 1.84-fold, respectively.

Keywords: grape pomace, solid-state fermentation, Ceriporiopsis subvermispora, polyphenolic compounds

#### Green extraction techniques of bioactive components from cocoa shell Zelene tehnike ekstrakcije bioaktivnih komponenata iz kakao ljuske

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The food industry waste is often consisted from unedible parts, so-called by-products. Today, there are huge quantities of by-products that are discarded causing enormous economic problems by polluting the environment. The cocoa shell, as a by-product of the cocoa production industry, has also been discarded despite the fact that it contains some valuable bioactive components. Theobromine, caffeine and certain phenolic components migrate from cocoa bean to the shell, making it desirable raw material with a potential to be used in further processing. Apart from being eco-friendly, green extraction techniques are increasingly applied due to their simplicity and opportunity to preserve sensitive components

found in extracts. The aim of this study was to demonstrate how certain a type of extraction technique can give extracts with various bioactive components in different concentrations. Four green extraction techniques were used in this study: supercritical  $CO_2$  extraction, ultrasonic assisted extraction, cold atmospheric plasma assisted extraction and extraction using deep eutectic solvents. Those modern techniques gave better yields of bioactive components and showed better antioxidant activity of the obtained extracts than classical Soxhlet extraction. The bioactive components in the obtained extracts were quantified by High Performance Liquid Chromatography. Supercritical  $CO_2$  extraction gave higher yields for theobromine content, while ultrasonic assisted extraction with 50 % aqueous ethanol solution gave the highest results for caffeine content and antioxidative activity. The extraction with a mixture of choline chloride: oxalic acid and 50 % water gave at room temperature the highest total phenolic content. Cocoa shell, due to its nutritional value and bioactive components, has a potential to become a desirable raw material in a large spectrum of functional products and pharmaceutical products.

#### Keywords: by-product, cocoa shell, extraction, bioactive compounds

Acknowledgments: This work has been supported in part by the Croatian Science Foundation under the project "Application of innovative techniques of the extraction of bioactive components from by-products of plant origin" (UIP-2017-05-9909)

#### GC-MS and LC-MS profiles of the supercritical fluid extracts of black poplar (*Populus nigra* L.) buds Određivanje sastava superkritičnih CO<sub>2</sub> ekstrakata pupoljaka crne topole (*Populus nigra* L.) primjenom GC-MS i LC-MS tehnika

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The buds of black poplar (*Populus nigra* L.) are used in traditional medicine due to their antibacterial, antifungal, anti-inflammatory and antioxidant activities. These activities are attributed to both volatile and non-volatile compounds, so it is important to use adequate methods to extract these component groups. The method that could allow an efficient extraction of compounds without leaving traces of toxic solvents is SC-CO<sub>2</sub>, resulting in an extract that can be used for medical purposes.

Therefore, the black poplar buds were extracted using supercritical  $CO_2$  (SC- $CO_2$ ) under various conditions (pressure and temperature), and optimized based on the sum of the major, relevant

volatile and non-volatile compounds. The best extraction conditions at which the highest amount of both volatile and non-volatile components was extracted were 300 bar and 60 °C. The composition of the obtained extracts was determined by GC-MS and UHPLC-DAD-QqTOF-MS. The volatile profile of SC-CO<sub>2</sub> extracts was compared with the composition of essential oil (EO) obtained by hydrodistillation. The percentages of the major volatile compounds in the optimized SC-CO<sub>2</sub> extracts were: pinostrobin chalcone (20.8 %),  $\beta$ -eudesmol (9.5 %),  $\alpha$ -eudesmol (9.2 %), 2-methyl-2-butenyl p-coumarate (7.9 %), tricosane (6.3 %), pentyl-p-coumarate (6.1 %),  $\gamma$ -eudesmol (4.9 %), benzyl salicylate (4.7 %), 3-methyl-2-butenyl-benzoate (4.0%). In contrast,  $\beta$ -Eudesmol (10.9 %),  $\alpha$ -eudesmol (10.4 %), guaiol (5.7 %), 3-methyl-2-butenyl-benzoate (5.5 %),  $\gamma$ -eudesmol (5.5 %),  $\gamma$ -curcumene (4.7 %) and bulnesol (4.4 %) dominated in the EO, but providing much lower yield. The major compounds identified in the UHPLC profile of the optimized SC-CO<sub>2</sub> extracts were pinostrobin, pinocembrin, 3-O-pinobanksin acetate and methyl-butenyl p-coumarate (751.7; 485.6; 290.2 and 144.9 mg/100g of buds, respectively). The SC-CO<sub>2</sub> extraction under the optimized conditions was found to be useful for the efficient and simultaneous extraction of both volatile and non-volatile, bioactive compounds of poplar buds.

