### Arhiv za higijenu rada i toksikologiju

### **Archives of Industrial Hygiene and Toxicology**

2017;68(Suppl. 1)

### Abstracts of the Food Safety and Quality Congress with international participation

"New Achievements and Future Challenges"

Opatija, Croatia, 21 to 24 November 2017

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Cover page: Opatija

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Andrija Štampar Teaching Institute of Public Health





South East European Network for Food Safety and Quality Control – SEE-FSQC



University of Zagreb, Faculty of Agriculture



University of Zagreb, Faculty of Food Technology and Biotechnology

#### Under the high auspices of:

Kolinda Grabar Kitarović, President of the Republic of Croatia

#### Under the auspices of:

Ministry of Agriculture of the Republic of Croatia

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#### **Contents**

#### ZVONIMIR ŠOSTAR 9 WELCOME ADDRESS

- 10 PROGRAMME
- 17 Abstracts
- 18 Invited speakers
- 21 Oral presentations
- 22 HEALTH SAFETY AND FOOD QUALITY, EFFECTS ON HEALTH, HEALTH PROTECTION AND CONSUMER RIGHTS
- 30 PRODUCTION, STORAGE AND DISTRIBUTION OF FOOD, PACKAGING
- 31 RECENT TRENDS IN FOOD (NOVEL AND RAPID METHODS FOR DETERMINING FOOD QUALITY, INTEGRITY AND SAFETY, "NOVEL FOOD")
- 34 QUALITY MANAGEMENT IN PRODUCTION, STORAGE, AND DISTRIBUTION AS WELL AS IN LABORATORIES AND OTHER CONFORMITY ASSESSMENT BODIES
- 37 FOOD AUTHENTICITY AND INTEGRITY, ECOLOGICAL PRODUCTION AND FOOD FRAUD
- 38 ENVIRONMENTAL IMPACT ON FOOD SAFETY
- 40 RISK COMMUNICATION AND PUBLIC HEALTH
- 42 RISK ASSESSMENT AND RISK MANAGEMENT
- 45 OTHER RELATED TOPICS
- 49 Poster presentations
- 50 HEALTH SAFETY AND FOOD QUALITY, EFFECTS ON HEALTH, HEALTH PROTECTION AND CONSUMER RIGHTS
- 55 PRODUCTION, STORAGE AND DISTRIBUTION OF FOOD, PACKAGING
- 56 RECENT TRENDS IN FOOD (NOVEL AND RAPID METHODS FOR DETERMINING FOOD QUALITY, INTEGRITY AND SAFETY, "NOVEL FOOD")
- 61 QUALITY MANAGEMENT IN PRODUCTION, STORAGE, AND DISTRIBUTION AS WELL AS IN LABORATORIES AND OTHER CONFORMITY ASSESSMENT BODIES
- 63 FOOD AUTHENTICITY AND INTEGRITY, ECOLOGICAL PRODUCTION AND FOOD FRAUD
- 65 ENVIRONMENTAL IMPACT ON FOOD SAFETY
- 68 RISK COMMUNICATION AND PUBLIC HEALTH
- 69 RISK ASSESSMENT AND RISK MANAGEMENT
- 72 OTHER RELATED TOPICS
- 77 Index



Food Safety and Quality Congress with international participation "New Achievements and Future Challenges"

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#### WELCOME ADDRESS

Distinguished colleagues and dear friends,

It is a great honor and pleasure to extend my greetings to you on behalf of the Organizing and the Scientific Committee of the first Croatian Food Quality and Safety Congress with international participation – "New Achievements and Future Challenges", which took place from the 21<sup>st</sup> to the 24<sup>th</sup> of November 2017 in Opatija.

We are exceptionally honored by the interest the Congress has generated in only its first year, as well as the response and support from the European Commission's representatives health and food safety, the European Parliament, the European Food Safety Authority (EFSA), the US Food and Drug Administration's Europe Office, EUROLAB (Lisbon, Portugal), the Ministry of Science and Education, the Ministry of Agriculture, the Ministry of Health of the Republic of Croatia, the Croatian Food Agency, the Croatian Food Processing Competitiveness Cluster, the International Committee for Animal Recording (Rome, Italy), the National Food Agency (Uppsala, Sweden), the Faculty of Chemistry of the University of Warsaw (Poland), the Italian National Institute of Health, and other distinguished participants.

Based on the key assumption that food safety is the shared responsibility of countries, manufacturers, distributors, professionals, but also the consumers themselves, top lecturers and interlocutors spoke about professional and current topics. The Congress' program included topics from the domain of food safety and quality, with an emphasis on its effects on human health. Therefore, the discussions covered, among other things, food contaminants and new trends in their analysis, the protection of origin and geographic origin, organic production, product labeling and identification, environmental impact on food safety and quality, risk assessment and risk management, case studies of good manufacturing practices, and numerous other important and related topics.

In addition to plenary lectures by eminent speakers, the Congress also included oral presentations, online symposia, workshops, and poster sections: participants had the opportunity to meet the authors of the posters and discuss their work, as well as the latest findings in the domain of food safety and quality. Within the Congress, the European Centre for Laboratory Excellence held a seminar entitled "Challenges in Assessing Measurement Uncertainty". In total, there were 6 plenary lectures, 49 oral lectures, and 47 poster presentations from the country and around the world.

This Book of Abstracts is a supplement to the scientific journal "Archives for Industrial Hygiene and Toxicology", the official journal of the Institute for Medical Research and Occupational Health. We are thankful to the chief editor and the editorial board of the Archives for accepting to publish the invited lecturers' presentation abstracts, as well as other oral and poster presentations from the Congress.

On this occasion, we would also like to thank the President of the Republic of Croatia Kolinda Grabar-Kitarovic for accepting the high patronage, and thank the Ministry of Science and Education, the Ministry of Agriculture, the Ministry of Health, the Croatian Accreditation Agency, the State Office for Standardization and Metrology, as well as the Mayor of the City of Zagreb for accepting the patronage.

We are wholeheartedly thankful to our sponsors, whose support contributed to the organization of this Congress.

Finally, I would like to once again thank all participants for their overall contribution to achieving a common goal; in other words, for providing credible information on food safety and quality, and for providing guidelines and suggestions to improve the food safety and quality system – "from the field to the table".

Sincerely, Zvonimir Šostar, MD President of the Congress

#### KONAČNI PROGRAM / FINAL PROGRAM

#### UTORAK, 21. studenoga 2017. / TUESDAY, November 21, 2017

#### ${\bf SEMINAR\ EUROPSKOG\ CENTRA\ ZA\ LABORATORIJSKU\ IZVRSNOST\ (dvorana\ 1B)}$

EUROPEAN CENTER FOR LABORATORY EXCELLENCE COURSE (Hall B)

14:00 - 15:00	Registracija / Registration		
dvorana 1B / hall 1B			
15:00 - 16:30	IZAZOVI U PROCJENJIVANJU MJERNE NESIGURNOSTI / CHALLENGES IN THE EVALUATION OF MEASUREMENT UNCERTAINTY GAŠLJEVIĆ VIŠNJA		
16:30 - 17:00	Pauza za kavu / Coffee break		
17:00 - 18:30	IZAZOVI U PROCJENJIVANJU MJERNE NESIGURNOSTI – nastavak CHALLENGES IN THE EVALUATION OF MEASUREMENT UNCERTAINTY - cont. GAŠLJEVIĆ VIŠNJA		
19:30	Koktel dobrodošlice u lobbyu hotela Royal / Welcome cocktail in the hotel Royal lobby		

#### SRIJEDA, 22. studenoga 2017. / WEDNESDAY, November 22, 2017

08:00 - 18:15	Registracija / Registration		
dvorana 1A+1B+1C zajedno / hall 1A+1B+1C			
09:00 - 10:00	Svečano otvorenje Kongresa / Opening ceremony		
10:00 - 10:30	<b>ANDRIUKAITIS VYTENIS</b> , Europska komisija, Povjerenik za zdravlje i sigurnost hrane, Bruxelles, Belgija / European Commission, Health & Food Safety Commissioner, Brussels, Belgium		
10:30 - 11:00	Pauza za kavu + izjave za medije / Coffee break + statements for the media		
11:00 - 13:00	Plenarna predavanja / Plenary lectures Voditelj sekcije / Moderator: MISLAV TOGONAL		
11:00 - 11:30	DIFFERENT QUALITY OF SEEMINGLY SAME PRODUCTS IN THE EU <b>BORZAN BILJANA</b> , Europski parlament, Bruxelles, Belgija / Strasbourg, Francuska / European Parliament, Brussels, Belgium / Strasbourg, France		
11:30 - 12:00	THE RISKS AND BENEFITS OF COMMUNICATING ABOUT FOOD SAFETY <b>TABACHNIKOFF SHIRA</b> , Europska agencija za sigurnost hrane (EFSA), Parma, Italija / European Food Safety Authority (EFSA), Parma, Italy		
12:00 - 12:30	<b>McIFF COLIN</b> , Ravnatelj europskog ureda Američke agencije za hranu i lijekove (FDA), Bruxelles, Belgija / Director of the FDA (US Food and Drug Administration) Europe Office, Brussels, Belgium		
12:30 - 13:00	AN EUROPEAN VIEW ON THE NEED OF TESTING AND MARKET SURVEILLANCE ACTIVITIES TO ASSURE SAFETY AND QUALITY OF FOOD IN A GLOBAL MARKET  RIBEIRO ALVARO, Predsjednik EUROLAB-a, Lisabon, Portugal EUROLAB President, Lisbon, Portugal		

13:00 - 14:30	Pauza za ručak / Lunch break
14:30 - 16:15	Plenarna predavanja / Plenary lectures Voditelj sekcije / Moderator: MISLAV TOGONAL
14:30 - 14:45	SOUTH EAST EUROPEAN NETWORK FOR FOOD SAFETY AND QUALITY CONTROL – SEEN-FSQC <b>ŠOSTAR ZVONIMIR</b> , Nastavni zavod za javno zdravstvo "Dr. Andrija Štampar", Zagreb, Hrvatska / Andrija Štampar Teaching Institute of Public Health, Zagreb, Croatia
14:45 - 15:15	Ministarstvo poljoprivrede Republike Hrvatske / Ministry of Agriculture of the Republic of Croatia
15:15 - 15:45	COMMISSION REGULATION (EU) ON THE APPLICATION OF CONTROL & MITIGATION MEASURES TO REDUCE THE PRESENCE OF ACRYLAMIDE IN FOOD – GREAT CHALLENGES FOR FOOD BUSINESS OPERATORS VAZDAR RUŽICA, Ministarstvo zdravstva Republike Hrvatske, Zagreb, Hrvatska / Ministry of Health of the Republic of Croatia, Zagreb, Croatia
15:15 - 15:45	CHALLENGES IN INFORMATIZATION AND NETWORKING OF SANITARY INSPECTION AND OFFICIAL FOOD LABORATORIES <b>FRANIĆ ROMANA</b> , Ministarstvo zdravstva Republike Hrvatske, Zagreb, Hrvatska / Ministry of Health of the Republic of Croatia, Zagreb, Croatia
15:45 - 16:00	THE ASSESSMENT OF COMPLIANCE OF ORGANIC FOOD LABELLING WITH THE EXISTING LEGISLATIVE FRAMEWORK ON THE CROATIAN MARKET – COMPARISON WITH THE STATUS BEFORE CROATIAN ACCESSION TO THE EUROPEAN UNION LUŠIĆ DRAŽEN, Medicinski fakultet Sveučilište u Rijeci, Rijeka, Hrvatska / Faculty of Medicine, University of Rijeka, Rijeka, Croatia
16:00 - 16:30	Pauza za kavu / Coffee break
16:30 - 18:30	Plenarna predavanja / Plenary lectures Voditelji sekcije / Moderators: JELENA ĐUGUM, VERA KATALINIĆ-JANKOVIĆ, MIRJANA HRUŠKAR (zamjena SANDRA ŠIKIĆ)
16:30 - 17:00	REFERENCE SYSTEM FOR SOMATIC CELL COUNTING ACTUAL CHALLENGES WITH GLOBAL EQUIVALENCE WITH MILK ANALYSES <b>ORLANDINI SILVIA</b> , Međunarodni odbor za praćenje proizvodnosti domaćih životinja, Rim, Italija / International Committee for Animal Recording (ICAR), Rome, Italy
17:00 - 17:30	ANALYSIS OF PESTICIDE RESIDUES IN FOOD UISNG ETHYL ACETATE EXTRACTION AND DETECTION WITH GC- AND LC-MS/MS (SweEt ) INCLUDING THE APPLICATION OF THE SANTE QUALITY GUIDANCE DOCUMENT <b>PIHLSTRÖM TUIJA</b> , Nacionalna agencija za hranu, Upsalla, Švedska National Food Agency, Uppsala, Sweden
17:30 - 18:00	ON THE USE OF MODERN ANALYTICAL TECHNIQUES FOR THE CHARACTERISATION OF BIOLOGICALLY ACTIVE COMPOUNDS IN FOOD <b>BULSKA EWA</b> , Kemijski fakultet Sveučilišta u Varšavi, Varšava, Poljska / Faculty of Chemistry, University of Warsaw, Warsaw, Poland
18:00 - 18:30	MYCOTOXINS IN FOOD: A REAL CHALLENGE FOR THE AGRI-FOOD CHAIN <b>BRERA CARLO</b> , Talijanski nacionalni institut za zdravstvo, Rim, Italija <i>Italian National Institute of Health, Rome, Italy</i>

### ČETVRTAK, 23. studenoga 2017. / THURSDAY, November 23, 2017

08:30 - 18:00	Registracija / Registration
00.30 - 10.00	dvorana 1A+1B+1C zajedno / hall 1A+1B+1C
	Voditelji sekcije / <i>Moderators</i> : NATAŠA GLADOVIĆ, ANDREA GROSS BOŠKOVIĆ, ANA MARIA CRNIĆ (ADELA KRIVOHLAVEK-zamjena)
09:00 - 09:30	STATE OF PLAY IN NOVEL FOODS <b>BABIĆ IVONA</b> , Europska komisija, Uprava za veterinarstvo i sigurnost hrane, Bruxelles, Belgija / European Commission, DG Health and Food Safety Directorate (DG SANTE), Unit E2 – Food processing technologies and novel foods, Brussels, Belgium
09:30 - 09:45	PATULIN IN APPLE JUCES - RISK ASSESSMENT GROSS BOŠKOVIĆ A, Hrvatska agencija za hranu, Osijek, Hrvatska / Croatian Food Agency, Osijek, Croatia
09:45 - 10:00	LABORATORY TESTS AS SERVICE TO CERTIFICATION (CONTROL) BODIES FOR ORGANIC PRODUCTION <b>ŠPANIĆ CICELI N</b> , Hrvatska akreditacijska agencija, Zagreb, Hrvatska / Croatian Accreditation Agency, Zagreb, Croatia
10:00 - 10:15	RISK MANAGEMENT IN FOOD PRODUCTION THROUGH INTEGRATED MANAGEMENT SYSTEM <b>DINIĆ T</b> , Ledo d.d., Zagreb, Hrvatska / Ledo Inc., Zagreb, Croatia
10:15 - 10:30	RISK ASSESSMENT OF PESTICIDE RESIDUES IN FOOD  PROKURICA PAVLINIĆ I, Hrvatski Centar za poljoprivredu hranu i selo, Zagreb, Hrvatska / Croatian Center for Agriculture, Food and Rural Affairs, Zagreb, Croatia
10:30 - 11:00	Sponzorirano predavanje - Shimadzu (Zlatni sponzor) / Sponsored lectures - Shimadzu (Gold sponsor)  FOOD PACKAGING AND FOOD CONTACT MATERIALS - REGULATIONS AND ANALYTICAL SOLUTIONS  LEITNER E, Tehničko sveučilište u Grazu, Graz, Austrija / Graz University of Technology, Graz, Austria  CRISIS COMMUNICATION: LEARNING FROM MISTAKES - FAILURE IS
11:00 - 11:15	INSTRUCTIVE!  MIKRUT VUNJAK S, Hrvatska agencija za hranu, Osijek, Hrvatska / Croatian Food Agency, Osijek, Croatia
11:15 - 11:30	Diskusija / Discussion
11:30 - 12:00	Pauza za kavu / Coffee break
	Voditelji sekcije / <i>Moderators</i> : IRENA ŽUNTAR, DARIA VRANEŠIĆ BENDER, LEA POLLAK
12:00 - 12:15	Sponzorirano predavanje - Shimadzu (Zlatni sponzor) / Sponsored lectures - Shimadzu (Gold sponsor)  HOW SAFE IS SAFE? ANALYTICAL TOOLS FOR THE DETERMINATION OF CONTAMINANTS IN FOOD  OPPERMAN U, Shimadzu Europa GmbH, Duisburg, Njemačka / Shimadzu Europe GmbH, Duisburg, Germany
12:15 - 12:30	SALT CONTENT IN CURED SAUSAGES: TIME FOR REFORMULATION <b>POPOVIĆ M</b> , Medicinski fakultet Sveučilišta u Novom Sadu, Novi Sad, Srbija / Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

12:30 - 12:45	SODIUM CONTENT AND THE MOST IMPORTANT DIETARY SOURCES OF SODIUM IN A SAMPLE OF STUDENT POPULATION <b>GRABEŽ M</b> , Medicinski fakultet Sveučilišta u Banja Luci, Banja Luka, BIH / Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina		
	Sponzorirano predavanje - Alphachrom (Brončani sponzor) / Sponsored lecture - Alphachrom (Bronze sponsor)		
12:45 - 13:00	THE USE OF GC QTOF DERIVED ACCURATE MASS, HIGH RESOLUTION DATA FOR RESIDUE SCREENING IN FOOD COMMODITIES <b>UPTON J</b> , Agilent Technologies, Stockport, Ujedinjeno Kraljevstvo / Agilent Technologies, Stockport, United Kingdom		
	Sponzorirano predavanje - Kobis / Sponsored lecture - Kobis		
13:00 - 13:15	IMPORTANCE OF SAMPLE PREPARATION FOR CHROMATOGRAPHY ANALYSIS <b>KAZALAC A</b> , Kobis d.o.o., Zagreb, Hrvatska / Kobis Ltd., Zagreb, Croatia		
	Sponzorirano predavanje - Labena / Sponsored lecture - Labena		
13:15 - 13:30	PROTECT YOUR "BRAND" WITH VALIDATED RAPID METHODS - REAL-TIME PCR, CHROMOGENIC MEDIA FAST & EASY ALTERNATIVE METHODS FOR MICROBIOLOGICAL ANALYSIS JASTRZEBSKA-CZUBAK M, Bio-Rad Laboratories		
13:30 - 14:45	13:30 - 14:45 Pauza za ručak / Lunch break		
	dvorana 1A / hall 1A	dvorana 1B+1C / hall 1B+1C	
	Voditelji sekcije / <i>Moderators</i> : TOMISLAV BOLANČA, ANA ČOP, VESNA POPIJAČ	Voditelji sekcije / <i>Moderators</i> : NIVES GALIĆ, VLADIMIR PETROVIĆ, VESNA HRŽENJAK	
•••••	CELIAC DISEASE IN ADULT	PLANT TOXINS	
15:00 - 15:15	POPULATION: CHALLENGES IN COMPLIANCE TO THE GLUTEN-FREE DIET	IVEŠIĆ M, Nastavni zavod za javno zdravstvo "Dr. Andrija Štampar", Zagreb, Hrvatska / Andrija Štampar Teaching Institute of Public	
	VRANEŠIĆ BENDER D, KBC Zagreb, Zagreb, Hrvatska / University Hospital	Health, Zagreb, Croatia	
	Centre Zagreb, Zagreb, Croatia		
	Centre Zagreb, Zagreb, Croatia  NOVEL FOOD AND FOOD SUPPLEMENTS – SIMILARITY AND	MYCOTOXINS, HEAVY METALS AND PESTICIDE RESIDUES IN CROATIAN CEREALS	
15:15 - 15:30	Centre Zagreb, Zagreb, Croatia  NOVEL FOOD AND FOOD	MYCOTOXINS, HEAVY METALS AND PESTICIDE RESIDUES IN CROATIAN	
15:15 - 15:30	NOVEL FOOD AND FOOD SUPPLEMENTS – SIMILARITY AND DIFFERENCES POLLAK L, Hrvatski zavod za javno zdravstvo, Zagreb, Hrvatska / Croatian National Institute of Public Health,	MYCOTOXINS, HEAVY METALS AND PESTICIDE RESIDUES IN CROATIAN CEREALS KOVAČ M, Inspecto d.o.o., Đakovo, Hrvatska	

		•····	
15:45 - 16:00	FOOD BORN ALLERGENS AS A POSSIBLE PUBLIC HEALTH PROBLEM	MYCOTOXINS IN CORN PRODUCTS: FUMONISINS TOROVIĆ LJ, Medicinski fakultet Sveučilišta	
	LASIĆ D, Nastavni zavod za javno zdravstvo "Dr. Andrija Štampar", Zagreb, Hrvatska / Andrija Štampar Teaching Institute of Public Health, Zagreb, Croatia	u Novom Sadu, Novi Sad, Srbija / Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia	
	MONITORING OF SPECIFIC FOOD SAFETY AND QUALITY INDICATORS WITH ADDED VALUES AND PROMOTIVE HEALTH EFFECTS	DUBROVNIK-NERETVA COUNTY	
16:00 - 16:15	JURČEVIĆ PODOBNIK V, Nastavni zavod za javno zdravstvo PGŽ, Rijeka, Hrvatska / Teaching Institute of Public Health of Primorsko-Goranska County, Rijeka, Croatia	LJEVAKOVIĆ-MUSLADIN I, Zavod za javno zdravstvo Dubrovačko-neretvanske županije, Dubrovnik, Hrvatska / Institute of Public Health of Dubrovačko-Neretvanska County, Dubrovnik, Croatia	
16:15 - 16:30	MANAGEMENT OF SUSTAINABLE SUPPLY CHAIN - CHALLENGES AND BENEFITS	FOOD SAFETY APPLICATION OF TANDEM MASS SPECTROMETRY USING A NOVEL FLOW-BASED DESIGN OFION OPTICS	
	<b>VDOVIĆ N</b> , DNV GL Adriatica d.o.o, Zagreb, Hrvatska / <i>DNV GL Adriatica</i> <i>Ltd., Zagreb, Croatia</i>	BISI A, Perkin Elmer Italia, Milano, Italija / Perkin Elmer Italia, Milan, Italy	
	TECHNOLOGY OF CHEESE PRODUCTION AND RIPENING IN	GMO ANALYSIS IN CROATIA: CURRENT APPROACHES AND NEW CHALLENGES	
16:30 - 16:45	CHEESE MATURATION ROOMS AND POSSIBILLITIES OF CHEESE MITES PREVENTION  VEŠLIGAJ B, Dukat d.d., Zagreb,	HANZER R, Hrvatski centar za poljoprivredu, hranu i selo, Osijek, Hrvatska / Croatian Center for Agriculture, Food and Rural Affairs, Osijek, Croatia	
	Hrvatska / Dukat Inc., Zagreb, Croatia		
	EXAMINATION OF CONSUMERS ATTITUDES IN THE FBIH ON THE IMPORTANCE OF QUALITY	CONTROL OF ILLEGAL USE OF NATURAL SEX HORMONES IN FARMING ANIMALS  SAMARDŽIJA M, Veterinarski fakultet	
16:45 - 17:00	ASSIGNMENT  PAKLARČIĆ M, Zavod za javno zdravstvo SBK/KSB Travnik, Travnik, BIH / Public Health Institute SBK/ KSB Travnik, Travnik, Bosnia and Herzegovina	Sveučilišta u Zagrebu, Zagreb, Hrvatska Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia	
17:00 - 17:15	Diskusija / Discussion	Diskusija / Discussion	
17:30 - 19:00	Razgledavanje postera i pauza za kavu Coffee break and poster viewing		
20:30	Zajednička večera u hotelu Royal / Congress dinner at hotel Royal		

#### PETAK, 24. studenoga 2017. / FRIDAY, November 24, 2017 08.30 - 12.00Registracija / Registration dvorana 1A / hall 1A dvorana 1B+1C / hall 1B+1C Voditelji sekcije / Moderators: SANDRA : Voditelj sekcije / Moderators: BRANKO ŠIKIĆ, BORIS ŠARKAJ, MARIJA KOLARIĆ, VEDRAN POLJAK, BISERKA ROMIĆ BAJZEK BREZAK FIVE KEYS FOR RELIABLE TEST DETERMINATION THE ADULTERATION OF MILK WITH RESULTS AS A PREREQUISITE FOR MILK POWDER USING A MODIFIED QUALITY AND SAFETY OF FOOD SPECTROPHOTOMETRIC METHOD **DEVČIĆ JERAS A**, Nastavni zavod za javno 09:00 - 09:15 zdravstvo "Dr. Andrija Štampar", Zagreb, MIKULEC N. Agronomski fakultet Sveučilišta u Zagrebu, Zagreb, Hrvatska Hrvatska / Andrija Štampar Teaching Institute Faculty of Agriculture, University of of Public Health, Zagreb, Croatia Zagreb, Zagreb, Croatia MONITORING OF TRACE METALS PUBLIC DINING-ROOM: A PLACE OF A IN URBAN SOILS FOR HEALTHIER HEALTH-PROVED, SAFE AND QUALITY **CITY MEAL** 09:15 - 09:30 ROMIĆ M, Sveučilište u Zagrebu, **ŽUPAN A**, Ustanova "Dobri dom" Grada Zagreba, Zagreb, Hrvatska / City of Zagreb Agronomski fakultet, Zagreb / Faculty institution Dobri dom, Zagreb, Croatia of Agriculture, University of Zagreb, Zagreb, Croatia QUALITY STANDARDS IN PRACTICE METALS IN ORGANICALLY AND CONVENTIONALLY PRODUCED AS ESSENTIAL BASE FOR MARKET WINES IN CROATIA SURVIVAL AND POSITIVE IMAGE KRIVOHLAVEK A, Nastavni zavod za KOLEK SIROVINA K, Biovega d.o.o., 09:30 - 09:45 javno zdravstvo "Dr. Andrija Štampar", Zagreb, Hrvatska / Biovega Ltd., Zagreb, Zagreb, Hrvatska / Andrija Štampar Croatia Teaching Institute of Public Health, Zagreb, Croatia PHYSIOLOGICAL. CLEANING VALIDATION PROCESS ULTRASTRUCTURAL AND IN THE MANUFACTURE OF DIETARY PROTEOMIC RESPONSES OF **SUPPLEMENTS** TOBACCO SEEDLINGS EXPOSED KORČOK D, Abela Pharm d.o.o., Beograd, TO SILVER NANOPARTICLES AND Srbija / Abela Pharm Ltd., Beograd, Serbia 09:45 - 10:00 SILVER NITRATE BALEN B, Prirodoslovno-matematički fakultet Sveučilišta u Zagrebu, Zagreb, Hrvatska / Faculty of Science, University of Zagreb, Zagreb, Croatia HOW DOES THE RESISTANCE FOOD SAFETY - QUALITY -TO FUSARIUM HEAD BLIGHT **SUSTAINABILITY** EFFECT THE DEOXYNIVALENOL GLADOVIČ N, Atlantic Grupa, Droga ACCUMULATION IN WHEAT? Kolinska d.d., Ljubljana, Slovenija / Atlantic 10:00 - 10:15 ŠARKANJ B, Prehrambeno-tehnološki Group, Droga Kolinska, Ljubljana, Slovenia fakultet Sveučilišta u Osijeku, Osijek, Hrvatska / Faculty of Food Technology, University of Osijek, Osijek, Croatia IZAZOVI STANDARDIZACIJE 10:15 - 10:30 Diskusija / Discussion BILANDŽIJA A, Bureau Veritas, Rijeka, Hrvatska / Bureau Veritas, Rijeka, Croatia 10:30 - 10:45 Diskusija / Discussion

10:45 - 11:15	Pauza za kavu / Coffee break		
	Voditelji sekcija / <i>Moderators</i> : MARTINA IVEŠIĆ, GORDANA JURAK	Voditelji sekcija <i>  Moderators</i> : MATIJANA JERGOVIĆ, NATAŠA MIKULEC	
11:15 - 11:30	ENVIRONMENTAL HEALTH PRIORITIES AND PUBLIC HEALTH TRENDS IN CROATIA - RISK COMMUNICATION AND RISK MANAGEMENT JERGOVIĆ M, Nastavni zavod za javno zdravstvo "Dr. Andrija Štampar", Zagreb, Hrvatska / Andrija Štampar Teaching Institute of Public Health, Zagreb, Croatia	CONTROL AND INSPECTION OF MEAT AND MEAT PRODUCTS IN SLAUGHTERHOUSES IN CROATIA <b>LEGEN S</b> , Bioinstitut d.o.o., Čakovec, Hrvatska / Bioinstitut Ltd., Čakovec, Croatia	
11:30 - 11:45	PESTICIDE-HANDLING PRACTICE AMONG NON-PROFESSIONAL VEGETABLE GROWERS	EFFECTIVE FOOD WASTE MANAGEMENT AND ENVIRONMENTAL RISK REDUCTION	
	POLJAK M, Nastavni zavod za javno zdravstvo "Dr. Andrija Štampar", Zagreb, Hrvatska / Andrija Štampar Teaching Institute of Public Health, Zagreb, Croatia	KALAMBURA S, Veleučilište Velika Gorica, Velika Gorica, Croatia / University of Applied Sciences Velika Gorica, Velika Gorica, Croatia	
11:45 - 12:00	RISK ASSESSMENT OF DRINKING WATER FROM PUBLIC WELLS BIJELOVIĆ S, Medicinski fakultet Sveučilišta u Novom Sadu, Novi Sad, Srbija / Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia	THE ROLE OF NUTRITIONAL STATUS IN THE ELDERLY AND NUTRITIONAL- GERONTOLOGIC STANDARDS  TOMEK ROKSANDIĆ S, Nastavni zavod za javno zdravstvo "Dr. Andrija Štampar", Zagreb, Hrvatska / Andrija Štampar Teaching Institute of Public Health, Zagreb, Croatia	
:::::::::::::::::::::::::::::::::::::::	BENEFIT AND THE RISK OF SEA	THE ROLE OF MONITORING	
12:00 - 12:15	FOOD  POLJAK V, Hrvatski zavod za javno zdravstvo, Zagreb, Hrvatska / Croatian National Institute of Public Health, Zagreb, Croatia	NUTRITIONAL STATUS IN THE ELDERLY VIA NRS 2002/GeroS/CEZIH WEB SERVICE KOLARIĆ B, Nastavni zavod za javno zdravstvo "Dr. Andrija Štampar", Zagreb, Hrvatska / Andrija Štampar Teaching Institute of Public Health, Zagreb, Croatia	
12:15 - 12:30	Diskusija / Discussion	Diskusija / Discussion	
12:30 - 13:00	Zatvaranje kongresa / Congress closing		

#### **ABSTRACTS**

**INVITED SPEAKERS** 

### Different quality of seemingly the same products in the EU

Borzan B\*

European Parliament, Brussels, Belgium/Strasbourg, France

Croatian Member of European parliament Biljana Borzan will present her work on the issue of the dual quality of seemingly identical products in different EU Member States. She will examine the legality of such practices and analyse marketing and health claims. She will support her presentation with the results of the survey she conducted with the Croatian Food Agency proving that products in the Croatian market are often more expensive and of lower quality than their supposedly identical counterparts on the German market. Her survey focused on foodstuffs and detergents and toiletries. It is the first survey in the EU to include non-food products. The survey was a strong impulse in setting the dual quality issue as a top EU policy priority. Also, several weeks after the public presentation of the results, one company withdrew the products of lower quality from the Croatian market. Dr Borzan will underline the main achievements regarding the issue and present future perspectives.

KEY WORDS: dual quality; EU Parliament; food and non-food products; quality control

### Mycotoxins in food: a real challenge for the agri-food chain

Brera C\*\*

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Mycotoxins are secondary metabolites of different mould genera, the most important being Aspergillus, Penicillium, and Fusarium. The adverse impact of these natural contaminants on the safety and yield of production chains still remains a burden even despite the adoption of tailored risk management actions aimed at preventing or at least minimising their presence in feed and food. This condition is destined to increase in the near future as a result of global warming leading to more favourable conditions for mould growth and mycotoxin production in many more geographical areas. So far, the prevalence of chronic effects vs. acute effects, at least in developed countries, and quite poor risk communication contributed to lessening the risk perceived by the final consumer. Due to their intrinsic toxic properties, mycotoxins were classified by the IARC as hazards belonging to group 1 (aflatoxin B1 and aflatoxin M1), group 2b (ochratoxin A) and group 3 (Fusarium-toxins). Worldwide, mycotoxins are present in foodstuffs as a result of a direct contamination of mainly plant origin foods and/or indirectly, in animal origin foods, as a result of the carry-over from feedstuffs. Additional routes of exposure are inhalation and dermal contact concerning workers who can be exposed to environmental contaminated dusts on a daily basis. Furthermore, one of the most underestimated alarming aspects has to do with the exposure of 3-10 yearold children for which no specific legislative provisions exist and, apparently, no effective and feasible remedy can be found. The promotion of virtuous production chains, starting from a systematic adoption of innovative and promising preventive activities (bio-control, binders, forecasting models) up to more targeted measures in the first and second transformation phase beyond informative risk communication actions could provide the right answer in preventing or at least minimising the phenomenon on a more powerful basis.

KEY WORDS: feed and food contamination; moulds; occupational exposure

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### On the use of modern analytical techniques for the characterisation of biologically active compounds in food

Bulska E\*

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Much attention has recently been given to the investigation of processes that take place in plants and animal tissues on the food market. Several analytical scenarios were tested here with regard to element transport as well as bi-transformation via examination of the presence of specific compounds in various tissues. Total content of elements of interest was determined by inductively coupled plasma mass spectrometry (ICP-MS). High-performance liquid chromatography (HPLC), coupled to ICP-MS, was used for the evaluation of the chemical speciation for compounds identified by known standards. In case of unknown species, the Orbitrap technology was validated and applied for the structural analysis of the extracted compounds. The isotopic profiles and isotopic ratio were monitored with the use of high-resolution ICP-MS. In this case, species-specific isotopic dilution MS procedure was used towards definite measurements for the certification of the chemical standard. Additionally, several imaging techniques were used to determine the spatial distribution of the element of interest over the tissue and solid samples. Images from confocal and light microscopy were compared with the distribution maps obtained by LA ICP-MS, while XANES was used for *in vivo* studies of the presence of selected species in plant cells.

KEY WORDS: analytical methods; bioactive compounds; functional food

### Reference system for somatic cell counting actual challenges with global equivalence with milk analyses

Orlandini S<sup>1\*\*</sup>, Van den Bijgaart<sup>2</sup>

International Committee for Animal Recording (ICAR), Rome, Italy<sup>1</sup>, QLIP, Zutphen, the Netherlands<sup>2</sup>

In a globalizing world, analytical results play a major role in free fair trade. However, worldwide equivalence of analytical results cannot be ensured "only" by producing standardized analytical methods. For some parameters, standardized reference materials are lacking and the reference method shows limited performance. It is there that the reference system should serve to optimally safeguard equivalence. This presentation explains what a reference system is and why the parameter of Somatic Cell Counting (SCC) was chosen as the first example for implementation as well as why this represents a typical problem. The project, its outcome, and future plans will be illustrated.

KEY WORDS: joint IDF/ICAR group; relation between reference and routine method; raw milk analysis; SCC; somatic cell counting

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# Analysis of pesticide residues in food using ethyl acetate extraction and detection with GC- and LC-MS/MS (SweEt) including the application of the SANTE quality guidance document

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The Swedish National Food Agency has employed a multi residue method for pesticide residue analysis in food since 1989. The method is based on extraction with ethyl acetate comprising about 500 different types of pesticides in one extraction. This very unique selectivity of ethyl acetate is the basis to use it as an almost universal extraction solvent in pesticide analysis, coupled to no or only very limited clean-up after extraction and prior to analysis. The determination is done by LC-MS/MS and GC-MS/MS. These techniques are sensitive and provide unambiguous identification and quantification. The multi residue method (SweEt) has been revised continuously resulting in a fast, robust, and simplified methodology for analysis of pesticide residues in food. In a search for alternative multi residue method for food, the SweEt method has shown to be a reliable and straightforward alternative in all kinds of food. The official control of pesticide residues in food and feed within the EU is carried out according to the guidelines SANTE 11945/2015 "Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed", the aim of which is to reach a harmonisation of quality assurance measures. These guidelines are acknowledged by the Codex Alimentarius which develops harmonized international food standards and guidelines to protect the health of the consumers and ensures fair practices in the food trade. Quality control guidelines should continuously be taken into account in new developments in analytical technology. The key objective is to find a cost-effective quality assurance system to guarantee quality and comparability in the results generated by laboratories.

KEY WORDS: EU guidelines; food quality; quality control

### A European view on the need of testing and market surveillance activities to assure safety and quality of food in a global market

Ribeiro AS1\*\*, Nieuwenhuis D1,2, Martin L1,2

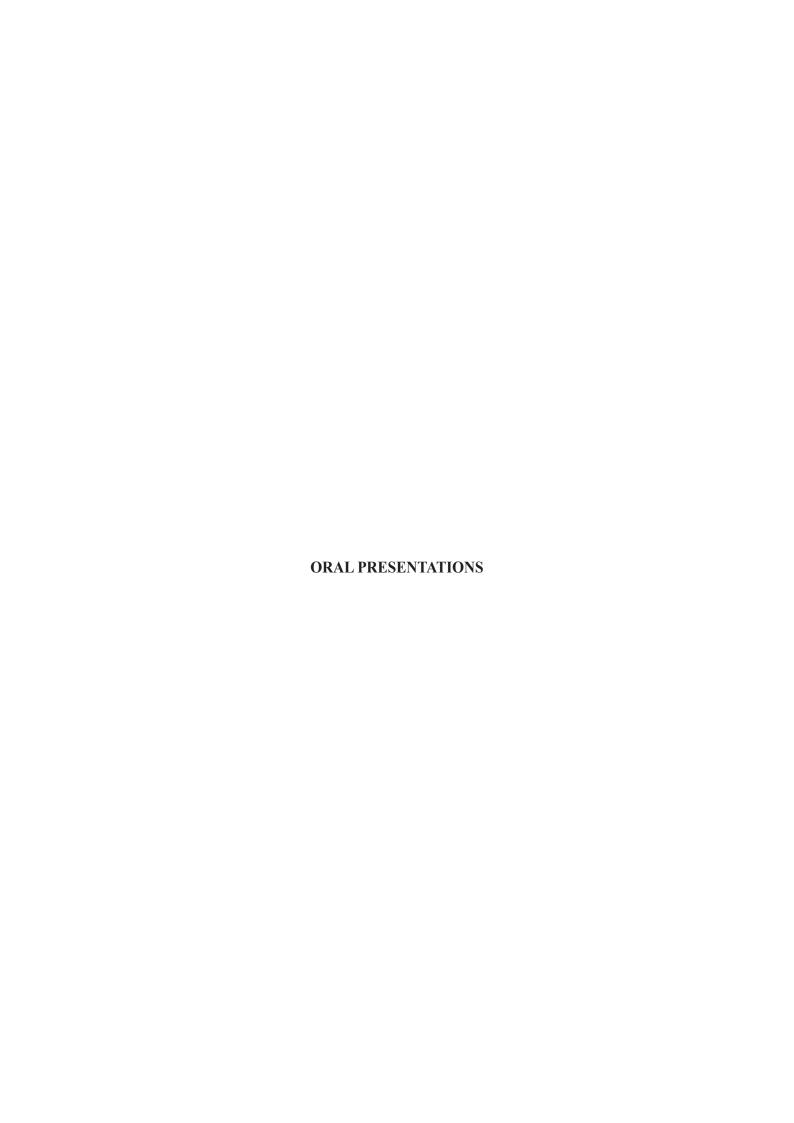
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The food industry is one of the major economic activities worldwide. Today, safety and quality are critical issues and a priority for industry stakeholders, regulatory agencies, and consumers. The increase of safety regulations related to the consumer perception of risk drives this industry to become more aware of the need to establish and upgrade programmes and actions aiming to reduce risk factors. These programs are key in assuring correct approaches to new safety challenges of diverse nature, e.g. chemical, microbiological, and environmental contaminants, requiring complex analytical methods and skilled and competent entities, able to provide services that fulfil requirements of standards and legal regulations recognized by society. The development of market surveillance is a relevant tool in the global market to provide safety and quality assurance to modern society expectations regarding risk, information access to consumers, health, fair competition, fight fraud, and crime, among many others. The laboratories and entities that provide testing, inspection, and certification (TIC Sector) have an important role in this process as they are able to contribute to market surveillance through independent accredited activities, being recognized by the European Commission and by other stakeholders. At the European level, the cooperation established among EUROLAB, CEOC International, and its members is an added value to the food industry and to the global market. It provides legislation and regulation monitoring, the harmonization of practices, the networking of Conformity Assessment Bodies (CABs) and Inspection Bodies at technical, economic, and political level. In addition, it promotes dialogue with other stakeholders and the development of guidance documents and supports CABs and Inspection Bodies accreditation and quality assurance.

KEY WORDS: consumer expectations; inspection; quality control

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### Sodium content and the most important dietary sources of sodium in a sample of student population

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High dietary sodium intake is an important public health issue. It is a risk factor of stroke and cardiovascular diseases, as well of osteoporosis, kidney disease, and stomach cancer. Knowledge about sodium content and food sources contributing most to sodium intake can be useful in reducing salt intake. There is no country-specific sodium reduction policy in Bosnia and Herzegovina. The aim of this study was to determine the sodium content in food and to assess its sources contributing the most to the sodium content in the nutrition of student population in the city of Banja Luka, Bosnia and Herzegovina. Students (n=176) of the University of Banja Luka completed a questionnaire on their gender, age, faculty attended, and the food frequency dietary recall. The sodium content in foods was calculated from the data obtained by analysing the salt content in foods (in 55 food items). This analysis was based on the Mohr method and titration with AgNO<sub>3</sub>. The sodium intake of 92 % (95CI 87.00-95.30) of surveyed students exceeded the recommended sodium intake. The NaCl content was highest in processed meat products, from 1300 mg NaCl 100 g<sup>-1</sup> in pate to 5030 mg NaCl 100 g<sup>-1</sup> in bacon; 1580 mg NaCl 100 g<sup>-1</sup> in "ćevapi"; and 1290-1990 mg NaCl 100 g<sup>-1</sup> in bread and bakery products. The food groups that contributed the most to the total sodium intake of the students were processed meat products (29.37 %), fast food (28.84 %), and bread and bakery products (24.21 %). High sodium content in processed food contributed to the high sodium intake in student population. Multisectoral collaboration between the food industry, health authorities, and government is required to raise public awareness and to lower salt consumption of the population in Bosnia and Herzegovina.