Keywords: Populus nigra L., sesquiterpenes, phenolics, supercritical  $CO_2$  extraction, phytochemical profiles

#### Application of matrix population models in biology Primjena matričnih populacijskih modela u biologiji

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Main reasons for the investigation of population dynamics are understanding, predicting and describing population growth, estimating population extinction risks, and improving control methods. For vectors, such as mosquitoes, studying population dynamics can be useful for other reasons, such as combining population models with the epidemiological models or predictions, as well as for the planning and optimisation of control methods. Matrix population models have long been used in modelling and describing the dynamics of different populations. These types of discrete mathematical models enable modelling of age-structured populations or

populations structured by developmental stages, i.e. predicting the number of individuals of a certain age or developmental stage after a selected time interval. In this study, matrix population models were constructed for two mosquito species, *Aedes vexans* and *Culex pipiens*, and also for three different ecological categories of earthworms, epigeic, endogeic and anecic. Using constructed models, it is possible to investigate population dynamics of modelled species and perform various simulations that enable the determination of population responses to environmental stress or climate change; to investigate and optimise control measures; to detect the most vulnerable developmental stages. Both population models, mosquito and earthworm, are constructed entirely based on the available literature and experimental data. Models are also forced by environmental factors relevant for modelled species (mosquito models: air temperature, rainfall, Danube and Drava water levels, photoperiod; earthworm models: soil temperature and soil humidity). The main aim of this study is to present various possibilities of mathematical models and their application in biology and ecology.

Keywords: matrix population models, mosquitoes, earthworms, climate change, population dynamics

#### Zooplankton taxonomy, trophic structure and food web interactions Taksonomija i trofička struktura zooplanktona te interakcije unutar hranidbene mreže

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Zooplankton are an essential component in inland waters, as they represent a link between primary producers and higher consumers. The aims of the study were to determine the influence habitat heterogeneity on community of assemblage, to detect grazing potentials of specific feeding guilds, and to investigate several food-web interactions. Zooplankton samplings are generally conducted on a monthly basis since 2006 and ongoing in different areas of the Kopački rit Nature Park. Each sampling occasion included measurements of several limnological variables as well: water temperature, dissolved oxygen, conductivity, pH, transparency, ammonia, nitrite, nitrate,

organic nitrogen, total nitrogen, total phosphate, and chlorophyll *a*. So far, 124 species of Rotifera, Cladocera and Copepoda were determined in the area of Kopački rit, with the discovery of two new species in the territory of Croatia. The results show that heterogeneous environments under variable hydrological conditions in floodplain areas support the development of different zooplankton assemblages that express within-group dissimilarities. During the isolation of the water bodies, algivorous rotifer species co-dominate with bacterivores and predators, suggesting an increased biodiversity and food web interactions during undisturbed conditions. In contrast, throughout the inundation period, trophic structure and grazing potential change, with algivorous being the dominant feeding guild. Variable grazing intensity serves as a good indicator of a shift in the trophic state of the water bodies and points to cascades in other components of the food web. Vertical layering also influences the strength of the interactions among the planktonic food web constituents. The transfer of organic matter to higher trophic levels at the surface water layer is dominated by herbivory, while at the bottom water layer the main route flows through bacterivory and omnivory. Overall results show the importance of hydrology in reshaping zooplankton assemblage and the usefulness of appropriate zooplankton groups as biological markers in natural habitats.

Keywords: Rotifera, Cladocera, Copepoda, floodplain, Danube River

#### Structure and development of periphytic ciliate community in a Danube floodplain lake Struktura i razvoj zajednice trepetljikaša perifitona u jezeru poplavnog područja Dunava

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is a complex community Periphyton of autotrophic and heterotrophic organisms, detritus, and mineral particles present in various substrata in aquatic environments. Studies on this type of communities are fundamental since they play an important ecological role inside the aquatic ecosystems. Ciliates, a remarkably successful group of microorganisms existing in almost all freshwater ecosystems, are important constituents of periphytic communities, as one of the main consumers of bacteria, algae, other protozoans and detritus, thus participating in biomass transfer to higher trophic levels. Considering the lack of information on ciliated protozoans in a temperate floodplain periphyton,

we studied the colonization and succession dynamics of these microorganisms on artificial substrata (glass slides) exposed in Lake Sakadaš (Danubian floodplain) during 2010 in two experimental series, from spring until winter and from summer until winter. Floods, different in their extent and duration, strongly influenced the lake conditions during the study period, consequently affecting the composition and growth dynamics of the ciliate community. Colonization and succession of ciliates in periphyton occurred through several phases, different in the structural and functional composition of ciliates and influenced by diverse environmental factors. The results showed that periphyton formation and ciliate colonization are more rapid during the summer period than in spring, and that the ciliate community becomes stable earlier under summer conditions. Ciliates reached a rather high abundance and diversity in periphyton formed on artificial substrata in a floodplain lake, with sessile peritrich species as dominant. The trophic structure of periphytic ciliates was characterized by the bacterivorous and bacterivorous filter-feeding species, important for carbon and energy transfer from the planktonic to the periphytic food web. This research contributed to a better understanding of taxonomic and functional diversity of periphytic ciliates and clarified their ecological role in riverine floodplain ecosystems.

Keywords: Ciliophora, growth dynamics, artificial substrata, functional groups, hydrology

# Physiological response of *Lemna gibba* L. when exposed to ionic colloidal silver Fiziološki odgovor vrste *Lemna gibba* L. na djelovanje ionskog koloidnog srebra

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Duckweeds are widely distributed aquatic vascular plants with many advantages as test organisms. Due to their small size and simple structure, they are easily cultivated in laboratory conditions. Lemna plants are representatives of primary producers in several standardised protocols (ISO, OCED, and US EPA) for determining phytotoxicity of different environmental chemicals in aquatic ecosystems. Silver is a non-essential element for living organisms and one of the most toxic metals in aquatic ecosystems. It is now recognised that silver concentration in the biosphere is increasing due to anthropological activities, such

as photographic industry, electronics and electrical applications, and nowadays, nanotechnology. The aim of this study was to determine the physiological changes in duckweed *Lemna gibba* L. induced by a treatment with ionic colloidal silver in full strength Pirson-Seidel's nutrient solution and in a nutrient solution with decreased nitrogen and phosphorus concentrations. Silver accumulation in plants disrupted nutrient balance, caused damage to the photosynthetic apparatus, oxidative stress and growth inhibition. Oxidative stress, evident from the increased accumulation of hydrogen peroxide and lipid peroxidation end-products, indicated an insufficient antioxidative response of *L. gibba* plants. The intensity of oxidative stress depended on the concentrations were lower, silver was toxic to *L. gibba* plants in nutrient solutions with decreased nitrogen and phosphorus concentrations. The observed physiological changes in *L. gibba* plants were the result of silver toxicity, since decreasing the nutrient concentrations to 50 % and 25 % in the medium did not cause significant oxidative stress. In plants treated with ionic colloidal silver in the nutrient solution with 10 % of nitrogen and phosphorus, the combined effect of metal toxicity and decreased nutrient concentration was evident.