KEY WORDS: Banja Luka; Mohr method; salt intake

### Mycotoxins, heavy metals, and pesticide residues in Croatian cereals

Kovač M<sup>1\*\*</sup>, Bulaić M<sup>1</sup>, Jakovljević J<sup>1</sup>, Nevistić A<sup>1</sup>, Kovač T<sup>2</sup>, Šarkanj B<sup>2</sup>

Inspecto Ltd., Đakovo<sup>1</sup>, Faculty of Food Technology, University of Osijek, Osijek<sup>2</sup>, Croatia

The quality and safety of cereals is of great importance since they are still the world's most important source of food and feed. Cereal safety can be endangered by numerous contaminants, including biological, chemical, and physical, which can enter the food chain during food and feed production, storage, and transport. Treatment of crops with pesticides may leave residues in products of plant and animal origin. Natural contaminants, such as mycotoxins, may also appear in food and feed, while heavy metals are introduced into products through water, air, or soil. The toxicological relevance of the above mentioned contaminants and residues is well established; however the total health impact of combined exposure to various pesticide residues, heavy metals, and mycotoxins remains unknown. Monitoring the incidence of these pollutants is crucial for health risk assessment, especially if considering the possibility of their synergistic effect. As systematic investigations of their occurrence in Croatian cereals are still lacking, this research aims to monitor the contamination level of nation-wide crops by mycotoxins, heavy metals, and pesticide residues. All major cereals grown in Croatia (corn, wheat, barley) and products thereof were collected from all Croatian counties. The regulated mycotoxins were determined by "dilute and shoot" method using highly sensitive LC-MS/MS. Heavy metals were analysed by microwave digestion and AAS, while pesticide residues were determined using QuEChERS sample preparation and both LC-MS/MS and GC-MS/MS. All used methods were validated and legislation criteria requirements were assured. The highest mycotoxin incidence was found in corn samples, where the most of regulated mycotoxins were present. Fusarium mycotoxin deoxynivalenol was observed to be the most frequent mycotoxin in all collected samples, having the concentration above LOQ in 40.8 % of samples. Mycotoxin concentrations higher than allowed were observed in nine corn samples. Pesticide residues and heavy metals were determined in the most significant samples taking into account the heterogeneity of sample contamination by mycotoxins (high or low level). The obtained data pointed to the higher incidence of mycotoxins rather than other two contaminant groups, however the importance of consistent control of all three stated contaminants in different foodstuffs and feedstuffs should not be overlooked.

KEY WORDS: contamination; crops; feed; food

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### Availability and safety of hemp food products in Slovenia

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National Institute of Public Health<sup>1</sup>, Nutrition Institute<sup>2</sup>, Agricultural Institute<sup>3</sup>, Institute for Quality Research and Development<sup>4</sup>, Ljubljana, National Laboratory of Health, Environment and Food, Maribor<sup>5</sup>, Slovenia

In the European Union, the cultivation of *Cannnabis sativa* varieties is granted provided the tetrahydrocannabinol (THC) content does not exceed 0.2 %. The aim of our study was to describe the availability of hemp in conventional foods and food supplements on the Slovenian market, to determine the occurrence of biologically active cannabinoids, and to address the potential risks caused through human dietary exposure. A national database and software tools were used to determine the availability of conventional foods, food supplements, and other products with hemp on the Slovenian market. The content of cannabinoids in a representative sample of foods was performed with analytical methods based on the LC/MS/MS technique. Acute dietary exposure to CBD and THC was estimated combining different scenarios for various population groups. The established acute reference dose (ARfD) of 1  $\mu$ g kg $^{-1}$  bw was used for THC and determined for CBD. Altogether, 75 conventional foods and 13 hemp-containing food supplements were purchased on the market in Slovenia. Cannabinoids – cannabidiol (CBD), THC, cannabinol (CBN), cannabigerol, cannabidivarin, and tetrahydrocannabinolic acid were identified and quantified. The results of exposure and risk assessment will be presented. The use and consumption of hemp food products in Slovenia is increasing and concerns have been raised about the safety of hemp products on the market. The results of our study will help to effectively manage the potential risks associated with hemp in food.

KEY WORDS: acute dietary exposure; Cannnabis sativa; LC/MS/MS technique

### **Examination of consumers' attitudes in the FBiH on the importance of quality labels**

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Consumers want quality and safe food. Higher quality and product safety is ensured by additional controls of production. Products that are subject to additional controls are marked by special signs of quality. These labels guarantee a higher quality of products to consumers and allow them to decide more easily when buying. This is true because the products bearing such a label indicate that they in no way deviate from the minimum quality which is valid for this type of product. The aim of this study was to examine and point to the importance of the labels of controlled quality among consumers in FBiH. The survey was conducted from February to May 2017 in the FBiH area, on a sample of 1568 respondents. An anonymous survey questionnaire was used, which was designed for the purpose of this research. The questionnaire consisted of 17 questions. The data obtained were analysed on the basis of age, gender, professional qualification, place of residence, buying habits, satisfaction with purchased products, as well as their views on the significance of the award of quality labels on food. Of the total number of respondents, the highest proportion (33.41 %) is of those who believe that quality labels confirm the quality and safety of the product. When asked whether they knew that an independent laboratory testing stood behind the quality label, the most common answer was yes (57.90 %). There is a large proportion of those who have more confidence in the products that carry the label of quality (58.03 %). When it comes to choosing among the products that cost more due to the quality label, the majority of respondents chose fresh fruits (45.21 %) as their first response, followed by milk and milk products (35.33 %), and eggs as the third answer (25.06 %). Consumers find it essential to be additionally assured when choosing and consuming food products. They believe that quality labels on products represent a confirmation of safe and quality food products, and that these thus provide additional security in terms of food safety and quality.

KEY WORDS: controlled quality; food; safe product

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### Salt content in cured sausages retailed in Novi Sad, Serbia

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Faculty of Medicine, University of Novi Sad<sup>1</sup>, Institute of Public Health of Vojvodina<sup>2</sup>, Novi Sad, Serbia

The excessive use of salt has been recognised as one of the leading factors causing hypertension and its adverse consequences. Processed food is the major contributor to high-salt intake. The highest level of salt is observed in cured meat products and sausages, ensuring preservation and microbial safety, as well as taste and texture, and accounting for over 20 % of the total salt dietary intake. The aim of this paper was to determine the salt content in industrially and hand-crafted dried sausages retailed in Novi Sad, a city located in the Pannonian Region with a high consumption of cured sausages. In total, 50 hand-crafted (19) and industrially produced (31) samples of cured sausages were collected at the "green" market and in retail shops. Ion chromatography was performed for determination of sodium content in the water extract of food samples. Results were expressed as a percentage of sodium chloride. The British Food Standard Agency (FSA) salt content profile is used for the classification of the samples: the low, medium, and high salt content,  $\leq$ 0.3 g,  $\geq$ 0.3–1.5 g, and  $\geq$ 1.5 g  $\leq$ 100 g<sup>-1</sup>, respectively. The average salt content in the controlled samples of retailed cured sausages was  $\leq$ 3.48±0.87 g  $\leq$ 100 g<sup>-1</sup>, ranging from 1.83 to 5.64 g  $\leq$ 100 g<sup>-1</sup>. Comparison of salt content in two groups of samples with the Student's t-test showed a statistically significant difference (p<0.05). The average salt content in industrially produced sausages (3.83±0.75 g  $\leq$ 100 g<sup>-1</sup>) was statistically significantly higher than in hand-crafted sausages (2.90±0.75 g  $\leq$ 100 g<sup>-1</sup>). The established salt content in all controlled samples is considered high, when applying the FSA criterion. Gradual salt reduction in all food products is urgently needed. A simple sodium reduction does not necessarily have a negative effect on safety and shelf life. In the absence of national standards, it is possible to apply the British FSA recommendation to limit the salt content in sausages to 1.4 %.

KEY WORDS: British Food Standard Agency; hypertension; sodium

### Mycotoxins in corn products: fumonisins

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Faculty of Medicine, University of Novi Sad<sup>1</sup>, Institute of Public Health of Vojvodina<sup>2</sup>, Novi Sad, Serbia

Occurrence of fumonisins, a group of structurally related mycotoxins primarily produced by *Fusarium verticillioides* and *F. proliferatum*, was investigated in corn products. A total of 56 samples (46 flours, 10 flakes), all produced and marketed in Serbia, was collected in 2015-2016 from retail stores. Analysis included fumonisin B1 (FB1) and B2 (FB2), and comprised the preparation on immuno-affinity columns, derivatisation with *o*-phthalaldehyde reagent, and HPLC-FLD determination. The study revealed a widespread occurrence of fumonisins in corn flours, with only minor differences between the sampling years regarding the percentage of contaminated samples: in total, 97.8 % for FB1 and 91.3 % for FB2. Overall mean contamination levels were 164.0 mg kg¹ for FB1 and 43.2 mg kg¹ for FB2 (207.1 mg kg¹ as sum of FB1 and FB2). Around two-fold reduction of mean contamination level was noted in 2016 compared with 2015. Vast majority of the samples (overall 89.1 %) exhibited contamination lower than a half of the maximum allowed level (ML) of 1000 mg kg¹ for total fumonisins. None of the samples from 2016 exceeded the ML (maximum of 467.2 mg kg¹), as opposed to one sample from 2015 with 1468.5 mg kg¹ of fumonisins (overal 2.2 % of non-compliant samples). Within the group of corn flakes, FB1 was detected in 80.0 %, and FB2 in 60.0 % of the samples, with mean at 89.8 and 29.1 mg kg¹, respectively. The highest level of total fumonisins (579.4 mg kg¹, with 434.2 mg kg¹ of FB1), was below the ML for corn-based breakfast cereals, set at 800 mg kg¹. Occurrence of fumonisins in food is of interest not only for regulatory purposes but also for population health risk assessment – fumonisins are associated with a wide range of toxic effects, and the liver and kidney are the most sensitive target organs. Corn flours are traditionally used in ethnic meals, and nowadays increasingly consumed by people on gluten-free diet.

KEY WORDS: British Food Standard Agency; hypertension; sodium

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### Mushroom metabolites in the control of mycotoxin contamination of food

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Mycotoxins are secondary metabolites produced by fungi toxic to humans and animals. Due to their toxicity, the legislations of almost all countries have limited the concentration of most important mycotoxins in food and feed commodities. The awareness that different chemicals, largely used in agriculture to control pests, could present both environmental problems and health hazards, led to large limitation of their use. Since 2014, the European Community has banned about 50 % of chemicals used in agriculture. In plant production systems, chemicals were also the main tool used against fungal pathogens and against the contamination of food/feed by mycotoxins. Today, the EC encourages research on control and/or detoxification strategies, which would use more environmentally friendly "green" approaches and eco-compatible tools. The preferred methods for controlling mycotoxin contamination are largely preventive and the use of biocontrol agents is one of the strategies. Different mushroom metabolites showed a potential to become control agents for fungal growth and mycotoxin production. Particularly, the exo-polysaccharides from medicinal mushroom *Trametes versicolor*, an edible and nontoxic basidiomycete, showed some interesting potential in mycotoxin control. Biocontrol agents from *T. versicolor*, such as polysaccharide Tramesan®, oligosaccharides from its partial hydrolysis and protein fractions, are proposed to counteract the growth of some mycotoxigenic fungal species (*Aspergillus flavus, A. carbonarius, A. ochraceus*), and mycotoxin synthesis (aflatoxin B1, AF; ochratoxin A, OTA). The inhibiting effect on germination, fungal growth, and toxin synthesis of these compounds was evaluated. Furthermore, some aspects of their mechanism of action is proposed and discussed. Results indicated that biocontrol agents from *T. versicolor* could be a new more eco-compatible tool for the control of biosynthesis of several mycotoxins in food and feed chain.

KEY WORDS: biocontrol agents; fungi; fungal growth

### Monitoring of specific food safety and quality indicators with added values and promotive health effects

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Economic nationalism is steadily increasing on a global scale and this is reflected in the food production segment. Consumers tend to consume locally-produced food strongly underscoring its safety aspect but also its nutritional and health value. In order for the manufacturer to have confirmation that his products meet the highest standards of safety, quality, and promotional health effects, it is necessary to offer and implement specific additional laboratory food analyses. Food monitoring is carried out in cooperation with food business operators by evaluating and assessing the safety and quality of specific food categories. In addition to the legally prescribed parameters that ensure the health and quality of food products, the parameters that represent the added value of the product are tested. Research on specific parameters of certain categories of food (eg. food frauds, food supplements, added sugar in honey, vitamin and mineral content in natural juices...) contributes to the positioning of more quality and safer products on the market. A systematic approach to ensuring safe and quality food is more important than ever. Rapid detection of specific analytical parameters in food presents the challenge and commitment of participants in the food safety and quality assurance chain. Supporting the increased availability of food with promotional health components will have significant effects on the general health of the population. New technologies and equipment enable quick results and help assess the fulfilment of the requirements for safety and quality of food. It is necessary to carry out further research on the effects of substances in food on human health including the parameters indicating the health promotion effect.

KEY WORDS: analytical parameters; consumers; market

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### Microbiological assessment of ready-to-eat vegetables in Dubrovnik-Neretva County

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The aim of this study was to assess the microbiological quality of ready-to-eat vegetables offered by local food business operators in Dubrovnik-Neretva County in the period 2011-2017. Vegetables were randomly selected and sampled in hotels, restaurants, and stores. Both packaged and unpackaged products were sampled. Analyses were conducted according to the Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs and the Croatian National Guidelines on microbiological criteria for foodstuffs. A total of 126 samples were analysed for *Enterobacteriaceae*, *E. coli*, Coagulase-positive staphylococci, sulphite-reducing *Clostridia*, yeasts/moulds, *Listeria monocytogenes*, and the presence of *Salmonella* spp., using respective ISO methods. The results showed that 68 (54 %) samples were unsatisfactory, mostly because of their high level of *Enterobacteriaceae* and yeasts/moulds. Unsatisfactory levels of *Enterobacteriaceae* were found in 55 (43.7 %) samples, yeasts/moulds in 45 (35.7 %) samples, Coagulase-positive staphylococci in four (2.8 %) samples, and *E. coli* in three (2.1 %) samples. Among the 126 samples, *Enterobacteriaceae*, *E. coli*, Coagulase-positive staphylococci and yeasts/moulds were 3.20–6.48, 3.60–5.81, 3.26-4.65, and 3.08–5.48 log CFU g<sup>-1</sup>, respectively. Sulphite-reducing *Clostridia*, *L. monocytogenes*, and *Salmonella* spp. were not detected in any of the samples. The most contaminated vegetables were lettuce, followed by tomatoes, cucumbers, and cabbage. All unsatisfactory samples were either sliced or grated. In 2017, seven samples of packaged ready-to-eat leafy green vegetables were also analysed. Four out of seven samples (57.1 %) were unsatisfactory due to contamination with *Enterobacteriaceae* (4-5 log CFU g<sup>-1</sup>), although the products were labelled as ready-to-eat with no need for washing. Although pathogens were not isolated, our study shows poor microbiological quality of vegetables and raises certain concerns regarding the safety of these products. Proper washing

KEY WORDS: Enterobacteriaceae; E. coli; Coagulase-positive staphylococci

### Coeliac disease in adult population: challenges in complying with a gluten-free diet

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Coeliac disease is an autoimmune enteropathy caused by gluten in genetically predisposed individuals. In coeliac disease, immune activation results in intestinal damage i.e. chronic inflammation of the small intestinal mucosa and a wide range of clinical manifestations. The condition may develop at any age. Although burdensome and restricting, the only treatment for individuals with celiac disease is a strict life-long gluten-free diet. While the term "gluten-free" implies complete elimination of all sources of gluten, in reality this is not possible due to contamination of foods with trace amounts of gluten. Hence the term "gluten-free" indicates a diet that contains gluten at such a low level (< 20 mg day¹) as to be considered harmless. Strict adherence to a gluten-free diet is challenging, with recent reports suggesting that adherence rates range from 42 % to 91 %. Perceptions of cost, effectiveness of gluten-free diet, knowledge of gluten-free diet, and self-effectiveness at following this regimen are the main factors contributing to compliance. Forty-three adults with coeliac disease (38 females and five males), with mean age 43.5, were monitored at the Unit of Clinical Nutrition, University Hospital Zagreb, in order to assess their nutritional status and compliance with gluten-free diet. All the patients had clinically, serologically, and histologically confirmed coeliac disease in adulthood and mean duration of gluten-free diet was 4.4 years (1-16 years). According to the serological testing of tissue transglutaminase (tTG), 27 % patients were not compliant with gluten-free diet, or were not responding to the diet. Mean BMI was 23.0 and 32 % patients were diagnosed with anaemia. All the patients who were monitored at the outpatient clinic were provided tailor-made training on gluten-free diet, therefore, the adherence rate was high. Nevertheless, future research is needed to develop resources to help patients with coeliac disease to follow a strict gluten-free diet. In contrast to children, up to 30 % ad

KEY WORDS: anaemia; BMI; trace amounts

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### Foodborne allergens as a possible public health problem

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Available literary data shows an increasing phenomenon of foodborne allergies and intolerances worldwide. The purpose of this research is to point to the need of establishing better controls on this matter. All food samples were provided from small and medium-sized food producers in Croatia and from third countries. All samples were processed products in original packaging and were analysed in accordance with EU legislation, with particular focus on non-marked allergens on the label. The ELISA (immunoenzymatic) technique was been used for laboratory analysis on the presence of known allergens. A total of 144 samples were analysed, most of them for gluten presence (51 %), almond presence (13 %), followed by milk, soybean, peanuts, and hazelnut. No detected allergen presence was found in 80 samples (55 %), but 21 samples (15 %) were considered non-compliant with the pertaining label and legislation. A total of 69 samples had a confirmed presence of allergens above the limit of method quantification (40 % > LOQ) or at the threshold of LOQ (8 %). The most frequent non-labelled allergens quantified were gluten (29 % above 20 ppm), followed by hazelnuts (24 %), and almonds (24 %). The obtained results indicate the presence of certain allergens in food products that have not been highlighted on the labels with another text style (mandatory by the EU Regulation No. 1169/2011). Clear guidelines have been provided for manufacturers to indicate a possible presence of these product allergens in the obligatory citation ("May contain..."). A quantitative probabilistic risk assessment needs to be determined whether these measurable results represent a potential risk to consumer health. These results stress the need to include additional cross-contamination control of allergens during routine self-control. Additionally, there is a need to establish targeted official monitoring in the Republic of Croatia in retail sector.

KEY WORDS: ELISA; consumer health; food producers; self-control

# Commission Regulation (EU) on the application of control and mitigation measures to reduce the presence of acrylamide in food – great challenges for food business operators

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Acrylamide is produced as a process contaminant in food from asparagine and sugar at temperatures higher than 120 °C. Its potentially carcinogenic properties were known even before a decade when EFSA issued the Acrylamide Declaration (2005) based on the risk assessment conducted by JECFA, concluding that acrylamide from food was danger to human health. EFSA did not have enough data to deliver its opinion and therefore the European Commission adopted the first Commission Recommendation No. 207/331 on the monitoring of acrylamide, and requested follow-up of the Member States over a three-year period. Over a decade, enough data has been collected and EFSA made a scientific opinion on acrylamide in food in 2015, concluding that current levels of acrylamide exposure of all age groups were worrisome due to its cancerous effects. Following this conclusion, the European Commission began adopting a legislative act immediately applicable in all Member States and for all food business operators. In July 2017, Commission Reg. (EU) on the application of control and mitigation measures to reduce the presence of acrylamide in food entered into force. Annex I to the Commission Regulation sets out the measures that subjects must take to reduce the levels of acrylamide in food. A whole range of processing chain measures, ranging from agronomic measures, raw material selection, ingredient and/or recipe modifications, treatment temperature, and other measures that an entity must take to reduce acrylamide is prescribed. In addition, the Commission Regulation prescribes an obligation for operators to carry out an analysis of their products with appropriate sampling. With the entry into force of the Commission Regulation, food business operators will have to carry out sampling and analysis at least once every year for each product for which the level of acrylamide must be controlled, and for problematic products even more frequently. In addition, they will have to keep all the records of the measures taken. Furthermore, all analyses carried out upon the Ministry of Health's request will be submitted annually, as well as the details of the implemented technological measures for problematic products that exceeded the reference value levels. The new Commission Regulation in Annex III prescribes analysis requirements for monitoring and laboratories, while Annex IV prescribes new benchmark levels that have been reduced compared to the previous levels for almost all products. By adopting this Regulation, the European Commission directly prescribes the measures in the HACCP systems of the entities to be undertaken, intervenes and prescribes the frequency of analysis as well as the delivery of data to the competent authorities. Its implementation will be challenging for both food business operators and inspection bodies.

KEY WORDS: EFSA; HACCP; reference values

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### Control and inspection of meat and meat products in slaughterhouses in Croatia

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Concerning safety of food of animal origin, it is important to highlight the role of authorised veterinarians in providing food chain information that accompany animals to slaughter. The aim is to present their broader role in vaccination of animals, sampling for disease testing, tuberculosis testing, disinfection, animal identification and registration, movement registration, animal welfare, and other measures included in the Order on measures to protect animals from infectious and parasitic diseases and financing thereof. Further, we will explain the role of control bodies accredited according to HRN EN ISO/IEC:17020 and official veterinarians in slaughterhouses and meat processing. We will present the official tasks performed by the control bodies and these are: control of the animal health certificate, tasks related to audit and control of GMP, GHP, and HACCAP, ante mortem control, verification of compliance with animal welfare standards at slaughter, post mortem control, sampling within the monitoring programme, identification marks and traceability requirements controls and other tasks delegated to the control body. The official veterinarians in slaughterhouses participate in the control of food business operators and supervise the performance of the control body. The described meat and meat product inspection system is based on Regulations 882, 853, 854, The Food Act, and Veterinary Act.

KEY WORDS: GHP; GMP; HACCAP

### Effective food waste management and environmental risk reduction

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It is an indisputable fact that the growth of the human population has led to an enormous growth in consumption and, consequently, waste generation. Waste, the consequence of all our activities, is the loss of resources such as energy and raw materials. Among the types of waste, food waste is one of the major problems of today. It can be viewed from different perspectives: environmentally and ethically. Ensuring the highest possible usability of food is the goal of every producer, but the question is why so much food is being discarded. Efficient management can only be achieved if the entire production, procurement, storage, and sales process meet set standards. The aim of the paper is to analyse how the management of food waste can be effectively achieved as well as to detect effects to the economy.

KEY WORDS: economic impact; environmental pollution; waste management legislation

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### Epidemiological data on foodborne diseases

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The most important food-borne diseases included in the List of Communicable Diseases of national interest are: salmonellosis, campylobacteriosis, dysentery, yersiniosis, alimentary toxic infection, enterocolitis, and viral gastroenterocolitis. Based on the data from the Communicable Diseases Registry, the largest number of reported cases has been categorized as alimentary toxic infection and enterocolitis (syndromes). The number of reported salmonellosis cases has been decreasing over the past 15 years, whereas the number of reported campylobacteriosis cases has been rising. The decreasing number of reported cases of dysentery speaks in favour of a beneficial epidemiological situation regarding diseases related to poverty, lack of hygiene and sanitation, or lower educational background. Nonetheless, the 2017 data show there is a strong need for continuous preparedness to treat and prevent the spread of foodborne diseases, since there have been several isolated cases of imported typhus and a minor hepatitis A epidemic in Europe. Croatian data on food-borne communicable diseases and epidemics are included in the EU summary report on trends and sources of zoonoses, zoonotic agents, and food-borne outbreaks issued by the ECDC and EFSA. The most recent report published in December 2016 contains data for 2015. Despite the decreasing number of epidemics in general, the number of people getting sick during a specific food-borne epidemic is increasing, mostly in cases of viruses (Norwalk and rota virus). In 45 % of foodborne epidemics the causes are untraceable, whereas 23 % are transmitted through meat and 6 % through eggs. Since eggs and egg-containing products are the most common means of disease transmission in case of Salmonella both in Croatia and other European countries, it is necessary to continuously remind all population groups of the importance of hygienic preventive measures such as properly washing one's hands during preparation of meals, adequate thermic treatment of food items, and proper handling of ready-made foods.

KEY WORDS: epidemics; food-borne diseases; salmonellosis

### Management of a sustainable supply chain - challenges and benefits

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Management of food and beverage supply chain becomes an increasingly important element of consumers' and general stakeholders' trust. Sustainable products are not only those which meet elementary food safety requirements; they are the results of responsible business, production, and distribution practices, taking care not only of own sites and process risks, but also of those of their supply chain. DNV GL, as one of the leading companies providing independent assessments and certifications, organised a series of surveys worldwide in order to understand how companies in food and beverage sector approach supply chain management and to which extent sustainability of their chain is in their focus. In our lecture, we shall focus on presenting the most interesting findings of surveys on supply chain management, addressing today's supply chain management readiness for the future, together with specific insights on RSPO (Roundtable on Sustainable Palm Oil) as an example of how markets', customers', and consumers' pressures can influence the change in perceptions of supply chain. Conclusions will be based on the findings of international surveys ran from 2013 onwards. They show that supply chain sustainability is attracting increasing attention driven by the fact that 80 % of companies are facing pressure from their customers to demonstrate sustainability of their supply chains, and 33 % of them see new, stricter regulations as the main driver. Concerning the RSPO example, the main aim of setting it up as a not-for profit organisation that unites seven sectors of palm oil industry (producers, processors, traders, manufacturers, retailers, investors, and NGOs) was to reduce the negative impacts of palm oil cultivation on the environment and communities. Its activities resulted in 2.46 million hectares, 2400 companies, and 4100 facilities already RSPO certified, which means that all of them produce, use, and/or sell sustainable palm oil. Our paper will show how world leading companies face these supply chain sustainabilit

KEY WORDS: companies; consumers; markets

## Technology of cheese production, ripening in cheese maturation rooms, and the possibilities of preventing cheese mites

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Cheese, as a high value product rich in proteins, is often an ideal substrate for the development of unwanted microorganisms, mould, and mites. All of the above can have a bad effect on the cheese appearance, taste, and durability. Because we are to discuss about long ripening (hard) cheeses, the question is what can we do in order to prevent the bad effects on such expensive and nutritious products. In this manuscript, the emphasis is to be put on the appearance and the effect of cheese mite *Tyrophagus casei* (Oudemans, 1990) in the whole process of cheese production. Apart from the quality of milk as a raw material, the sanitation in the entire technological process of cheese production is very important as well. Good manufacturing and hygiene practice are essential in the prerequisite programmes. In the technology of cheese production, it implies a clean environment of the production facility, cleanliness of rennet rooms, mechanical and microbiological purity of cheese vats, which are used for the souring of milk, curd processing, cheese brining, adequate equipment for mechanical cleaning and washing of the vats, pest control and, finally, hygiene and microclimate in cheese maturation rooms. We must not ignore good hygiene practice, which includes employee's (cheesemaker's) personal hygiene, workwear hygiene, cleaning processes, mechanical and chemical disinfection of all containers, appliances, machinery and equipment used in the making of cheese.

KEY WORDS: good manufacturing and hygiene practice; proteins; souring

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### Food safety application of tandem mass spectrometry using a novel flow-based design of ion optics

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The increased awareness of people of the factors affecting the quality of their life urges the need for increased controls on environmental and food quality and safety; the number of analysis is growing year after year, more parameters must be controlled, and the cut-off concentration of target products keeps lowering. In the last months, Europe faced Fipronil outbreak affecting not only the market of fresh eggs but also the whole chain of products using egg derivatives in food industries. To speed-up the workflow, simplified sample preparation is required leading to lower cleanup and increased concentration of the matrix injected, and there is a need to increase the selectivity of the analytical technique; given these requirements, tandem mass spectrometry can provide the required sensitivity and selectivity. In 2017, analytical instruments are required to provide increased sensitivity even with simplified sample preparation, better sample throughput, and less down-time than in the past and this can be achieved through a novel ion optics design that makes the tandem mass spectrometer more sensitive and at the same time more rugged and less prone to matrix contamination. After a quick description of the novel flow-based design we will go through several applications on food contaminants including a quick method for the detection of Fipronil in eggs, antibiotics in infant formula, and pesticides in food. All reviewed applications include sample preparation, analytical conditions for UHPLC-MSMS analyser, results, and validation.

KEY WORDS: Fipronil; matrix; sample

### GMO analysis in Croatia: current approaches and new challenges

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Since the beginning of commercialisation of genetically modified plants in 1994, we have been dealing with the challenges of control and monitoring through policies and laboratory analysis. An increasing number of genetically modified crops, along with the development of new types of genetic modifications, pose a challenge for enforcement laboratories. Reliable analysis is needed to ensure the implementation of national and EU regulation. In Croatia, as well as in the European Union, real-time polymerase chain reaction (real-time PCR) is still the method of choice for detection, identification, and quantification of genetically modified organisms in authorised laboratories. The method is applicable to a large number of matrices, from raw materials to highly processed samples. It provides high level of specificity and sensitivity but as the number of authorised GM events and their complexity have increased, the method has become more and more time-consuming and, consequently, expensive. Use of a primer pair and a probe, which amplifies a specific target, makes this method suitable for all authorised GM events, but the presence of unauthorised evets can only be insinuated. The necessity to face the challenges has led to the development of new detection approaches and new methods such as multiple targeting PCR, digital PCR, next generation sequencing, and others. Although the current approach meets our legislation requirements, it is expected that implementation of new methodologies will be needed in the near future.

KEY WORDS: EU legislation; laboratories; monitoring; PCR

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#### **Plant toxins**

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Different kinds of food products, such as cereal products, herbal teas, and honey, can be contaminated with various toxic substances originating from plants and fungi. Recently, plant toxins have drawn attention of researchers, as these are substances that appear in certain plants that are widespread and hence food contamination may occur. Some of the plant toxins that are known to have a negative impact on human body are tropane, ergot, and pyrrolizidine alkaloids. Currently, maximum permitted levels of these plant toxins do not exist (except tropane alkaloids in baby food) but can be expected soon as they are identified as a priority under the European Food Safety Authority. Considering the very complex and diverse composition of food, large number of plant toxins, and the effort to achieve the lowest limits of detection and quantification, only the methods with mass spectrometric (MS) detection provide the prerequisites to analyse plant toxins at ppb or ppt levels in food, either in combination with gas chromatography (GC) or high performance liquid chromatography (HPLC). Coupling of (UPLC) HPLC instruments with tandem mass-spectrometry (MS/MS) has become the method of choice for the analysis of plant toxins in recent years. The National Reference Laboratory for Mycotoxins, as part of Andrija Stampar Teaching Institute of Public Health, regularly monitors the development of mycotoxins in food and feed and provides scientific and technical assistance to the Ministry of Agriculture and the Ministry of Health in the development and implementation of a coordinated mycotoxin control programme, including plant toxins.

KEY WORDS: gas chromatography; high performance liquid chromatography; tandem mass-spectrometry

### The use of GC QT of derived accurate mass, high resolution data for residue screening in food commodities

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Accurate mass with high resolution measurements can be of value in certain instances to the food residue laboratory. The systems can deliver a good level of targeted analysis whilst at the same time opening up the opportunity for suspect screening, unknown analysis or true discovery type applications. With this in mind, the data produced needs careful investigation to meet the requirements of the food regulatory authorities. In this work, we show how routine data deconvolution tools will enhance target compound confidence by using the deconvoluted mass spectrometric data. Furthermore, the co-elution criteria of the SANTE guidelines place an emphasis on co-elution measurement of the chemical entity ions. For unknowns analysis, the use of an advanced 3D deconvolution tool shows how an enhanced chemical entity detection allows for successful spectral library searching or interpretation. Data shows the detection of pesticides and other food contaminants following a simple QUECHERS based extraction.

KEY WORDS: co-elution criteria: contaminants: deconvolution tool

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### Novel food and food supplements – similarity and differences

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Based on the development of awareness of self-healing, the growing development of new technologies and product formulations in the food industry, especially in the field of dietary supplements, has set in. Nutrition supplements are subject to the Food Act and all regulations and directives related to this area. With the emergence of new EU Regulation No. 2015/2283 on new food and ingredients of new food, new legislative provisions and regulations have been introduced. All food or its ingredients that are not significantly used in human nutrition in the European Union until 15 May 1997 are categorized as Novel Food or a Novel Food Ingredient. They are currently covered by Regulation (EC) No 258/97 of the European Parliament and of the Council on novel foods and novel food ingredients, which became directly applicable by the accession of the Republic of Croatia to the European Union. The new Regulation 2015/2283 comes into force at the beginning of 2018. With the goal of positive impact and health improvements with the expected physiological effect on human health, dietary supplements are all the more powerful. This influence depends on the type and amount of biologically active ingredients used. When formulating a product, it is important to pay attention to whether any of the ingredients in the new food or the ingredients are new foods. If they fall into this category, it is necessary to carry out and ensure appropriate risk and safety assessment procedures as well as notification in both the Republic of Croatia and the EU. The role of producers, control institutions and competent ministries is to point out and follow the legislative provisions relating to new foods, establish conformity of labeling of products, and check any health claims. In the Republic of Croatia, the competent authority for novel food is the Ministry of Health, which has authorized the Croatian Institute of Public Health as a body responsible for conducting professional activities and advising on the preparation of a novel food risk assessment report since 2013.

KEY WORDS: dietary supplements; food legislation; nutrition; quality control

### Food packaging and food contact materials – regulations and analytical solutions

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It is a fundamental requirement of all legal bodies as well as of any hygiene management system (e.g. DIN EN 15593 2008, ISO22000 or ISO/TS 22002, BRC/IoP, IFS...) that there is no transfer of chemicals into packaged goods, as this may endanger the end user at the point of sale under normal or foreseeable conditions of use. The Regulation (EC) No. 1935/2004 on materials and articles intended to come into contact with food defines the requirements for all food contact materials in general. The European Framework Regulation (EC) No. 1935/2004 includes all materials and articles intended to come into contact with food. The latter regulation covers not only the packaging material (plastic, paper, metal,...) but also converting machines, pipes, bond-conveyors, tanks, cutting boards, and other kitchenware. It states that any article intended to come into contact with food must be sufficiently inert to preclude substances from being transferred to food in quantities that may endanger human health or to bring about an unacceptable change in the composition of the food or deterioration in its organoleptic properties. However food contact materials contain many substances that can migrate into food, therefore the Regulation (EC) No. 1935/2004 is accompanied by specific measures for controlling the legal provisions depending on the type of food contact material. Besides many "traditional" analytes (like heavy metals, PCP, PCB, PAH, etc.), especially UV stabilizers, photosensitizers for printing inks and varnishes, dyes, and endocrine disruptors are also intensely discussed in any food contact application and in relation to their impact on consumers. Further to the above mentioned substances, unknown substances can enter the process chain and must be identified and quantified to ensure the product quality (Non-Intentionally Added Substances, NIAS). NIAS are impurities in the substances used for manufacturing or reaction intermediates formed during the production process or decomposition or reaction products occurring in the final product. Generally, it is accepted that only compounds <1000 Dalton are considered NIAS because substances with a higher molecular weight are regarded as inert and more difficultly migrate due to their larger size. Very often the volatile composition can be complex and therefore demands sensitive and selective methods. The combination of gas chromatographic separation with mass spectrometric detection is still the gold standard. The optimisation process for analytical methods is always a compromise between speed, selectivity, sensitivity, and cost. A proper analytical process has all three relevant parts (sample preparation, separation and detection) optimised. It should be kept in mind that there are no ideal sample preparations and measuring instruments available on the market, so each method should be checked carefully if the instrumentation and the methods fulfil the requirements in terms of sensitivity, reproducibility, and accuracy. Several examples of analytical strategies will be discussed in this presentation.

KEY WORDS: PAH; PCB; sample preparation

### Laboratory tests as a service to certification (control) bodies for organic production

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For the purpose of certification decision-making process and surveillance activities, certification (control) bodies use the results of laboratory tests. Such tests have the purpose of proving the absence of active substances that are not allowed by legislation relevant to organic production. According to the legislation, the required sampling (soil, cereals, processed products, etc.) is performed by competent personnel of the certification (control) body. Laboratories preforming this type of testing and certification (control) bodies performing certification activities in organic production are authorised by the competent authority, the Ministry of Agriculture. One of the criteria for authorisation is a valid accreditation status for both laboratories and certification (control) bodies. The experience in performing accreditation of laboratories and certification bodies triggered frequent discussions concerning the responsibilities for defining the scope of the required testing, interpretation of the criteria for applicable LOQ, influence of measurement uncertainty, and formulation of statements of compliance. This presentation will highlight the relevant requirements of the standards HRN EN ISO/IEC 17025, HRN EN ISO/IEC 17065, and applicable legislation; criteria for the selection of testing laboratories; expected changes in the new standard HRN EN ISO/IEC 17025.

KEY WORDS: HRN EN ISO/IEC 17025; HRN EN ISO/IEC 17065; legislation

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### Five keys to reliable test results as a prerequisite for quality and safety of food

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The aim of this paper was to point out five keys to reliable test results in testing laboratories and their implementation in Andrija Štampar Teaching Institute of Public Health (NZZJZAŠ). Each industrialised country in the world has standardisation, metrology, accreditation, and certification of its management systems in place, all in line with internationally recognised standards. For achieving success on national and international markets, it is crucial that testing laboratories are accredited according to EN ISO/IEC 17025:2007. Accreditation under this standard gives service users the assurance that the stated specifications and requirements of the world market are satisfied. The five keys that the Centre for Integrated Management System (Centre) implemented to provide reliable test results are: 1. Management of Test Methods: 2. Internal Quality Control Measures; 3. External Quality Control – Interlaboratory Comparison (MLU); 4. Analysis and Evaluation of Quality Control Measures; 5. Making a Five-Year Plan for Interlaboratory Comparisons. Improvements that the Centre introduced in the management of test methods were a platform for an easier and more effective control of quality assurance measures, but also for their analysis and evaluation. Introducing the Register of Interlaboratory Comparisons has made it possible to analyse and evaluate the results of interlaboratory comparisons for each test method in a quick and easy way. In 2016, NZZJZAŠ test laboratories completed 45 MLUs, which included 247 parameters. 74 parameters were tested in food. The analysis and evaluation of quality control measures facilitated the planning and preparation of a Five-Year Plan for Interlaboratory Comparisons. NZZJŽAŠ has proven its competence in conducting complex food analyses, not only by successfully participating in interlaboratory comparisons but also by continuously improving quality assurance of its test results by consistent application of a quality management system.

KEY WORDS: EN ISO/IEC 17025; interlaboratory comparisons; NZZJZAŠ; quality assurance

### Quality standards in practice as an essential base for surviving and developing a positive image on the market

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Biovega company was founded in 1994 as a unique business concept that connects retail and education with production and wholesale activities, along with the passion of owners and employees for healthy lifestyles. All the projects and pioneer business activities lead to the development of an organic market niche in Croatia. We offer 100 % organic products in our bio&bio stores. Ecological origin and environmental quality standards are confirmed by the method of cultivation, processing of raw materials, packaging, and distribution to customers. All of the products are subject to strict quality controls, both external and internal, and have all required certificates. The rise in quantity, which has been brought about by a rapid development of organic market in Croatia and globally, is now also faced with the imperative of maintaining quality. This requires continuous strict controls of organic production and products but also the acknowledgement and understanding that organic business operations must contribute to the overall sustainability and that their development must bring benefits to customers, the economy, and the environment in general. Recently, Biovega was in the middle of food safety affair related to imported eggs from Holland. Only thanks to its excellent structure of controlling process, Biovega managed to save customers from consuming possibly unsafe products, to alarm public about this affair, to proactively cooperate with institutions, and to save company from a threatening damage of losing its positive image. Biovega company is certified according to ISO 9001, HACCP as well as the IFS standards. IFS currently comprise eight standards, which have been developed for and by the stakeholders involved in all parts of the supply chain. All standards are process standards, which help users when implementing legal provisions regarding food and/or product safety, and provide uniform guidelines on food, product safety, and quality issues.

KEY WORDS: environment; organic market; sustainability

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### Cleaning validation process in the manufacture of dietary supplements

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Cross-contamination in the manufacture of food supplements represents one of the most important risk factors and possibilities for it to occur are numerous due to technical or organisational imperfections. Insufficient cleaning of manufacturing equipment, poorly designed manufacturing facility or manufacturing processes and an inappropriate ventilating system are all possible causes of contamination, which is transferred via personnel or material. The risk increases if the same manufacturing facility and equipment is used for the manufacture of many different products. In most cases, for the purposes of financial savings and to increase productivity, manufacturers decide for multiproduct facilities: facilities where different products are manufactured using the same equipment. Due to the development of partnerships and contract manufacturing for economic reasons, the number and diversity of products manufactured using the same equipment can significantly increase. Manufacturer's responsibility is to overcome or reduce the risks of cross-contamination using different measures in the manufacture of dietary supplements, as well as in the larger "lifecycle" of a product. This is also important for the company's reputation and the risk of potential financial loss. The subject of this research is the validation process for the cleaning of the equipment used in the manufacture of dietary supplements. A detailed description of all phases of the cleaning validation in the manufacturing facility of Abela Pharm d.o.o. is provided. The purpose of this research is to describe the principles of documented validation of the quality of cleaning in order to meet good manufacturing practice (GMP) requirements. Based on defined research objectives and according to the subject of the research, a hypothetical research framework was established. After performing the cleaning validation of the manufacturing equipment, it was confirmed that after the production of the next product manufactured after the selected worst case the production line was not contaminated with the residues of cleaning agents and that there was no crosscontamination.