Keywords: model organism, silver, nutrient, oxidative stress, synergistic effect

#### Macular thickness changes in diabetic patients after uncomplicated phacoemulsification cataract surgery Promjene debljine makule u dijabetičara nakon nekomplicirane operacije mrene metodom ultrazvučne fakoemulzifikacije

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The purpose of this study was to analyse macular thickness changes after uncomplicated phacoemulsification cataract surgery in the early postoperative period in diabetic patients, using optical coherence tomography (SD-OCT). This prospective study included 55 patients divided in two groups. All patients underwent uncomplicated phacoemulsification cataract surgery at the Department of Ophthalmology, University Hospital Centre Osijek, in the period from October of 2017 to January of 2018. The patients were followed up 1 month after cataract surgery. Full ophthalmological examination was performed on all the patients, the best corrected visual acuity

(BCVA) using the Snellen chart was measured, and they all underwent SD-OCT macular thickness measuring of the eye planned for surgery. The first control follow-up was performed one week after and the second one month after cataract surgery. At each control follow-up, BCVA was determined, a complete ophthalmological examination was performed, and an SD-OCT measuring of the macular thickness of the operated eye was recorded. With this prospective study, we wanted to investigate the effect of uncomplicated phacoemulsification cataract surgery on macular thickness changes in the group of diabetic patients and a control group of patients in the early postoperative period. There was a statistically significant difference in macular thickness before surgery and during the second control follow-up in the group of diabetic patients ((p = 0.0045137)) and between the first and the second control in diabetic group patients ((p=0.0069024)). There was also a statistically significant difference in macular thickness in the control group of patients before surgery and duringthe second control followup  $((p = 5.2657 \times 10^{-4}))$  and between the first and the second control follow-up ((p=0.01285)). Uncomplicated phacoemulsification cataract surgery causes macular thickness changes in the early postoperative period in both groups of patients. Statistically significant differences in macular thickness were observed one month after the surgery, compared to preoperative macular thickness and compared to macular thickness seven days after the surgery, in both groups of patients.

Keywords: macular configuration, phacoemulsification, diabetes, postoperative period, SD-OCT

#### Desmuramyl peptide derivatives – synthesis and adjuvant activity Derivati desmuramil peptida – sinteza i adjuvantska aktivnost

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Muropeptides, also called peptidoglycans, are fragments of unique polymers that build up the cell wall of bacteria. Muramyl dipeptide (MDP), N-acetylmuramyl-L-alanyl-D-isoglutamine, is the smallest structural unit of peptidoglycans showing the immunostimulating (adjuvant) activity. Dipeptide core of MDP is the essential structure required for adjuvant activity, therefore, MDP analogues lacking the hydrophilic carbohydrate moiety, desmuramyl peptides, are explored. Numerous derivatives with different groups at C- and N-terminus of the L-Ala-DisoGln moiety are known. We have previously prepared desmuramyl peptides containing bulky and lipophilic adamantane attached to the L-Ala-

D-*iso*Gln which showed significant immunomodulating activity *in vivo* in the mouse model. Furthermore, the influence of mannosylation on the immunostimulating activity of adamantyl desmuramyl peptides was investigated. The obtained results indicate that attachment of mannose on the adamantly tripeptide increases the activity of the parent compound, probably because of the binding of mannosylated peptides to mannose receptors present on immunocompentent cells (such as macrophages and dendritic cells), which are considered to be pattern-recognition receptors. They are responsible for the binding of mannosylated antigens or other relevant biologically active molecules containing mannose, thus affecting the immune reactions. Our current research includes developing methodology for the stereoselective synthesis of di- and tri-antennary mannose derivatives of desmuramyl peptides. We believe that the increase of the number of mannose subunits will enhance the strength of overall mannose – receptor interactions. This research will contribute to better understanding of adjuvant activity of mannosylated desmuramyl peptides and development of more effective vaccine formulations, such as liposome formulations, which can be prepared by the insertion of the adamantane group to the membrane lipid bilayer.