KEY WORDS: cross-contamination; equipment; GMP

### Public dining-room: a place of a health-proved, safe, and quality meal

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The institution of Zagreb Dobri dom is a unique institution in Europe that provides daily meals for social care users in Zagreb. 3,500 meals are prepared daily (or 1.2 million yearly), which is a total of 16 million since 2004 when the institution was founded. The purpose of our work is to analyse the quality of meals in public dining-rooms, which is, together with health-proved and safe meal, and a satisfied user, the general end result. We also want to elaborate the quality of the entire service by comparing the results of lab analyses and users' survey. The paper presents the results of analyses of an authorised lab concerning the microbiological control of the meals, water quality, and drinking water in order to establish the meals' nutritional value. Received data has been compared with the mark given in the survey that involved 399 subjects. Results of these analytic reports show that all collected samples were in conformity with legal provisions. Since 2004, there has not been a single case of meal contamination during preparation and distribution. Results of nutritional analyses show that meals were prepared according to the daily advised inputs and that the share of meal ingredients was satisfactory and balanced. Survey results, though, show lower grades for the meals quality and offer (3.6), portion size (3.7), while cleanness, equipment, personnel hospitality, and their commitment to work are highly graded (4.7). Analytical report results also show that there is a concern about health and nutritional value of the meals and the sanitary-hygienic terms in the institution. Thus, we can conclude that there is still room for improvement.

KEY WORDS: authorised lab; nutritional value; portion size

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### Determination of adulteration of milk with milk powder using a modified spectrophotometric method

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Milk production by adding milk powder or selling reconstituted milk powder as raw, pasteurised or UHT milk is a serious problem the dairy industry faces today. Adulteration, besides creating unfair market competition, misleads the consumer. The development of methods, in order to prevent product market failures that do not comply with legal regulations, is one of the key challenges to protecting society. The aim of this paper was to modify the spectrophotometric method for determining drinking milk adulteration. The method includes milk degreasing, coagulating milk proteins by adding acetic acid, and heating the formed curd in the presence of phosphomolybdic acid, which results in its blue staining. The intensity of the blue colour is proportionally increased by the addition of reconstituted milk powder and is measured spectrophotometrically at 620 nm. For semi-quantitative determination of the amount of added milk powder, the calibration milk samples with known composition, produced from a mixture of raw or pasteurised or UHT milk and 0; 5; 15; 35, and 65 % (v/v) reconstituted milk powder, were prepared. The curd of unadulterated milk samples coloured greenish while the curd of adulterated milk samples with added milk powder coloured light to dark blue depending on the percentage of added milk powder. The molecular modelling methods were used to predict and clarify the staining mechanism of formed curd and phosphomolybdic acid. The applied and modified spectrophotometric method was found to be suitable for the detection of milk adulteration by addition of milk powder.

KEY WORDS: curd; degreasing; pasteurised milk

## The assessment of compliance of organic food labelling with the existing legislative framework on the Croatian market – comparison with the status before Croatian accession to the European Union

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Food labelling represents basic communication between food business operators and consumers. It is an important part of marketing and a part of the information and consumer protection system. Besides general food labelling legal requirements, organic food must comply with specific legislation pertinent only to organic food. In this study, the labels of 164 (2017 study) and 123 (2013 study) organic food products were assessed as to their compliance with the existing legal framework (Croatian, EU), based on a meticulously elaborated evaluation form. The analysed products were divided into two categories - processed and unprocessed organic foods because of the fact that certain labelling parameters (nutritional value, ingredient list) are related only to processed food. The results of 2017 were compared with the results of the comparable earlier (2013) research (before Croatia joined the EU). The results showed that only 9.8 % of the evaluated organic labels fulfilled completely the food labelling requirements. Furthermore, a decrease of 0.9 % in noncomplying labels was observed when compared to the earlier 2013 study, when only 8.9 % of the evaluated organic labels fulfilled all the labelling requirements. Most common errors included the improper labelling materials (hags, tags), only partial information about addresses of food business operators, lack of the nutrition declaration, improper lot number, non-translated place of origin of organically grown ingredients, and omission of data about the quantity of organically produced ingredients. The 2017 results confirmed that the most frequent reason of labelling non-compliance was the choice of unsuitable labelling material which made the labels illegible (27.4 %). Non-compliances reflected also the negligence in the translation of nutrition declarations (13.6 %), place of origin of organically grown ingredients (49.4 %), as well as in the misuse of non-permitted labelling words for organic product as a replacement for the permitted words "ceo", "bio", and "organic

KEY WORDS: food business operators; nutritional value; tags

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### Monitoring of trace metals in urban soils for a healthier city

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Urban soil environment is subjected to pollution through numerous pathways: atmospheric deposition from traffic and industry, waste deposits, heating, and many more. Specific and highly diversified land use in urban area makes the monitoring of soil quality an extremely complex programme. Accumulation of toxic substances in soils, including trace metals, can directly influence public health. Metals are chemically very reactive in the environment, which results in their mobility and bioavailability to living organisms. People can be exposed to high levels of toxic metals by breathing air, drinking water, or eating food that produced in widely spread urban gardens. Besides anthropogenic sources, trace metals can be also found in the parent material from which the soils developed. Whether the said inputs will become toxic and to what degree are these mobile depends on a number of factors: specific chemical and physical trace metal characteristics, soil type, land use, geomorphological characteristics within the soil type, and exposure to emission sources. Processes that control the mobility, transformation, and toxicity of metals in soil are of special importance in the soil root developing zone – the rhizosphere. For this reason, there is a considerable interest in understanding trace metal behaviour in soil, with a special emphasis on the way they build up in soil and on the processes by which plants take up metals. The City of Zagreb has recognised the importance of soil quality monitoring as an important tool in estimating the hazards to the vital roles of the urban ecosystem. It has also found vital to collect relevant data for the development of quality standards for both policy makers and citizens and citizen associations. Geochemical soil survey of the City of Zagreb has triggered several studies so far, which resulted in an action undertaken by the city. The ongoing programme aims to provide a database for environmental health risk evaluation for the Croatian capital.

KEY WORDS: anthropogenic sources; pollution; trace metal

### Metals in organically and conventionally produced wines in Croatia

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Wine is a popular and widely consumed alcoholic beverage and economically important product. Daily consumption of wine in moderate amounts contributes significantly to the needs of human body for essential elements, while As, Cd, and Pb are known as potentially toxic. Consuming and growing organic food, including wine, is increasing and so is the need to determine elements in organically produced wines. The aim of this work was to assess the impact of organic wine production on the levels of metal residues in the samples collected from the most important Croatian winegrowing regions. Eleven locations with organic wine production were selected in different winegrowing regions of Croatia (Central and Northern Croatia, Istria, Northern Dalmatia, Vis Island). In each location, wine samples from both, organic and conventional wine producers were collected and analysed for metal content (15 organic wine samples and 16 conventional wine samples). Flame atomic absorption spectrometry (AAS) was used for the determination of Zn, Fe, and Cu and inductively coupled plasma mass spectrometry (ICP-MS) was used for the determination of Na, Al, Cr, Ni, As, Cd, Sn, Pb, and Ag content in wines. All wines contained significantly lower levels of residues in comparison with maximum residue limits (MRLs), with the exception of one organically produced wine containing excessive amounts of copper (1043  $\mu$ g L-1) and Zn (5706  $\mu$ g L-1) exceeding the current MRL. There were no significant differences in the mean concentrations of analysed metals between organically and conventionally produced wines with the exception of Pb and Mg being significantly less represented in organic wines.

KEY WORDS: copper; ICP-MS; maximum residue limits

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### How does the resistance to fusarium head blight affect the deoxynivalenol accumulation in wheat?

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Fusarium head blight (FHB) is a wheat disease that affects numerous wheat cultivating regions, including Croatia, reducing the safety and quality of wheat and wheat-derived food. The most occurring mycotoxin produced by *Fusarium* spp. is deoxynivalenol (DON), the immunotoxic mycotoxin, harmful to humans and animals. Its concentration is regulated in raw cereals, food, and feed, therefore it is important to control wheat infections if cereals are to be used in the food and feed industry. The aim of this work was to compare wheat varieties grown in Croatia in terms of their resistance to FHB. 25 varieties were tested and divided into four subgroups according to their declared resistance: resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S). The test was carried out on the field by using natural contamination as a control and inoculation with *Fusarium culmorum* as a high contamination scenario. The resistant varieties did show the best resistance to high contamination with *F. culmorum* (median DON concentration 328 μg kg¹), and natural contamination (median DON concentration 50.0 μg kg¹). Moreover, on average, the varieties did show resistance to FHB and DON accumulation, but there were several exceptions. In the high contamination scenario, the highest sensitivity was detected in the MS variety with a maximum DON concentration of 6663 μg kg¹, and in the control group, the most susceptible variety was the MR variety with a maximum DON concentration of 580 μg kg¹. This suggests that there is a need for further research and harmonisation of statements on the susceptibility to FHB of the varieties and DON accumulation to ensure further food and feed safety and quality.

KEY WORDS: accumulation; food; mycotoxin

### Pesticide-handling practice among non-professional vegetable growers

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In November 2015, the Croatian Government adopted new regulations on the mandatory education for pesticide users as an action plan for achieving the sustainable use of pesticides. This study was carried out to determine the pesticide handling practice and safety procedures among non-professional vegetable growers in Hrvatsko Zagorje, the northwest region of Croatia, before providing education. A convenient sample of 116 non-professional pesticide handlers from Hrvatsko Zagorje were enrolled in the survey conducted using an anonymous questionnaire. The data collected included the social-economic status of respondents, pesticide-use safety, handling practices, and pesticide hazards knowledge. Data were analysed using SAS software package. Results indicate that only 12.07 % of respondents dispose unused pesticide mixtures properly and 39.66 % dispose empty pesticide containers properly. The majority of respondents (96.55 %) use at least one or two pieces of safety protective equipment during pesticide application, but only 7 % of them use high-level protective equipment. At least 39.66 % of respondents suffered from at least one health symptom associated with pesticide handling. The results of the research indicate that the respondents were not taking appropriate measures in their pesticide-handling practice and their action can affect the safety of the environment and their personal health. Therefore, efforts to increase knowledge of pesticide handling among non-professional pesticide handlers by the Croatian Government are important and should have an effect on safer pesticide handling practices in the future.

KEY WORDS: environment; health; protective equipment

### Environmental health priorities and public health trends in Croatia – risk communication and risk management

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The World Health Organization defines environmental health as those aspects of human health and diseases determined by factors in the environment. It includes both direct pathological effects of chemicals, radiation, and some biological agents and often indirect effects on health and well-being of the broad physical, psychological, social, and cultural environment, which includes housing, urban development, land use and transport. Risk assessment and risk communication for sustainable water and food safety systems must be supervised by national public health authorities. These stakeholders and areas represent an integral part of a proper risk management policy. Database searches were conducted in an iterative manner to retrieve the articles related to literature on risk communications in public health, as well as the policy literature related to the determinants of environmental health. Potentially relevant studies were included or excluded based on journal citation index, titles, abstracts, and full paper content. Search terms included: risk assessment, risk communication, health impact assessments, and health risk assessments. The Croatian National Environmental Protection Plan 2016-2023 includes an introduction to the health impact assessment (HIA) method. This method provides a qualitative and quantitative assessment, health impacts communication, expert recommendations, and corrective measures to prevent negative health effects. The definition of national priority strategic projects and formation of multidisciplinary teams depending on the type of environmental influences on human health, both for HIA ex ante or for HRA (Health Risk Assessment) after possible exposure to environmental factors, are important measures with a potential to encourage the development of new communication action plans. Use of these powerful tools will have a significant impact on the public health system's economic determinants and the burden of diseases from environmental risks. Problems in risk communication often arise because of the differences in perspectives between specialist and public opinions. Therefore, it is essential to establish environmental health teams in Croatia based upon audit and analytical-advisory services. A funded service within the institutes of public health will help in the promotion of a preventive approach and implementation of new risk assessment and risk communication methods.

KEY WORDS: corrective measures; health impact assessment; World Health Organization

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### Challenges in informatisation and networking of sanitary inspection and official food laboratories

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The computerisation of sanitary inspection began in 2015 by introducing the Central Information System for Sanitary Inspection (CISSI). The main goal of computerisation was to provide a unique database of all state, county, and border sanitary inspectors and IT linkage with other institutions. Alongside the central application that provides IT support to the business processes of sanitary inspection, it also provides linkage with the PIN Tax Administration System, the web service for data exchange with freight forwarders, the E-citizens System, and the Ministry of Health Laboratory Information System (eQMS::LIMS). All official food laboratories which work with Sanitary Inspection have access to the Ministry of Health eQMS::LIMS System for the purpose of entering analysis results of official samples. In accordance with food safety regulations, all EU member states must submit annual reports on the results of the official food sample analysis to the European Food Safety Authority (EFSA). With the integration of the Central Information System for Sanitary Inspection (CISSI) and the Ministry of Health Laboratory Information System (eQMS::LIMS), it is possible to automatically generate reports according to EFSA requirements. Prior to the implementation and integration of CISSI and the Ministry of Health eQMS::LIMS, reports on the results of the analysis were collected by reference official laboratories for a particular area of food analysis and reports were manually drafted according to the SSD models defined by the European Food Safety Agency. Data and reports on analysis results for each area of analysis submitted to EFSA are subject to changes and adjustments for each reporting year, which also requires continuous adaptation of IT systems. In order to improve the quality of data and reports submitted to the IT System of the European Food Safety Agency (DCF – Data Collection Framework), both Central Sanitary Inspection Information System (CISSI) and the Ministry of Health Laboratory Information System (eQMS::LIMS) have taken over the EFSA data classification. Interconnection of official laboratories and the Ministry of Health provides a database of all the official samples and the automatic creation of reports in accordance with EFSA requirements, but official laboratories are currently recording analysis results in two laboratory systems, their own laboratory system and the Ministry of Health eQMS::LIMS system. In the future, unique classification or data mapping could solve this problem of entering the same data into two IT systems.

KEY WORDS: computerisation; database; food analysis

### Crisis communication: learning from mistakes - failure is instructive!

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In recent years, we have witnessed several food- or feed-related incidents in Europe. With the process of globalization, open borders, and the expansion of new communication tools and channels (online media, social networks, etc.), the flow of goods and information have become much faster. Therefore, during recent incidents it became clear that a more effective, accurate, and proactive reaction from potentially responsible authorities is required. Furthermore, they are highly communication-sensitive and demand a special communication approach. In that context, we can talk about crisis communication. Although each situation seems to be specific and it may seem to require a different approach, there are some rules that have to be followed and can be applied to any incident and/or crisis situation. This paper will reflect on the negative examples and myths about crisis communication in order to emphasise what should (not) be done in times of crisis. Whether we speak about industry or public authorities, a crisis is a test of the reputation of responsible communications holders. Therefore, a reaction to it is pivotal and with an inadequate crisis-reaction, one's reputation can be irretrievably destroyed. But can we profit from the crisis? Is it possible to turn inconvenience into opportunity and come out as a winner?

KEY WORDS: communication failure; food-related incident; industrial safety

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#### Patulin in apple juices – a risk assessment

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Patulin is a mycotoxin produced by certain types of moulds such as Penicillium, Aspergillus, and Byssochlamys, which can grow on various foods including fruit, cereals, and cheese. It is toxic to many biological systems, but its significance in causing disease in humans and animals has not yet been fully explained. Due to insufficient knowledge of patulin carcinogenicity, the International Cancer Research Agency (IARC) classified it into Group 3 - "Not classified as carcinogenic to humans". The current provisional maximum tolerable daily intake (PMTDI) for patulin is  $0.4\,\mu g\,kg^{-1}\,BM$ , and according to Commission Regulation (EC) No. 1881/2006, the maximum permitted amount of patulin in apple juice is 50  $\mu g\,kg^{-1}$ . Apples and apple juice are the most important sources of contamination with patulin in human nutrition, especially juices produced by pressing fresh apples. Till 2014, the Ministry of Health carried out the monitoring of patulin in selected products and from 2014 this has been done by the Croatian Food Agency (HAH) in order to build a risk assessment on a multi-annual basis. Within three years of monitoring, HAH collected data for a total of 122 apple juice samples. In 21.3 %, patulin was quantified. Data on apple juice consumption were obtained from the National Food Conservation Survey of the Republic of Croatia and totalled 131 instances of consumption of this type of juice, accounting for 6.55 % of the total population. The computer model "Improrisk 1.3.4" was used to evaluate consumer exposure. Exposure estimate has shown that no apple juice consumer is exposed to patulin in an amount exceeding the toxicological limit or PMTDI of 0.4  $\mu g\,kg^{-1}$  BM. In the worst-case scenario, exposure is 0.025  $\mu g\,kg^{-1}$  BM, which is 16 times lower than PMTDI for patulin, and therefore the risk of apple juice consumption may be considered as negligible.

KEY WORDS: Croatian Food Agency; exposure; fruit

### Risk management in food production through an integrated management system

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Risk management in food production is one of the most effective tools for covering all the complex requirements of a manufacturing company. Ledo d.d. has been the largest producer and distributor of ice cream and frozen food in Croatia and the region since 1958 when the first industrial ice cream Snjeguljica was produced. To maintain this position and market competitiveness, it was necessary to adapt to new market demands. Quality assurance and management systems play a key role in the control of food safety, and they also define, describe, and improve all business processes of the company. Quality Management System (ISO 9001), Food Safety (HACCP, IFS, BRC), Environment (ISO 14001), Energy Efficiency (ISO 50001), and Religious Standards (Kosher and Halal) are the systems that have been built into a unique integrated management system in Ledo d.d., which presents now a sound basis for effective risk management. We have identified the risks in the field of quality assurance and environmental management through the risk analysis of raw materials, trade products, our own products, and packaging materials and we used these to develop sampling plans and environmental aspects. We will present the methods of risk analysis we have implemented. Requirements of all the above mentioned standards bring about benefits to the entire system and by implementing these we set the bar slightly higher in the self-control system. This is how the British Retail Consortium (BRC) request for risk analysis of food fraud resulted in our own methodology development, which we use for the overall control of raw materials and products where our own checklist is one of the tools for the assessment of suppliers. Integration of all systems has been successfully done across the entire company, which results in a high level of success in certification audits, while at the same time represents an invaluable help in successful business operations.

KEY WORDS: food fraud; risk analysis; quality assurance

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#### Benefits and risks of seafood consumption

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Seafood represents a major part of animal protein consumption in many parts of the world, accounting for 16.7 % of the global population's intake of animal protein and 6.5 % of all protein consumed. Fish proteins can represent a crucial nutritional component in some densely populated countries where the total protein intake levels may be low. Of 136 million tonnes globally utilised as human food, 27 % is consumed fresh and the rest is processed as frozen, salted, dried, smoked, or canned. Being the fastest growing food production system in the world, aquaculture produced 66.6 million tonnes in 2012, adding to a global increase from 9.9 kg per capita fish consumption in the 1960s to 19.2 kg in 2012. This development has been driven by a combination of population growth, rising incomes, and urbanisation and has been facilitated by the strong expansion of fish production and more efficient distribution channels. As seafood is high on the list of foodborne diseases and sources of chemical hazards, safety concerns related to the intake of raw fish are considerable. While scombroid food poisoning can result from eating rotten fish, seafoodborne parasitoses are either caused by ingestion of viable cestodes, trematodes, and nematodes, or can elicit allergic reactions following the sensitisation to the parasite's antigen, as is the case of Anisakis spp. In respect of chemicals, bioaccumulation results in a higher concentration of methyl-mercury and arsenic in sea organisms compared to the surrounding environment, while the misuse of veterinary drugs in aquaculture is an important hazard necessary to address during the implementation of the HACCP programme on farms. Although fish and fish products were the most alerted category in 2015 in the EU Rapid Alert System for Food and Feed, the hazards related to contamination or survival of biological hazards during processing are well-defined and can be controlled by applying Good Manufacturing Practice (GMP), Good Hygiene Practice (GHP), and a well-designed HACCP-programme in

KEY WORDS: Good Hygiene Practice; Good Manufacturing Practice; food poisoning

#### Risk assessment of drinking water from public wells

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The aim was to identify microbiological, chemical, and physical hazards in drinking water from public wells, which can have an impact on human health, and to evaluate the results of routine analyses of drinking water safety differently, not considering the national legislative, but rather risk management approach. Drinking water from 20 public wells in South Backa District (SBD) was sampled and analysed during 2016 at the Institute of Public Health of Vojvodina according to the accredited, standardised, and proposed national methodology. The hazards were identified according to international recommendations. Risk assessment was done by applying the semi-quantitative approach, which classifies the likelihood and consequence of an identified hazard in terms of risk as low, medium, high, and very high. Among 218 drinking water samples, only 10 % was in accordance with national regulations. The identified hazards were thermotolerant coliforms (especially *Escherichia coli*), enterococci (genus *Streptococcus*), *Pseudomonas aeruginosa*, *Proteus* species, and nitrates above the proposed level. In two thirds of controlled public wells, the risk was rated as high with an influence on morbidity of sensitive population and in one third as medium, causing the changes in the choice of drinking water sources. The proposed risk assessment methodology enables an easier understanding of information and clearly rates the risk caused by drinking water from public wells. The largest challenge in managing drinking water safety is to systematically prioritise risk assessment in the Republic of Serbia.