*Keywords: peptidoglycan, desmuramyl dipeptide, adamantane, D-mannose, immunostimulating activity, peptide synthesis* 

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#### Synthesis of heterocyclic compounds with biological activity in deep eutectic solvents

### Sinteza heterocikličkih spojeva s biološkom aktivnošću u eutektičkim otapalima

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Deep eutectic solvents (DESs) are novel generation of green solvents which have proven to be a convenient media for many synthetic routes. They are often characterized as environmentally friendly and sustainable mixtures of nontoxic and cheap components which are combined together from a eutectic mixture. Hydrogen bond acceptors (HBAs; quaternary ammonium or metal salt) are often combined with hydrogen bond donors (HBDs; amides, carboxylic acids, alcohols and sugars) in different ratios to obtain DES with desirable physical and chemical characteristics. Hereby, we describe our achievements in the field of

heterocycle synthesis in DESs. So far, we have found DESs to be very effective in synthesis of novel coumarinyl Schiff bases, Knoevenagel condensation of rhodanine with aromatic aldehydes, synthesis of thiosemicarbazides, and triazoles from isothiocyanates and coumarinyl hydrazide, synthesis of 2-mercaptoquinazolinones from anthranilic acid and isothiocyanates. At present, a screening of 20 different deep eutectic solvents has been carried out to determine a convenient DES and temperature for the synthesis of quinazoline derivatives from anthranilic or substituted anthranilic acid.

Keywords: deep eutectic solvents, Knoevenagel condensation, rhodanine, quinazolinone

#### Mechanism of action of newly synthetized *N*-9-sulfonylpurine derivatives Mehanizam djelovanja novosintetiziranih *N*-9-sulfonilpurinskih derivata

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Two new N-9-sulfonylpurine derivatives (SPD 1 and SPD12) containing nucleobase and sulfonamide pharmacophores were designed to induce cell death in tumour cells by interacting with cellular molecules and organelles. Flow cytometry was used to determine the effect of the tested compounds on the cell cycle, apoptosis, mitochondrial potential, and the accumulation of ROS in the treated cells as well. Selected compounds caused the accumulation of cells, mostly in the subG0 phase of the cell cycle and the induction of apoptosis in almost 55 % of the treated cells.

Furthermore, SPD1 and SPD12 induced increased accumulation of ROS and mitochondrial disruption in more than 80% of cervical adenocarcinoma (HeLa) and Burkitt lymphoma (Raji) cells. The mechanism of action of the selected derivatives is achieved by the promotion of apoptosis and accompanied with impaired mitochondrial potential, as well as increased ROS accumulation. Purine derivatives with the styrylsulfonyl group are good candidates for further investigation of antitumor effects *in vivo*.

Keywords: sulfonylpurine derivatives, apoptosis, ROS.

#### An equilibrium study of flavan-3-ols adsorption onto β-glucan Ravnotežna istraživanja adsorpcije flavan-3-ola na β-glukanu

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Flavan-3-ols belong to the group of polyphenolic compounds which showed numerous potentially positive bioactivities. One of the polyphenol bioactivities which is completely not investigated is the interaction of polyphenol with food compounds such as lipids, proteins, and dietary fibres. To obtain more information about these interactions, they can be studied through the adsorption process to the equilibrium state at constant temperature. The aim of this study was to conduct adsorption between procyanidin B1 and procyanidin B2 and  $\beta$ -glucan at different temperatures (25 °C, 37 °C, 45 °C, at pH 5.5) and different pH values (pH 1.5 and 10.0, at 37 °C). The non-linear and linear adsorption models

like Freundlich, Langmuir, Dubinin-Radushkevich, Temkin and Hill were applied on the results of adsorption processes like  $c_{e_i}$  which is the polyphenol concentration in the solution at equilibrium (mol/L) and the  $q_e$  which is the amount of polyphenols adsorbed per g of  $\beta$ -glucan at equilibrium (mol/g), and the constants of these models were determined. Also, the thermodynamic parameters, like Gibbs energy change  $\Delta G$ , standard enthalpy change  $\Delta H$  and standard entropy change  $\Delta S$ , were also determined. Kinetic models of pseudo-first and pseudo-second order were also determined. The results showed that the adsorption of flavan-3-ols onto  $\beta$ -glucan was favourable, and higher for procyanidin B1. Also, the adsorption was best described by the pseudo-second order model.