KEY WORDS: coliforms; hazard; human health

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#### Risk assessment of pesticide residues in food

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"Pesticide residues" refers to residues either in or on the products of plant or animal origin and in animal feed that stem from the use of plant protection products, biocides, and agents used in veterinary medicine. To protect consumers from exposure to unacceptable levels of pesticide residues in food and animal feed, maximum residue limits (MRLs) have been set as a safety limit according to EU Regulation 396/2005. The increasing dose, number of applications, or noncompliance with the post-harvest interval led to increased quantity of crop residues, which suggests an improper use of plant protection products. In case of using the active substance of pesticide on a crop for which it has not been registered, pesticide residues can be found. In case of exceeding MRLs, risk assessment is carried out using EFSA PRIMo model, which uses a set of relevant parameters and gives a final result based on four different parts: hazard identification potentially harmful health effects of chemicals are identified by toxicological studies; hazard assessment - estimated acceptable daily intake (ADI); exposure assessment - evaluation of intake of residues through food; risk characterisation - comparison between estimated and acceptable daily intake. Food is considered safe for consumption if the estimated intake of harmful substances does not exceed the ADI or ArfD (acute reference dose) values. When exposure assessment is taken into account, besides data on the concentrations of residues, data on consumption of a particular type of food has also to be taken into account, bearing in mind the nutritional habits of a particular population. Risk assessment is a very important part in the area of food safety and every new finding opens up space for improving the entire system "from stable to table".

KEY WORDS: consumption; EFSA PRIMo model; plant protection products

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### Control of illegal use of natural sex hormones in farming of food animals

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The most important representatives of the three groups of natural steroid sex hormones (NSSHs) used as growth promoters in intensive livestock industry are: 17B-estradiol, progesterone, and testosterone. Their effects on the growth and improvement of the organoleptic properties of meat are very positive, because they had been applied in the farming of food animals long before their negative side effects were revealed. Long-term use of NSSHs has toxic, mutagenic, teratogenic, and cancerous effects on animals. As a result of anabolic application, the accumulation of residues occurs in animal tissues and the elimination of NSSHs is performed by body fluids in the environment, but also in milk. Consumers could be exposed to NSSHs by consuming food products of animal origin (milk, meat, eggs), causing even more severe side effects and harmful consequences in humans than in animals. Because of the mentioned reasons, the anabolic use of NSSH was banned in the EU in 1988. Their application is limited only to the appear to treat reproductive and gestation disorders. For determining concentrations of NSSH in food of animal origin, it is essential to know the physiological concentrations of these hormones, in order to know if the results are the consequence of abuse or within physiological referent values. As physiological values can vary and are dependent on many factors, when considering the obtained results it is important to take into account anamnestic information and data regarding feeding regime and accommodation of animals. Today's control systems are more detailed and diagnostic tests are more precise, so the results are more reliable and animal food products are safer for consumers. Considering the constant development of new synthetic substances and the possible abuse of so-called "cocktails" with low levels of active substances with anabolic effects, the continuous monitoring of their use is essential in food animal farming and in slaughterhouses for their residues in meat samples.

KEY WORDS: abuse control; farm animals; food of animal origin; natural steroid sex hormones

#### **Food safety - quality - sustainability**

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In Atlantic Grupa (AG), responsibility is an integral part of the company's business and development strategy and there is a high level of awareness of the need to exert company's own influence to improve general conditions in the social environment. Already in 2012, strategic corporate functions, through materiality assessment, agreed on the scope of our future sustainable development, at the same time when the company decided to report on the progress according to the Global Reporting Initiative standards. Within this frame of sustainable development, we find quality systems, such as product safety, quality assurance system, environmental, and energy management systems, to be crucial. Through these sub-systems, we can ensure continuous improvement and manage some of the most significant environmental and social impacts of our company. Key performance indicators are the drivers towards the progress in a given area. However, key indicators are not long-term fixed, they need to be well understood and dynamically adopted to reach the variable expectations of all different stakeholders. When managing risks in product safety, the logical approach is to first align with world best practices and then upgrade these with our own knowledge. Conformity with legislation and zero mistakes are common goals for all our products. The AG's robust quality system, highly supported with internal functional educational programmes, ensures proper resilience. More details are presented in the lecture. When managing product quality, starting from product design, one should be aware that every product, technological process, brand, or market is specific. Marketing teams strive to properly define the product or brand quality, adjusted to the expectations of different groups of our customers (buyers, shoppers, consumers). It is a common goal to align products and labels with new emerging trends of environmental and social awareness such as healthy eating habits. Further, every business or brand has to "translate" this common goal into its own specific performance indicators, which drive the teams of technologists to develop innovative products.

KEY WORDS: brand; key performance indicators; product safety

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#### Challenges of standardisation

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Consumers and authorities increasingly demand that the food we eat offers high levels of quality and security. Companies involved in the production, packaging, handling, transportation, and sale of food and food products, or in the agricultural commodities industry, must comply with these demands and implement standards to access valuable consumer markets. HACCP system covers the requirements for prerequisite programmes and 6 HACCP principles. Implementing an effective system is a legal obligation (Codex Alimentarius (CAC/RCP 1-1969, Rev. 4-2003). ISO 22000 is the first international standard for a food safety management system. It covers Interactive communication, System Management and Hazard control. FSSC 22000 is fully based on ISO 22000, technical specification for Prerequisite Programmes (PRPs), and additional scheme requirements. IFS and BRC are food quality and safety standards published by retail trade groups. Any company wishing to supply its food products to those retailers must meet the required standards. GLOBALG.A.P. is an internationally recognised set of farm standards dedicated to Good Agricultural Practices (GAP). For consumers and retailers, this certification is reassurance that food reaches accepted levels of safety and quality, and that it has been produced sustainably. Organic Certification is an internationally recognised set of standards aimed at proving that agricultural and food products have been produced with the emphasis on the protection of soil and water, enhancement of biodiversity, and responsible use of energy and natural resources. MSC is an independent non-profit organisation founded with the aim of transforming the seafood market to a sustainable basis by: reversing the decline in global fish stocks; delivering improvements in the marine environment; contributing to securing fishers' livelihood. Three schemes of geographical indications and traditional specialities, known as protected designation of origin (PDO), protected geographical indication (PGI) and traditional specialities guaranteed (TSG), promote and protect names of quality agricultural products and foodstuffs.

KEY WORDS: good agricultural practices; HACCP; MSC

## Physiological, ultrastructural, and proteomic responses of tobacco seedlings exposed to silver nanoparticles and silver nitrate

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The small size of nanoparticles (NPs), with dimensions between 1 and 100 nm, results in unique chemical and physical characteristics, which is why they are being implemented in various consumer products. Therefore, one of the important concerns is the potential detrimental impact of NPs on environment. As plants are a vital part of the ecosystem and the first component of the food chain, the investigation of NPs phytotoxic effects is of particular interest. In this study, we investigated the potential phytotoxicity of commercial citrate-coated silver nanoparticles (AgNPs) on tobacco (*Nicotiana tabacum*) seedlings and compared it with the effects of the same AgNO<sub>3</sub> concentrations. The Ag content was higher after exposure to AgNPs compared to AgNO<sub>3</sub> of the same concentration. However, elevated ROS formation, MDA and protein carbonyl content, as well as DNA damage was recorded in AgNO<sub>3</sub>-treated seedlings, which suggests a direct uptake of AgNPs by tobacco cells and high stability of applied AgNPs. Furthermore, the activity of antioxidant enzymes, particularly superoxide dismutase (SOD) and ascorbate peroxidase (APX), was in general induced in all AgNP treatments. The upregulation of SOD and APX was additionally confirmed by proteomic analysis. This indicates that seedlings are exposed to oxidative stress but are successfully coping with it. Moreover, this ultrastructural and proteomic study revealed that photosynthesis was the biological process on which exposure of tobacco seedlings to both types of treatments had the most significant impact, although this was more pronounced in the AgNO<sub>3</sub>-treated seedlings. All these findings indicate that AgNPs can induce phytotoxic effects but are less detrimental for tobacco seedlings compared to AgNO<sub>3</sub>.

KEY WORDS: Nicotiana tabacum; oxidative stress; superoxide dismutase

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#### The role of nutritional status in the elderly and nutritionalgerontologic standards

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In the elderly, nutritional status is of utmost importance for the occurrence of the geriatric domino effect in the treatment of geriatric patients with the predominant 4I + 1P syndrome (immobility, loss of independence, instability, and incontinence) with a fifth component (unfavourable therapeutic outcome with polypharmacy). This especially refers to geriatric underweight patients where it is associated with increased morbidity and mortality rates. The authors, experts engaged in the development of the Nutritional Guidelines for the Elderly in Croatia, conducted continuous training on the implementation of the Guidelines and the monitoring of nutritional status in the elderly via NRS 2002/GeroS/CEZIH web service, all this with a support from professional societies and relevant institutions. The objective of the Guidelines is to support the nutritional status assessment in the elderly at risk of malnutrition, to define specific nutritional needs of the elderly, and to aid in choosing the most appropriate nutritional support. Malnutrition is considerably more frequently recorded in old people's homes, where it ranges from 19 % to 30 %, depending on the category of geriatric patients; hospitalised elderly individuals are most frequently exposed to malnutrition, i.e. up to 65 % of those in deep old age ( $\geq$ 85 years). The Guidelines present specific nutritional needs and recommendations for energy and nutrient intake. Preparation and planning of meals should be consistent with the nutritional-gerontologic standard, in accordance with physiologic requirements of the elderly and with diseases that require specific nutritional adjustments.

KEY WORDS: malnutrition; geriatric patients; old people's homes

#### Monitoring nutritional status in the elderly via NRS 2002/ GeroS/CEZIH web service

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Inappropriate nutritional status in persons older than 65 in particular manifests as deterioration of their health condition and functional ability, as well as increased mortality and geriatric consumption. Therefore, the NRS 2002 web service has been implemented in the GeroS/CEZIH gerontologic information system; besides the Nutritional Risk Screening 2002 (NRS-2002) as a fast method of nutritional screening, the NRS 2002 web service includes focused gerontologicpublic health indicators such as age group, sex, primary and secondary diagnoses in geriatric patients, basic anthropometric measurements, functional ability, etc. Functional ability (physical and mental) of the elderly is one of the key determinants that is assessed, monitored and analysed in healthcare of the elderly. The innovative program of the NRS 2002/GeroS/ CEZIH web service is used by physicians and other healthcare professionals in hospitals, family medicine teams, 'geriatric' nurses in old people's homes, and home visiting nurses in primary healthcare. The gerontologic-public health analysis of focused determinants of monitoring via NRS 2002/GeroS/CEZIH web service (1 March 2015 – 31 August 2017, Croatia) in individuals aged >65 (N=375; geriatric and gerontologic insurees) estimated the functional ability based on physical mobility and mental independence according to entities/site of nutritional screening. Results of the gerontologic-public health analysis of focused determinants of monitoring via NRS 2002/GeroS/CEZIH web service (1 March 2015 – 31 August 2017, Croatia) in individuals aged >65 (N=375; geriatric and gerontologic insurees) according to entities/site of nutritional screening showed that most of them were recorded in general/family medicine (n=141), whereas only one person was recorded in the Gerontologic Centre. Analysis of functional ability based on physical mobility revealed that most of the subjects were in the category of completely mobile (n=158) and according to mental independence in the category of completely independent (n=186). Results of the final screening revealed that 94 (25 %) subjects were in the group of nutritional risk (≥3). Study results point to a high prevalence of the gerontologic-public health problem of malnutrition in the elderly because data obtained via NRS 2002 web service revealed nutritional risk in as many as onequarter of the subjects. The NRS 2002/GeroS/CEZIH web service proved highly useful in timely determination of nutritional risk in persons aged >65, with concomitant monitoring of the defined and targeted determinants (e.g., functional ability) that are important in individualised gerontologic approach. In healthcare of the elderly with inappropriate nutritional status, evaluation of the indicators of therapeutic outcomes is of utmost importance, which is enabled promptly, in real time, by use of the NRS 2002/GeroS/CEZIH web service.

KEY WORDS: gerontologic; malnutrition; NRS 2002

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#### **Physical Activity and Nutrition**

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The aim of this paper was to demonstrate the relevance of choosing a proper and healthy nutrition plan appropriate for the type and intensity of a chosen physical activity. It is essential to adjust the quantity and type of nutrients carried into the body either through food or through fluids before, during, and immediately after a certain type of physical activity in order for them to be in accordance with the aim of the training. The intake of proteins, carbohydrates and fats are most frequently associated with physical activity. Proteins are made up of amino acids linked by peptide bonds and while only some 20 types of amino acids can be found in proteins in food and in the human body, the number of amino acids found generally in nature substantially exceeds that number. Physical activity requires an increase of protein intake. According to numerous authors, the recommended protein intake for an adult equals 0.8 g kg<sup>-1</sup> of a person's body mass (BM). Endurance trainings require an increase up to between 1.2 and 1.4 g kg<sup>-1</sup> of BM, whereas power and strength trainings require an increase of protein intake up to between 1.2 and 1.7 g kg<sup>-1</sup> of BM. The mentioned protein intake should be properly distributed during the day; however, the greatest benefits are achieved when carried in immediately after the physical activity. In more simple terms, carbohydrates are the basic source of energy of the anaerobic and aerobic metabolism, and as such, they represent the main nutrient for preparations for endurance exercises. Glycogen in the liver and in the muscles (carbohydrate reserves) presents a restrictive factor during long-term physical activity. The average recommended carbohydrate intake is between 40 and 60 % of the overall quantity of keal during a day, while this recommendation is somewhat higher for the physically active population. Carbohydrate intake before physical activity has several benefits, among which, the most important is to resupply the stock of muscle glycogen that was spent during the previously completed activity. The general recommendation for carbohydrate intake before a certain activity is to carry in as much g kg<sup>-1</sup> of BM as the number of hours between the meal consummation and the beginning of the activity. During a certain type of physical activity carbohydrates are most often provided through beverages with 6 to 8 % of carbohydrates, which is the required amount for physical exercise lasting longer than 60 minutes. After finishing a physical activity, in order to ensure for a quality restoration of glycogen supplies, the optimal intake of carbohydrates equals between 1 and 1.5 g kg<sup>-1</sup> of BM during the first 30 minutes, as well as each two hours over 6 hours after the activity. Contrary to proteins and carbohydrates, not as many studies were conducted when it comes to the role of fats in nutrition of athletes and physical activity in general. Common recommendations and guidelines that apply for the general population are equally utilized for the physically active population. Furthermore, physical activity increases the need for micronutrient intake (vitamins and minerals) because of the increased consumption, as well as due to the building, renewal, and maintenance of lean body mass, however, nutritional supplements containing micronutrients are justified only in specific situations. Water is the essential and quantitatively most important nutrient although the need for fluid intake does not substantially increase during activities of shorter duration and lower intensity. To conclude, the effects of physical activity are directly related to the amount and quality of food that we carry into our body before, during, and after physical activity. The ratio between proteins, carbohydrates, various micronutrients, and regular hydration of the body significantly contribute to the quality of results of physical activity.

KEY WORDS: carbohydrates; hydration; nutritional recommendations; proteins; training



## Prevalence and antimicrobial resistance of Gram-negative bacteria isolated from food samples in the Zenica-Doboj Canton, Bosnia and Herzegovina

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Gram-negative bacteria have been increasingly detected in environmental samples in different countries and have gained considerable attention worldwide. The aim of this study was to present the prevalence, antimicrobial resistance and distribution of Gram-negative isolates collected from food samples in the Zenica-Doboj Canton, Bosnia and Herzegovina, during May to December 2015. All of the food samples were analysed according to ISO standards. The antibiotic susceptibility of all of the isolates was determined using the disk diffusion method. The production of ESBLs was determined by double-disk synergy test. Among the food samples (n=1603), 61 (out of 91 positive samples, 67.0 %) were detected as Gram-negative isolates. The results showed that 25 (out of 91; 27.5 %) positive samples were collected from milk and dairy products, 20 (22.0 %) from cakes and ice cream, and 19 (20.9 %) from meat and meat products. The most prevalent Gram-negative bacteria isolated from food samples were *Klebsiella* spp. (n=19; 31.1 %), non-fermenters (n=9; 14.7 %), *Escherichia coli* (n=8; 13.1 %), *Proteus* spp. (n=8; 13.1 %), *Citrobacter* spp. (n=8; 13.1%), *Salmonella* spp. (n=6; 9.8 %), *Morganella morganii* (n=2; 3.3 %) and *Enterobacter* spp. (n=1; 1.7 %). All of the isolates showed low resistance to amoxicillin/clavulanic, cefuroxime, cefpodoxime and aztreonam, except non-fermenters with a range of 50-70 %. Beta-lactamases were detected by phenotypic test in eight (13.1 %) and chromosomally or plasmid AmpC beta-lactamases in 30 (49.2 %) Gram-negative isolates. Environmental surfaces are important reservoirs of Gram-negative bacteria, which can be transmitted to humans through the food chain. It is globally important to monitor the hygiene of food samples and detect the sources and modes of the bacterial spread that produced beta-lactamases.

KEY WORDS: antibiotics; beta-lactamase; food safety; hygiene

### Control of histamine in samples of fish and its products as food risk

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Histamine poisoning is a foodborne disease characterized by a variety of symptoms similar to allergic reactions: diffuse redness of the face with itching around the mouth, redness around the eyes, sweating, nausea, vomiting, cramps in the abdomen, heart palpitations, and chest pressure. Histamine is a biogenic amine that occurs in fish tissue by the decarbocylation of free histidine by the exogenous decarboxylase exempted from a microorganism. Statistics on the incidence of histamine poisoning are not available (for Bosnia and Herzegovina and Republika Srpska) due to often mild and unrecognized symptoms and inadequate systems of reporting foodborne diseases. The aim of this study was to determine the frequency of the presence of histamine in the food group "fish and fish products". Samples of fish and fish products (n=109) were delivered from a Republika Srpska market. The histamine content was analysed using HPLC (High Performance Liquid Chromatography). The results of the analyses were interpreted in accordance with regulations. Within the study, 17.64 % of unsafe samples were found. Increased histamine concentrations were found in samples of fresh marine fish-salmon; canned sardines with vegetables, and canned salmon in marinade. The risk of histamine poisoning can be reduced by implementing good hygiene practices in accordance with the Hazard Analysis Critical Control Point (HACCP) system, which must be established by everyone in the chain of handling fish and fish products. It is also necessary to establish monitoring procedures for fish and products with high levels of histidine in order to ensure a higher level of health safety. In accordance with EU legislation, fish must be stored and handled at the appropriate temperature (ice surface) as soon as possible after being caught. This way, the contamination and growth of bacteria that affect the production of histamine are avoided, since histamine is thermostable and is not destroyed by conservation or smoking.

KEY WORDS: allergy; fish contamination; food monitoring; food safety

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#### Quality control of milk from milk vending machines

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The aim of this study was to determine the physicochemical and microbiological quality of milk sampled directly from 36 milk vending machines in Zagreb, the Zagreb County, and Sisak, from December 2015 to January 2017. The content of milk fat, protein, lactose, and total solids was determined by infrared spectrometry (ISO 9622). The freezing point of milk was determined by thermistor cryoscope method (ISO 5764). The hygienic quality of milk was determined on the basis of somatic cell count by fluoro-opto-electronic method (ISO 13366-2) and the total bacterial count by flow cytometry method (ISO 21187). Determination of physicochemical composition and hygienic quality of milk were conducted in the Reference Laboratory for Milk and Dairy Products, Department of Dairy Science, Faculty of Agriculture, University of Zagreb. Additional microbiological analysis included the determination of psychotropic bacteria (ISO 6730), lactic acid bacteria (ISO 15214), *L. monocytogenes* (ISO 11290-2), *S. aureus* (ISO 6888-3), *E.coli* (ISO 16649-2), *Y. enterocolitica* (ISO 10273), *Pseudomonas* spp. (ISO 11059), were conducted at the Department of Hygiene, Technology and Food Safety, Faculty of Veterinary Medicine, University of Zagreb. The results showed that the physicochemical properties of milk complied with the Regulation on Determination of Raw Milk Composition (OG 27/17). The microbiological quality of milk was poor since over 60 % of the analysed samples contained more than 100,000 bacteria mL-1. The results also showed variation of the microbiological quality of milk, with a tendency of improvement in the majority of samples. Due to potential risks to human health from raw milk and based on our results it can be concluded that systematic quality control of milk from milk vending machines is necessary. This systematic quality control would increase the level of competitiveness of domestic products on the market, as well as protect the interests of the consumers.

KEY WORDS: consumer safety; food safety; microbiological analysis; quality control

### The effect of changing the pH of the solution on the migration of aluminium from zeolite

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This case study was performed to evaluate the effectiveness of pH change on the migration of aluminium (Al) from zeolite, which shows good properties as an adsorbent and ion exchanger and is often used as an oral supplement to adsorb toxic elements. However, zeolites can be a potential source of elements that, in certain quantities, can endanger the health and safety of humans and animals. Three commercially available zeolites for oral consumption from Serbia and one from Greece were prepared according to the manufacturer's instructions for use and tested. In order to determine the reproducibility of the test, the experiment was repeated after a few days. In this case, for each individual repetition pattern, it was performed in several samples at pH 2 (6 h migration) as well as at a variable pH from 2 to 8 (at pH 2, 2 h and after changing to pH 8, 4 h; total of 6 h of migration) at a temperature of 37±2 °C. The amount of extracted Al was significantly different when we compare the values obtained at pH 2 and pH 8. The extracted amount of Al from the zeolite at pH 2 in all four of the samples ranged from 0.8 to 20 mg per single dose, and the amount of Al at pH 8 drastically decreased in the migration solution (0.03-0.6 mg per single dose). The reason for this may be its reabsorption from the solution by zeolite or precipitation. The amount of extracted Al at pH 2 was not negligible compared to the tolerable weekly intake for this element, which is 1 mg kg<sup>-1</sup> b.w. All this suggests a need for further studies for zeolite effects not only as an adsorbent and ion exchanger but as a source of chemical elements due to leaching in the gastrointestinal tract under aggressive conditions.

KEY WORDS: consumer safety; oral supplements; toxicity of commercially available products

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### Nutritional quality of meals sampled in public preschools in Belgrade

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Constant exposure and access to energy rich and nutritionally deficient food as well as the increasing prevalence of obesity and other conditions related to unhealthy diets are particularly important in childhood due to the possible long-term health effects. Considering that the preschool period (up to age 6) is key for adopting healthy eating habits and a growing number of children is attending preschools, these facilities have become very important for the adoption of healthy nutritional habits. The study aimed at assessing the quality of diet in public preschool facilities in Belgrade through the comparison of macronutrient content (protein, fat, and carbohydrates) and energy value of sampled meals with norms. The data on the macronutrient composition of meals were obtained through chemical-bromatology analysis of sampled whole-day meals (breakfast, lunch, and a snack) in public preschools in Belgrade from 1 January 2012 to 31 December 2016. Data were processed by specially designed software for collection and analysis of food samples from public preschools. Data were analysed using descriptive and inferential statistics. The average energy value of the sampled meals (n=3331) during the period was 3826 KJ (1 to 3 years) and 4896 KJ (4 to 7 years). Meal samples generally met the defined norms in terms of protein and carbohydrates in both age groups. In terms of fat content, nearly half of the sampled meals in both age groups were below the lower normative limit for fat. In this aspect, greater discrepancies from the normative were determined in the 1 to 3 years age group. The quality of meals in terms of the most parameters over the selected period generally fulfilled norms and leaves room for further improvements, especially with regard to fat content.

KEY WORDS: children's nutrition; dietary habits; preschool children

## Determination of benzimidazole and levamisole residues in animal liver tissues using liquid chromatography-tandem mass spectrometry

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Benzimidazoles and levamisole are veterinary drugs widely used in the prevention and treatment of parasitic infections in agriculture and aquaculture. The group of benzimidazole drugs comprises albendazole, albendazole sulphoxide, netobimin, febantel, fenbendazole, oxfendazole, mebendazole, flubendazole, thiabendazole, triclabendazole, and oxibendazole. Residues of benzimidazoles and levamisole in liver can occur due to incorrect treatment, because of excessive amounts of the drug in relation to the recommended dosage, due to non-compliance to the withdrawal period, or because the animals consume contaminated food. For most benzimidazole residues in samples, the marker is defined as the sum of the proportions of the drug and/or the most common or the most persistent metabolites. During the metabolic degradation of benzimidazole drugs in animal organisms, one or more major metabolites of a particular drug are produced, which have been identified in the liver. In order to protect the health of consumers, the European Union has established maximum residue levels (MRLs) of benzimidazoles and levamisole in liver (Commission Regulation 37/2010). For the determination of benzimidazole and levamisole residues in liver, the confirmatory method of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is used, validated according to Commission Decision 2002/657/ EC. A total of 62 samples of animal liver were collected during 2016 and analysed for benzimidazole and levamisole drug residues. None of the samples exhibited concentrations above the maximum level, which indicated that animal liver in Croatia is safe for consumption.

KEY WORDS: food contamination; food safety; veterinary drugs; quality control

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### The assessment of quality and safety of used frying oils in restaurants and fast food facilities

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Deep frying is one of the most frequent ways of food preparation, both in households and in fast food facilities. The oils are often used repeatedly without taking into account the length of use and formation of harmful compounds throughout the degradation processes that take place during frying (hydrolysis, oxidation, polymerization). Harmful products are absorbed into the prepared food and then ingested. The influence of used frying oils on chronic disease incidence has been the subject of numerous studies. Most countries have specific regulations and guidelines on the use and handling of frying fats and oils with diverse control parameters; however, in the Republic of Croatia, no such regulation exists. Therefore, in an attempt to evaluate the true situation, 255 used oil samples were sampled in various catering facilities in the Split-Dalmatia County in the period 2010-2014. The oil samples were analysed for free fatty acids (FFA) content (HRN EN ISO 660:2010), peroxide value (PV) (HRN EN ISO 3960:2010), the most common indicators of possible degradation processes in fats. FFA and PV levels exceeded the maximum permissible values set for fresh refined oils in 42.7 %. and 32.1 % of samples, respectively. Furthermore, in 9.8 % of samples, PV was several times higher than in fresh oil. Considering that primary oxidation products (expressed through the PV) are unstable and rapidly break down to secondary oxidation products, the level of oxidation in the analysed samples was certainly higher. The obtained results indicate the necessity to establish national regulations and guidelines on the use and handling of frying fats and oils. It is important to develop systematic control over the quality of frying oil in catering facilities, which should include additional analyses.

KEY WORDS: deep-fried food; food safety; free fatty acids; peroxide

### Labelling and consuming dry fruits – hidden intake of sucrose

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Healthy diets and recommendations on the consumption of certain types of food, especially dry fruits, are very common and well accepted. In its new guidelines, the WHO suggests reducing the daily intake of free sugar for adults and children to below 10 % of the total energy intake, based on scientific evidence that this will decrease the risk of obesity and tooth decay. The term free sugars refer to the amount of sugar (glucose, fructose, and sucrose) added to food during production or preparation and the sugars naturally present in certain food types (honey, syrups, fruit juices, and fruit juice concentrates). According to the WHO, most sugars introduced into the body are "hidden" in prepared products usually not considered sweet. Labels such as "dry fruit" can be misleading for the customer and conceal added sucrose, even if it is (mostly on the back of the packaging or label) properly indicated in the ingredients. Therefore, in 32 samples of dried, candied and fresh fruit from the market, the fructose, we analysed glucose and sucrose content in order to determine whether sugar was added. The analyses were performed on HPLC, RI detector, column: Shodex Asahipak, NH2P-504E. Fructose, glucose, and sucrose ratios were determined in each type of fruit, in fresh and dry form. The obtained results indicated the addition of sucrose in certain types of "dry fruit", more precisely in "dry cranberry", "dry papaya", some samples of "dried apricots", and banana chips. In samples of raisins, dried figs, dried plums, and some dates, no sucrose supplements were proven. The biggest issue with hidden sucrose intake are incorrect labels with inaccurate names that fail to provide information that the product is candied instead of dried. A particular issue are products sold in bulk under the name "dry fruit" without the list of ingredients on the label.

KEY WORDS: consumer safety; false labelling of food; WHO guidelines

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### Incidents of histamine intoxication in Slovenia – lessons learned

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The aim of this study was to evaluate all incidents of histamine intoxication from tuna meat from 2012 to 2016 and assess the need for preventive measures. For this purpose, we traced back epidemiological and analytical data. Analysed epidemiological data were collected as part of a routine reporting system of foodborne diseases and outbreaks. Analytical data on histamine content in the analysed foods collected on the investigated incidents were also included. There were 4 outbreaks of histamine intoxication in Slovenia in 2016, one cluster in 2015 as well as one in 2012, with a total of 21 persons affected. Urgent antihistaminic and supportive therapy relieved symptoms in a few hours. Complications or deaths did not occur. Histamine concentrations in tuna meat samples were below 200 mg per 1000 g. Different sources of potentially toxic tuna meat were discovered. Close cooperation and coordination of caterers, medical personnel, epidemiologists, and sanitary health officers is necessary for obtaining leftover of suspected tuna meat for laboratory analyses and verifying the source of intoxication. The reporting of such cases linked to tuna and other foods to the reporting system of foodborne diseases should be mandatory because of the potential severity of the intoxication. Cases should be investigated thoroughly to find the possible source of intoxication and suggest preventive measures for the future. A possible cause for the increase of histamine intoxication could also stem from the illegal practices in the tuna industry. În July 2016, the European Commission received information that the tuna industry used unauthorised additives to enhance the colour of the tuna, selling tuna of low quality as high quality tuna. After using nitrite, histamine concentrations are impossible to verify.

KEY WORDS: disease outbreaks; disease prevention; foodborne diseases; illegal industry practices; tuna fish

#### Foodborne outbreaks in Slovenia

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The aim of the study was to evaluate the epidemiological characteristics of foodborne outbreaks in Slovenia, all of which were reported by regional epidemiologist. Outbreaks caused by ingestion of drinking water are also considered foodborne since drinking water is defined as food. Data on the causative agents, foodstuffs implicated, setting, places of origin of problem, and contributory factors were collected through investigating specific outbreaks. The outbreaks for which the causative agent was the norovirus were excluded because of the high possibility of contact transmission. There were 200 foodborne outbreaks in Slovenia from 2002 to 2016. The most common causative agent was Salmonella. A total of 5791 persons were infected, 663 were hospitalized, and 11 died. The male to female ratio was 1:1. Most outbreaks were defined as "possible" with descriptive epidemiological evidence only. Route of transmission was probably food but the foodstuff implicated in the outbreaks was mostly not identified. In the outbreaks in which the food was identified, the most common products were broiler meat. A prompt response to notification and epidemiological investigation of any case of suspected foodborne outbreak is needed to prevent further spreads of disease. Foodborne outbreaks should be investigated thoroughly to find the possible source of infection and suggest preventive measures for the future. Close cooperation and coordination of caterers, medical personnel, epidemiologists, and sanitary health officers is necessary for obtaining leftover of suspected food for laboratory analyses and verifying the source of the infection. Molecular diagnostics can prove the exact source of infection when in doubt. Storing food samples at the caterers for at least 48 hours after serving the meals should be mandatory for premises serving large numbers of meals.

KEY WORDS: disease prevention; disease transmission; epidemiology; food safety

#### Migration of toxic components from food contact materials

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The aim of this study was to present the results of sample analysis on food contact materials (FCM) during 2016. Samples were submitted by the Sanitary Inspection during import and monitoring and by private entities. The results obtained were compared with MDK values determined by reference legislation depending on the type of material from which they were made. In total, 577 samples were statistically processed. The parameter type was defined by the type of material from which the sample was made. In accordance with legislation, samples were categorized into those made of artificial mass, stainless steel, enamel, clay, ceramic and porcelain, glass, paper, wood, and other materials. The highest number of analysed parameters was determined by inductively-coupled plasma mass-detector (ICP-MS) and gravimetrically. From the total number of analysed samples, the most represented were artificial materials (44.71 %), other materials and articles (27.55 %), clay ceramic and porcelain articles (11.27 %), stainless steel (5.72 %), paper (5.20 %), glass (3.64 %), wood (1.39 %), and enamel (0.52 %). As many as 97.92 % of samples were rated compliant and 2.08 % were nonconforming. Among the non-conforming samples, 41.66 % was stainless steel (heavy metal migrations), 25.0 % artificial mass (sensory properties), 16.67 % clay ceramic and porcelain items (bad finish), 16.67 % other materials (sensory properties). Most samples were delivered by private subjects (77.48 %), state inspectors (15.59 %), and monitoring (6.93 %). The results obtained regarding the increased amount nickel and manganese, the lack of suitable sensory properties, and poor final processing indicate that manufacturers place on the market products that can be potentially hazardous to human health. Negative effects can be triggered by a certain type of food as well as certain risk factors (age group and individual health status). In the interest of consumer protection, it is necessary to conduct systematic market control.

KEY WORDS: artificial mass; heavy metal migration; ICP-MS; sensory properties; stainless steel

#### Detection of enterotoxin production from Staphylococcus aureus strains by automatized VIDAS system

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Staphylococcal enterotoxin (SE) is a product of Staphylococcus aureus. Staphylococcal food poisoning is caused by the introduction of a staphylococcal enterotoxin resistant to heating. Several types of these toxins (A, B, C, C1, C2, C3, D, E, F, H, G, I, etc.) have been identified and described thus far. The most common type of enterotoxin is SEA (in about 75 % of cases) followed by SED. The toxin develops more easily when prepared foods contaminated with this bacterium stay at room temperature for a longer period. Food can be contaminated by carriers or people with an active skin infection. The aim of this study was to determine the presence/production of enterotoxins in *Staphylococcus aureus* strains using VIDAS Staph enterotoxin II (SET 2). VIDAS Staph Enterotoxin II (SET) is an automatic qualitative test for use on VIDAS instruments for the detection of staphylococcal enterotoxins by ELFA technique (enzyme-linked fluorescent immunoassay). The suspension is made from isolated Staphylococcus aureus strains. A 500 µL suspension is placed in the sample well. The reagents for analysis are ready for use and are found in a closed reagent strip. All of the steps in the analysis are performed automatically. Once the analysis is complete, the results are analysed automatically and each sample is interpreted as positive or negative. Out of the 20 total examined Staphylococcus aureus strains, two exhibited the production of enterotoxin (10 %). The test was used as a screening test for the detection of staphylococcal enterotoxins in food. The presence of enterotoxins directly from isolated strains of Staphylococcus aureus was detected. This test can be also useful in hygienic-epidemiological studies.

KEY WORDS: ELFA; food contamination; staphylococcal enterotoxins

#### **Determination of allergenic residues of caseinates in** Croatian wines by enzyme-linked immunosorbent assay (ELISA)

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With consumer safety in mind, the European Union imposed an obligation of labelling allergenic substances used in wine production. Caseinates are used in the winemaking process as a fining agent for white or rosé wines to remove phenolic and tannin compounds, but such wines are not usually labelled for allergen presence ("contains caseinates"). Because of the mild solubility of caseinates in acidic or low pH solutions as wine, it could be assumed that they are completely removed during the production process, but their possible presence in treated wine cannot be completely excluded. Any residues could be a risk for individuals allergic to milk. Allergic reactions to milk proteins are most commonly associated with alpha S1-casein and its derivatives, such as caseinates. The aim of this study was to determine caseinate residues in samples of Croatian wine by Enzyme-linked immunosorbent assay (ELISA). Samples were randomly chosen during the procedure of official wine quality control in the process of obtaining a licence for marketing the wine. Selected wines of unknown oenological practices were analysed for the possible presence of allergenic caseinate residues. To exclude the possible impact of other wine compounds on caseinate activity, the following physicochemical parameters were measured: relative density, alcoholic strength, reducing sugars, ash, pH, total and volatile acidity, and free and total sulphur dioxide. The measurement of caseinates was conducted with commercial Enzyme-immunosorbent assays (LOD=0.25 mg kg-1; LOQ=0.5 mg kg<sup>-1</sup>). Samples of white and red wine, positive controls (spike), and negative controls (allergen free), were measured double-blinded. No detectable casein residues were found in the tested samples, regardless of the levels of the other physicochemical parameters measured. All of the tested wines were casein-free and safe for the customers.

KEY WORDS: allergies; casein; quality control

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#### Application of membrane filtration with the possibility of removing the LAB-AFM<sub>1</sub> complex from milk

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Previous scientific studies indicated a need for the further development of applying biological methods in the removal/ binding or biotransformation of mycotoxins in food and feed. Such methods should inactivate mycotoxins without affecting the nutritional value and organoleptic properties of the product being processed. In practice, synthetic and natural zeolites are most commonly used as fodder additives, which effectively eliminate aflatoxins through adsorption; however, the drawback is that necessary nutrients are also absorbed in the process. Therefore, the microbial population from fresh cow's milk and traditional dairy products such as cottage cheese and cream was isolated and identified with the aim of selecting bioprotective cultures as a biofixator for the removal of AFM, from milk. One hundred and fifty colonies of LAB were isolated and identified by API tests and ten strains were confirmed with molecular (PCR) methods. All of the confirmed strains (Lactobacillus plantarum SM1, Lactobacillus helveticus S9, Lactococcus lactis 5MS1, Lactobacillus plantarum SMB, Lactobacillus plantarum SMA, Lactobacillus plantarum SS1, Lactobacillus paracasei KM, Lactobacillus rhamnosus KM, Lactobacillus plantarum KM, Lactobacillus plantarum MM) were tested for possible removal/binding of AFM, from milk. Therefore, one of the goals of the study was to implement the practical application of membrane filtration with the possibility of removing the complex biofixator-AFM, from milk and preserving the nutritional value of milk. The amount of unbound AFM, was determined by HPLC.

KEY WORDS: aflatoxin elimination; aflatoxin M; food safety; lactic acid bacteria

#### A fast screening method for the determination of melamine in high protein food

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The purpose of this study was to find an easy, fast, and cost-effective method for the determination of melamine in high protein food, especially baby food and protein shakes, and analyse and examine the submitted samples if they had been adulterated by melamine. To this end, samples of baby food and protein shakes from world-known brands were analysed. The samples were homogenized and after extraction with a suitable organic solvent, a DSA-TOF (direct sample analysistime of flight) instrument was used to identify the possibility of melamine presence. Separation was achieved under 2 min. The detection was performed by DSA-TOF in positive polarity mode. Identification was based on an accurate mass measurement of the protonated ion. The results in this paper are based on a very small number of analysed high protein food samples. All of the analysed samples were negative for the presence of melamine. It is evident that melamine occurs in high protein food as an adulterant. Many studies have been conducted concerning the illegal addition of melamine as a cheaper substitute for food proteins. Due to a very small number of analysed samples, it would be wise to implement a study that would cover a large number of different products that can be ordered online and which probably do not undergo official controls.

KEY WORDS: baby food; DSA-TOF; food quality; protein shakes

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#### **Determination of corticosteroids using liquid** chromatography/time-of-flight mass spectrometry

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Undeclared corticosteroids in products intended for frequent use might cause serious side-effects. In order to prevent this or find the cause, it was essential to develop a method for quick detection and quantification. This work investigated the applicability of reversed-phase liquid chromatography coupled to time-of-flight mass spectrometry (LC-TOF-MS) for qualitative and quantitative determination of corticosteroids in different cosmetic products and dietary supplements. Nine corticosteroids were used: prednisone, prednisolone, hydrocortisone, dexamethasone, hydrocortisone acetate, triamcinolone acetonid, clobetasole propionate, betamethasone dipropionate, and alclomethasone dipropionate. Separation was achieved under 15 min on an Inertsil ODS-4 C18 column using gradient eluation with 5 mmol L<sup>-1</sup> ammonium acetate in water and acetonitrile as mobile phases at a flow rate of 0.3 mL min<sup>-1</sup>. The detection was performed with a TOF mass spectrometer in positive polarity equipped with an electrospray interface. Identification was based on accurate mass measurement of protonated ion [M+H]+ and LC retention time of the analyte compared to the standard. The method was validated in terms of specificity, precision, accuracy, detection and quantification limits, linearity, and recovery. The validation parameters met all acceptance criteria. An LC-TOF method was developed and validated for the rapid and simultaneous determination of nine corticosteroids. The proposed method can be used for testing different products such as dietary supplements and cosmetic products (creams and ointments). Regular and consistent monitoring of the use of corticosteroids is needed. However, additional efforts are needed to improve the extraction of analytes from different matrices.

KEY WORDS: cosmetic products; dietary supplements; LC-TOF-MS; quality control

#### The trend of chlorpyrifos concentrations in food samples exceeding the MRL

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The aim of this study was to analyse food samples on pesticide residues using multiresidue methods in order to define which pesticide had the most frequent occurrence in the analysed samples and why. The analysed samples included fruit and vegetables taken during official controls. The samples were prepared using QuEChERS method according to HRN EN 15662/2008 and analysed with GC-MS/MS. In total, 554 samples were analysed using multiresidue method. Of the 554 samples, 13 samples had pesticide residue concentrations above the maximum residue level (MRL). Pesticides identified and quantified above the MRL were bromopropylate and chlorpyrifos. The quantified pesticide residue concentrations ranged from 0.026 to 1.35 mg kg<sup>-1</sup>. Samples that contained pesticides above the MRL were pear (1), dried wild apples (1), leek (2), and apples (10). It is evident that chlorpyrifos concentrations above the MRL occurred more frequently than with other pesticides. With leek, the manufacturer was using the product with active substance chlorpyrifos which was not compliant for use on leek. In case of dried wild apples, the manufacturer mixed wild apples with conventional ones. The MRL for chlorpyrifos on apples changed in 2016 from 0.05 mg kg<sup>-1</sup> to 0.01 mg kg<sup>-1</sup>. The apple manufacturers were not aware about changes to the MRL or did not possess the proper training on pesticide usage.

KEY WORDS: fruit and vegetable safety; GC-MS/MS; pesticides

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#### Antimicrobial profiling of phytol and their multidrug resistance modulating effects

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Food contamination by multidrug resistant Gram-negative bacteria can be a major threat to the public. The prevalence of antibiotic resistance among Gram-negative foodborne pathogens and food spoilage bacteria has increased during recent decades; therefore, the food chain has been recognized as one of the key routes of antimicrobial resistance transmission from food to human bacterial populations. Consequently, a potential way to restore some of the resistance is to use plantbased compounds as sources of novel and promising natural antimicrobials and resistance modifying agents have attracted enormous scientific interest. Plant diversity constitutes an infinite pool of novel chemistry, making up a valuable source of highly effective phytochemicals to prevent the emergence of resistant bacteria in the food industry, by reducing or modulating the resistance and can thereby improve the efficiency of the processed food, ensure food quality and safety of the products, as well as human health. Nowadays, diterpenes are widely employed as fragrances and food additives. However, limited studies are available not only for the direct antimicrobial efficacy of diterpene alcohol such as phytol, but also for resistance-modifying agents. In this study, antimicrobial screening was performed using the agar-diffusion method against a panel of both clinically relevant antibiotic susceptible Gram-positive and antibiotic resistant Gramnegative bacteria that harbour resistance genes for the most commonly important antibiotics. In addition, phytol was then evaluated in the quantitative bioassay to determine the minimum inhibitory concentration (MIC) based on broth microdilution. Finally, in vitro activities of phytol were also performed to confirm their ability to restore the resistance of the clinically and environmental Gram-negative strains producing a wide range of β-lactamases from A and C molecular classes and a possible mechanism of action responsible for the antibacterial activity was also examined.