Keywords: adsorption models, flavan-3-ols,  $\beta$ -glucan, kinetic, thermodynamic

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#### Modified aqueous sol-gel route towards quaternary metal oxides containing tungsten

#### Modificirana vodena sol-gel metoda za sintezu kvaternarnih metalnih oksida na bazi volframa

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In modern times of technological development, there is a need for reducing the size and the density of data storage expanding components. Ferroelectricity and ferromagnetism are essential for achieving multifunctional properties. Complex metal oxides with the double perovskite type crystal structure Sr<sub>3</sub>Fe<sub>2</sub>WO<sub>9</sub> and Ba<sub>3</sub>Fe<sub>2</sub>WO<sub>9</sub> have been synthesized using a modified citrate sol-gel route. Dried samples were calcined at 600 °C and 950 °C in a furnace with air flow and at a temperature rate of 2 °C per minute. The calcined samples were characterized by Powder X-ray Diffraction (PXRD), Energy-Dispersive X-ray Spectroscopy (EDS), Transmission

Electron Microscopy (TEM), and Scanning Electron Microscopy (SEM), while magnetic ground state has been determined using SQUID measurement. At room temperature, the crystal structure of  $Sr_3Fe_2WO_9$  is cubic, space group Pm-3m (221) with the a = 3.9440 Å, whereas  $Ba_3Fe_2WO_9$  crystallizes in hexagonal crystal system with space group P63/mmc (194), a = 5.7593 Å and c = 14.1096 Å. The obtained results have revealed that synthesized materials are 96 wt. % pure and their average crystallite size has remained in the nanometre range, ranging from 15 to 27 nm despite the high calcination temperatures. Magnetic measurements have revealed that these materials possess ferrimagnetic properties.

Keywords: perovskite structure, sol-gel, ferroics, multiferroics, citrate route

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## Functionalized carbon nanotubes as a new sensing material for cationic surfactant determination

#### Funkcionalizirane ugljikove nanocjevčice kao novi senzorski materijal za određivanje kationskih tenzida

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Cationic surfactants (CATs) are used in a wide range of products for cosmetic, industrial, agricultural, and household applications, but they also have ecotoxic properties and because of that their determination is of great importance.

A new potentiometric sensor, sensitive to CATs, was prepared, containing multiwalled carbon nanotubes (MWCNTs) chemically modified with a sulphate group and a cetylpyridinium ion (CP) embedded in a plasticized PVC membrane. Graphite of spectroscopic grade was employed as a conducting substrate. The chemical modification of MWCNTs with CP cations significantly improved the most important analytical and practical sensor properties. The new sensor

showed a Nernstian response for CP (58.9 and 58.2 mV/decade in water and 0.01 M Na2SO4, respectively) and sub-Nernstian responses for the hexadecyltrimethylammonium ion (CTA) (56.6 and 55.5 mV/decade in water and 0.01 M Na2SO4, respectively) and Hyamine (52.1 and 49.7 mV/decade in water and 0.01 M Na2SO4, respectively). The detection limit for CP was  $1.2 \cdot 10^{-7}$  M (in water and 0.01 M Na2SO4), whereas those for CTA were  $2.5 \cdot 10^{-7}$  and  $3.2 \cdot 10^{-7}$  M (in water and 0.01 M Na2SO4, respectively), and that for Hyamine was  $1.5 \cdot 10^{-6}$  (in water and in 0.01 M Na2SO4). The sensor showed good selectivity for CP cations, compared to the inorganic and organic cations commonly present in commercial formulations.

Keywords: functionalized carbon nanotubes, cationic surfactants, potentiometric titration

#### Characterization of chlorogenic acids in different coffee varieties using electrochemical methods

#### Karakterizacija klorogenskih kiselina u različitim vrstama kave primjenom elektrokemijskih metoda

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The characterization of chlorogenic acids (CGAs) in different brands of coffee (green and roasted beans C. arabica and C. robusta, ground coffee Franck Guatemala and Flatcher Olympia, and instant coffee Nescafé Classic, Nescafé Espresso, Jacobs Monarch and Jacobs Intense) was investigated. Before the characterization, the electrochemical properties of the caffeoylquinic acids (CQAs), dicaffeoylquinic acids (diCQAs) and feruloylquinic acids (FQAs) were analysed usingsquare-wave voltammetry (SWV), differential pulse voltammetry (DPV) and flow through stripping chronopotentiometry (FTSCP). The electrochemical measurements of the CGAs

have shown that the different electrochemical reactions *via* different mechanisms of oxidation proceeded on the surface of the working electrode, in connection with the chemical structure of the CGAs. The characterization of the CGAs was performed using SWV, DPV, FTSCP and HPLC methods. The identification and quantification of the CGAs in different brands of coffee were performed usingthe SWV, DPV, FTSCP and HPLC methods. The results have shown that the highest total content of CGAs exists in the green coffee beans.

Keywords: chlorogenic acids, coffee, square-wave voltammetry, differential pulse voltammetry, flow through stripping chronopotentiometry

### **AUTHOR INDEX**

A Antunović, Zvonko, 5 Antunović, Manda, 4

#### B

Barać, Josip, 16 Bernardi-Opačak, Teuta, 19 Bijelić, Jelena, 21 Bijelić, Mirjana, 21 Bilić-Dujmušić, Nikolina, 7 Bucić-Kojić, Ana, 9

С

Castro-Montoya, Joaquín Miguel, 5

#### D

Dickhoefer, Uta, 5 Djerdj, Igor, 21 Djurkin Kušec, Ivona, 6 Domaćinović, Matija, 5 Drenjančević, Ines, 7

#### DŽ

Džijan, Snježana, 6

#### G

Galir Balkić, Anita, 13 Garnweitner, Georg, 21 Glavaš-Obrovac, Ljubica, 19 Guberac, Sunčica, 3 Gvozdanović, Kristina, 6

H Habuda-Stanić, Mirna, 8 Hackenberger Kutuzović Branimir, 12 Hackenberger Kutuzović, Davorka, 12 Hajduković, Mateja, 22 Horvatić, Janja, 15

#### I

Iljkić, Dario, 1

#### J

Jagličić, Zvonko, 21 Jakobek Barron, Lidija, 20, 23 Jakovljević, Martina, 10, 11 Jerković, Igor, 11 Jokić, Stela, 10, 11 Jukić, Marijana, 19

#### K

Klir, Željka, 5 Komar, Mario, 18 Kovačević, Vlado, 1 Kralik, Gordana, 7 Kralik, Zlata, 7 Kristek, Andrija, 4 Kuś, Piotr, 11 Kušec, Goran, 6

#### L

Lončarić, Željka, 12 Lončarić, Zdenko, 2, 4

#### Μ

Margeta, Vladimir, 6 Marković, Berislav, Matasović, Brunislav, 21 Matić (ex. Ćosić), Anita, 7 Matić, Josipa, 19 Matić, Petra, 20 Matić, Magdalena, 2 Matić, Suzana, 16 Mioč, Boro, 5 Miškulin, Maja, 10 Molkentin, Jeffery, 5 Molnar, Maja, 11, 18

#### Ν

Novoselec, Josip, 5 Nujić, Marija, 8

#### Р

Paurević, Marija, 17 Pavlović, Nika, 10 Petrović, Sonja, 3 Planinić, Mirela, 9 Pospišil, Milan, 4 Preller, Tobias, 21 Pivić-Kovačević, Ivana, 16

#### R

Rastija, Mirta, 1 Rašić, Lidija, 7 Rebekić, Andrijana, 3 Ribić, Rosana, 17

#### S

Salajpal, Krešimir, 6 Samardžić, Mirela, 22 Skoko, Željko, 21 Stanković, Anamarija, 21 Stupin, Ana, 7 Salinger, Željka, 16

#### Š

Šelo, Gordana, 9 Šeruga, Marijan, 23 Štefanić, Goran, 21 Štolfa Čamagajevac, Ivna, 2, 15

#### Т

Tišma, Marina, 9 Tomac, Ivana, 23 Tomić, Srđanka, 17

#### U Ukić, Šime, 20

#### V

Varga, Martina, 15 Varga, Ivana, 4 Vidaković, Josip, 14 Vila, Sonja, 3 Vlaičević, Barbara, 14 Vuković, Ana, 2 Vuković, Rosemary, 2, 7

#### Z

Zellmer, Sabrina, 21

#### Ž

Žinić, Biserka, 19



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