KEY WORDS: antimicrobial activity; antibiotic resistance;  $\beta$ -lactamases; diterpenes

#### Determination of ergot alkaloids in animal feed by liquid chromatography tandem mass spectrometry

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Ergot alkaloids are mycotoxins produced by fungi of the Claviceps species, mainly Claviceps purpurea, which can infect different cereals such as rye, wheat, barley, and corn. Since these fungi are widespread, there is great risk of food and feed contamination. Ergot alkaloid poisoning causes a disease called ergotism, and some of the symptoms are abdominal pain, nausea, hallucinations, and gangrene. Among the more than 40 known ergot alkaloids, the most common ones are ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine, and their corresponding epimers. No maximum levels of ergot alkaloids in food and feed have been legislated, although the acute reference dose for the sum of the ergot alkaloids is set at 1 µg kg<sup>-1</sup> b.w. There is ongoing research on their presence in food and feed, and the enactment of legislation for maximum levels of ergot alkaloids should follow in the near future. This paper presents the liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the determination of the 6 most common ergot alkaloids and their epimers in feed. The Dutch Institute of Food Safety (RIKILT) conducted an inter-laboratory validation study in order to standardize the method of analysis. Feed samples with known concentrations of ergot alkaloids were dispatched to all of the participants, including the Reference Laboratory for Mycotoxins within the Andrija Stampar Teaching Institute of Public Health. Samples were extracted with a mixture of methanol and water acidified with formic acid and purified by ultrafiltration. Analytes were quantified by multilevel standard addition. Chromatographic separation was achieved on a Gemini C18 column (150x20 mm, 3 μm) with mobile phases consisting of 1 mmol L-1 ammonia in acetonitrile and in water. Results showed good chromatographic separation and recoveries, as well as low levels of quantification. From these results it can be concluded that this method is suitable for the determination of ergot alkaloids in feed, as well as in other cereal-based products.

KEY WORDS: alkaloid epimers; food and feed contamination; LC-MS/MS; mycotoxins

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#### The nutritional and antioxidant properties of strawberry tree (Arbutus unedo L.) fruit

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Arbutus unedo L. grows in the Mediterranean as a wild evergreen shrub. It is used in folk medicine as an antiseptic, diuretic, and laxative, as well as for treating cardiovascular and gastrointestinal diseases. It has also shown significant antiproliferative properties and its health benefits are mainly attributed to phenolic compounds such as flavonoids, phenolic acids, and tannins. Literature data reveals the strawberry tree fruit as a good source of polyphenols, vitamin C, and dietary fibres as well as minerals with significant potassium, calcium, and phosphorus content. The dried fruit contains 42-52 % of sugar, dominantly fructose. Polyunsaturated fatty acids account for 58 to 66 % of the total fat content, with linoleic, linolenic, and oleinic acid dominant and a highly favourable  $\omega 3/\omega 6$  ratio (0.09 %). The aim of this study was to present literature data on the nutritional properties of *A. unedo* fruits and determine the polyphenolic content (total phenols, tannins, flavonoids, and phenolic acids) and antioxidative capacity of fruits collected at two different locations in Croatia (the islands of Mali Lošinj and Koločep). Antioxidant capacity was estimated by DPPH (2,2'-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), and FRAP (Ferric reducing antioxidant power) assay. Average content of total phenols, tannins, flavonoids, and phenolic acids in the extracts of liophylised fruit was 21.58 and 15.32 mg gallic acid equivalents per g d.w., 0.25 mg quercetin equivalents per g d.w., and 8.30 mg rosmarinic acid equivalents per g d.w., respectively. Strawberry tree fruit extracts showed good antiradical capacity (EC $_{50}$  222.31 mg L $^{-1}$  and 20.59 mg Trolox equivalents per g d.w. in DPPH and ABTS assays, respectively) and reducing power (0.28 mmol Fe(II) per g d.w.). Our overall results point to higher polyphenolic content and stronger antioxidant properties of strawberry tree fruit compared with other berries. Due to the presence of large quantities of bioactive compounds, strawberry tree fruit may have great potential as a functional food.

KEY WORDS: antioxidant activity; polyphenols; functional food; folk remedies

#### PCR in detection of *Listeria monocytogenes*

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Polymerase chain reaction (PCR) is one of the most important techniques in molecular biology. This method enables the in vitro synthesis of specific DNA fragments resulting in billions of copies in very little time. Real-time PCR is an advanced form of PCR enabling real-time monitoring of target DNA amplification. The basic principle of real-time PCR is the detection of fluorescence emitted by fluorophore which increases as a reaction proceeds. Today, real-time PCR has widespread applications, e.g. in medical diagnostics, DNA cloning for sequencing, DNA fingerprinting (used in forensic sciences and paternity testing) as well as in the identification of foodborne pathogens. *Listeria monocytogenes* is a Grampositive, non-spore forming, facultative anaerobic rod that grows between -0.4 and 50 °C. It is catalase positive and oxidase negative and expresses P-haemolysin which produces zones of clearing on blood agar. L. monocytogenes is widely present in plants, soil, and surface water samples, and has also been found in silage, sewage, slaughterhouse waste, milk of normal and mastitic cows, and human and animal faeces. Classical microbiological analysis, according to the ISO method, is very time-consuming and can last up to 6 days. PCR's advantages over classical microbiology are: high specificity and sensitivity, low possibility of false positive results, less time spent. However, there are also a few disadvantages: high cost of analysis and high risk of contamination. For now PCR is mainly used as a confirmation method or, due its speed, as a screening method.

KEY WORDS: DNA analyses; fluorescence; molecular biology; real-time PCR

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## Analysis of mechanical vibration energy and its effects on chemical changes in tomato fruits (*Solanum lycopersicum* L.) during road transport

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The transport of fruits and vegetables is a difficult task because these products are living tissue that continuously undergoes processes of life, which initially results in the development of quality characteristics desirable to the consumer but leads to spoilage later on. The changes in the quality of fruit and vegetables are influenced by factors such as temperature, humidity, and gas composition of the atmosphere in which they are stored. During transport, an additional factor is the impact in the form of mechanical vibrations. They can lead to mechanical damages in transported fruit and vegetables resulting in a reduction in quality and, in extreme cases, spoilage. In order to maintain the highest possible quality despite the long transport of fruits and vegetables, they are transported at an immature stage and they are matured just before sale. The aim of this study was to analyse the effect of mechanical vibrations on the chemical parameters of tomato fruits with regard to their maturation process. The tested parameters where: pH, total soluble solids, reducing sugars, and ascorbic acid. First, we measured the vibrations that occur during the real road transport of food and estimated the energy supplied to the transported products. Then a vibration simulator was constructed and we measured changes in the chemical parameters of the fruit under the influence of selected vibration frequencies at different times. The results of the study showed that tomatoes subjected to vibrations of 0.002-10.5 J kg<sup>-1</sup>, irrespective of their duration time, resulted in accelerated fruit ripening, while energies above 12 J kg<sup>-1</sup> had negative effects on fruit and caused damage due to mould and rot.

KEY WORDS: food spoilage; fruit and vegetable quality; vibration frequencies

### Food safety and food hygiene course attendees and knowledge about cleaning and sanitizing

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The aim of this study was to obtain insight into the knowledge and practices regarding cleaning and sanitising among food safety and hygiene course attendees as a prerequisite for the production of safe food. Analysis was performed using an anonymous questionnaire given to the attendees of the Food Safety Minimum Training during July 2017 regarding their knowledge and practices of cleaning and sanitising. The survey included 96 respondents (65 % women; 35 % men) aged 18-60. Of all the respondents, 44 % were in contact and directly handled food in their premises (food handlers). Two thirds of the respondents did not have any previous experience or education for food handling. Personal protective equipment (gloves) in cleaning and sanitising procedures were used by 79 % of respondents. Half of the respondents used appropriate detergents during cleaning procedures and one third of food handlers were not aware and did not use approved sanitising agents at appropriate concentrations. Half of the respondents thought that regular everyday cleaning and sanitising in food processing areas have a negative impact on their health. The analysed data show that the food handling occupation comprises a remarkable number of persons insufficiently educated for handling food in a safe way. The high percentage of respondents who did not use proper cleaning and sanitising agents or did not perform cleaning and sanitising on a regular basis indicates the need for regular education for food handlers (before they start work, and in regular intervals) in order to reduce the risks and behaviours associated with foodborne illnesses and outbreaks.

KEY WORDS: food handlers; food safety and cleaning education; sanitising agents

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### **Experience from the accreditation of conformity** assessment bodies in the food sector

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Reliable information related to food safety and quality is an important issue for all interested parties in the chain of food production, distribution, storage, and marketing, as well as for regulators who prescribe requirements and perform market surveillance of food. A credible assurance system of food safety and quality is based on reliable data delivered by laboratories and other conformity assessment bodies that declare their competence by accreditation according to internationally recognized criteria. This paper presents the activities of the Croatian Accreditation Agency (HAA) in the accreditation of laboratories and other conformity assessment bodies in the food sector, statistics about accredited conformity assessment bodies in Croatia, application of a flexible scope of accreditation, applicable European co-operation for Accreditation (EA) and HAA documents on accreditation, cooperation of the Croatian national accreditation body with regulators in drafting and implementation of food regulations, etc.

KEY WORDS: food quality legislation; food safety; laboratory quality assurance

### The quality of honey according to the project of voluntary labelling of honey with the label "Med iz Lijepe Naše"

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The project of voluntary labelling honey with the label "Med iz Lijepe Naše"("Honey from Our Beautiful Homeland") is a cooperation between the Croatian Agricultural Agency and the Croatian Beekeepers' Association that has been implemented since 2014. The main goal is to include as many beekeepers enlisted in the Beekeepers' Registry who sell their honey through some form of direct sales: exhibitions, fairs, beekeeping events, etc. For beekeepers, this way of labelling and control provides a higher level of security and increases their recognisability on the market. Also, labelling food with a trademark sends consumers a clear message that the product is produced in Croatia and that its quality has been tested in an accredited laboratory. The aim of this paper was to research how beekeepers make use of the project's benefits and to determine the quality of honey, all in order to develop future projects and development measures and increase the quality of honey production. This paper analyses data collected by the Department of Honey Quality Control at the Croatian Agricultural Agency; primarily data regarding collected samples and the number of producers since 2014. In the period between July 2014 and September 2017, the Department of Honey Quality Control tested 379 samples submitted by 233 producers within the project "Med iz Lijepe Naše", which is an average of 1.6 samples per producer. In 374 samples, all of the tested parameters were in line with applicable rules and Quality Certificates and the labels were issued. Five of the tested samples showed deviation from the set rules, mainly due to a quantity of water higher than prescribed. The control of tested parameters on samples awarded the Quality Certificate revealed the high quality of all of the tested parameters as well as great botanical diversity. There were 15 different types of honey determined, all from various areas of Croatia. However, according to the data provided by the Croatian Agricultural Agency, less than 1 % of members of the Croatian Beekeepers' Association submitted samples for testing, even though the project demonstrated clear benefits for them.

KEY WORDS: beekeeping; branding; Croatian Beekeepers' Association; quality control

### Characterization of Satsuma mandarin honey from the Neretva valley region

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The significant production of unifloral Satsuma mandarin (Citrus unshiu Marc.) honey is enabled by the orchards of the mentioned Citrus species in the Neretva valley. However, as knowledge regarding this type of honey is insufficient, the aim of this study was to establish its botanical origin and physicochemical and sensory properties. A total of 19 samples of mandarin honey collected from the mentioned area in the period from 2014 to 2016 were analysed. The pollen spectrum of the studied honey samples was determined by melisopalinological analysis, while the physicochemical parameters included determination of water content, electrical conductivity, pH value, and diastase activity. Along with Satsuma mandarin pollen, the pollen of the following nectariferous plant species were more represented in the pollen spectrum of the analysed honey samples: shepherd's purse (Capsella bursa pastoris), aster family (Asteraceae Taraxacum form), mediterranean buckthorn (Rhamnus alaternus), and oranges (C. sinensis). Nectarless plant species were represented by pollen originating from manna ash (*Fraxinus ornus*), holm oak (*Quercus ilex*), oak (*Quercus* spp.), orchard grass (*Dactylis glomerata*), and olives (*Olea europea*). The average water content in the honey samples was 17.5 %, electrical conductivity 0.23 mS cm<sup>-1</sup>, pH value 3.96, and diastase activity 9.72 DN. Sensory analysis revealed that the colour of Satsuma honey varied from light to dark yellow, while the crystallization was moderate but fast. The honey was characterized by an orange blossom odour with medium intensity. The taste was weak to medium stable with medium sweetness and poorly expressed acidity, while the aroma was medium persistent, floral, or fruity. Citrus honey is known in the world honey market for its specific sensory properties and takes up an enviable portion of the honey supply. Orange and lemon honeys are mostly present on the market, while a very small part is represented by mandarin honey. Therefore, due to the almost monocultural orchards of the Satsuma mandarin in the Neretva valley, the possibility of producing this rare honey type in this region should certainly be utilised.

KEY WORDS: citrus honey; melisopalinology; sensory analysis; quality control

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#### Olive oil – the benefits of its bitter and pungent taste

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Olive oil from *Olea europea* of the Mediterranean region has an extremely beneficial effect on the human organism, which is related to its quality. Both the oil and the fruit are some of the main components of the Mediterranean diet. The bitter and pungent taste of extra virgin olive oil, much like its extremely positive characteristic, derives from phenolic compounds (e.g., oleuropein) as natural antioxidants, whose effect directly protects oil from oxidation. Oleuropein brings to the fore pharmacological activities including antioxidant, anti-inflammatory, anti-atherogenic, anti-cancer, antimicrobial, and antiviral activity, as well as hypolipidemic and hypoglycaemic effects. In March 2017, we performed an organoleptic assessment of 30 samples of olive oil. Organoleptic evaluation was performed with the Panel test, according to the International Olive Council (IOC) COI/T.20/Doc.15/Rev. 8, 2015 method for organoleptic assessment of virgin olive oil. Each sample was tested by 12 evaluators during three separate evaluations. Results for properties that depend on phenolic compound content, bitterness, and pungency were analysed with regard to the intensity as well as variety by type: orkula, istarska bjelica, and buža. According to bitterness and pungency intensity, 57 % of the analysed samples were rated as medium, 37 % as intense, and 6 % as mild. The sort with the highest value of analysed properties was istarska bjelica. Each of the evaluated samples was rated as high quality olive oil. Different intensity of bitterness and pungency partially depends on variety. The examined oils were rich in phenolic compound content compared to the measured intensity. However, this investigation must be extended by the analytical measurement of phenolic compounds in order to reach a more precise conclusion.

KEY WORDS: antioxidant activity; Mediterranean diet; oleuropein; phenols

### The potential of coupling NIRS with chemometrics for determination of extra virgin olive oil geographical origin

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The synergy of near-infrared spectroscopy (NIRS) and chemometric classification methods for geographical origin discrimination was tested on a Croatian olive oil test sample set. For this purpose, 41 extra virgin olive oils from three different Croatian regions (Istria, Kvarner, and Dalmatia) were collected and analysed by NIRS. The obtained NIR spectra were pre-treated and analysed by two different chemometric methods: principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). The PCA from NIR data revealed the modest grouping power of extra virgin olive oils according to their geographical origin, although Istrian samples were grouped completely separately from other Croatian olive oils. Afterwards, we tried to achieve better insight into the predictive capabilities of measured NIR spectra for accurate classification of olive oils by PLS-DA. The PLS-DA model constructed using only significant NIR spectra variables (p<0.01) demonstrated the highest classification accuracy for origin prediction with R2 equal to 0.91 and Q2 equal to 0.85. The obtained results proved NIRS as a fast and non-destructive technique with high potential in the prediction of olive oil geographical origin.

KEY WORDS: food analysis techniques; PCA; PLS-DA; quality control

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### Silver nanoparticles affect germination and photosynthesis in tobacco

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Nanotechnology has an impact on the food industry in all stages of production, from farming to processing. Silver nanoparticles (AgNPs) are the most common nanomaterial on the market due to their unique antibacterial and antifungal properties, often used in agriculture as vehicles for the delivery of fertilizers and pesticides. Since plants are involved in the accumulation and biodistribution of many environmentally released substances, this can lead to an increased risk of human exposure to AgNPs through the food chain. This study investigated the effects of three differently coated AgNPs [citrate, polyvinylpyrrolidone (PVP) and cetyltrimethylammonium bromide (CTAB)] and AgNO $_3$  on the germination and photosynthesis of tobacco seedlings (*Nicotiana tabacum* L.). The silver content in plant tissue was also determined. Five concentrations (25, 50, 75, 100, and 150  $\mu$ M) of AgNPs and AgNO $_3$ , and two concentrations (10 and 25  $\mu$ M) of surface coatings were applied. Chlorophyll fluorescence parameters were measured with a fluorimeter using the saturation pulse method, while photosynthetic pigment content was analysed by high-performance liquid chromatography (HPLC). Silver uptake in plant tissue was determined with inductively coupled plasma mass spectrometry (ICP-MS). The results showed that positively charged AgNP-CTAB and CTAB coating have a negative effect on all of the parameters of seed germination, while AgNP-citrate and AgNP-PVP as well as AgNO $_3$  caused only a slight reduction of fresh weight and root length when applied at higher concentrations. Citrate and PVP surface coatings did not show any significant impact on seed germination. Photosynthesis efficiency was decreased in all of the AgNP and AgNO $_3$  treatments, indicating a negative impact on the photosynthesis efficiency was decreased in all of the AgNP and AgNO $_3$  treatments, indicating a negative impact on the photosynthesis efficiency parameters. Silver uptake was the highest in AgNP-CTAB treatment thus demonstrating that the phytotoxicity

KEY WORDS: antibacterial activity; HPLC; human exposure; ICP-MS; phytotoxicity

#### Cytochrome P450 3A4-mediated metabolism of flavonoids

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Flavonoids are a diverse class of compounds to which we are exposed through the vegetables and fruits we ingest. The human body perceives flavonoids as xenobiotics that should be metabolized and excreted. Most xenobiotics first undergo oxidation reactions primarily catalysed by cytochromes P450. A major enzyme involved in the oxidations of more than one third of xenobiotics is cytochrome P450 3A4. It is abundant in enterocytes and hepatocytes and serves to prevent xenobiotic absorption and facilitate elimination. The objective of this work was, based on Michaelis-Menten kinetic data, to determine the catalytic efficacy at which cytochrome P450 3A4 biotransforms flavonoids screened out of 30 representative flavonoids. The flavonoids were incubated in 2 to 200  $\mu$ M range with 5 pmol of baculosome enzyme system containing P450 3A4, NADPH reductase, and cytochrome  $b_5$  in water media containing phosphate buffer pH 7.4. The reaction was initiated by an addition of 15 % NADPH generating system. Incubation ran for 15 min in a shaking water bath at 37 °C and was stopped by acidified acetonitrile. The mixture was centrifuged and the supernatant was directly analysed. The structure of the metabolite was determined based on comparison of retention time, UV-Vis spectra, precise mass data based on TOF-MS, as well fragmentation pattern. As flavonoids are mostly UV active, quantification was based on DAD data. Data was plotted against the Michaelis-Menten curve from which kinetic parameters were determined. The analysed flavonoids had  $k_{cut}$  values ranging from 0.4 to 13.8 pmol pmol<sup>-1</sup> min<sup>-1</sup> and  $K_{m}$  values of 40-112  $\mu$ M. Catalytic efficacy was between  $(0.05-0.21)x10^6$  min<sup>-1</sup> M<sup>-1</sup>. This data indicates that some flavonoids undergo extensive metabolism mediated by cytochrome P450 3A4. As this enzyme presents the most significant enzyme for the metabolism of xenobiotics including drugs and toxic substances, it can be presumed that some flavonoids can interfere with the metabolism of other xenobiotics i.e. food-dr

KEY WORDS: biotransformation; enzymes; TOF-MS; xenobiotics

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### Accumulation of heavy metals in edible saprotrophic and ectomycorrhizal mushrooms

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Heavy metals cause serious problems in the environment and can accumulate in organisms, especially in higher fungi. The increasing concentrations of heavy metals in mushrooms also increase the importance of fungi as potential biological indicators of environmental pollution. However, extremely high concentrations of toxic heavy metals in mushrooms can have negative effects on human health. Therefore, the aim of this study was to determine the concentrations of heavy metals Ni, Cr, Pb, Cd, and Hg in certain edible species of saprotrophic and ectomycorrhizal fungi and the substrate collected in the region of Gorski kotar. This study used 87 samples from 10 species of wild edible mushrooms from seven different genera, five of which were saprotrophic species (Agaricus campestris (L) Fries, Armilariella mellea (Vahl. ex Fr.) Karst., Clitocybe inversa (Scop. ex Fr.) Pat., Clitocybe nebularis Batsch. ex Fr., Macrolepiota procera (Scop. ex Fr.) Sing.,) and five ectomycorrhizal fungi (Lactarius deterimus Groger, Boletus edulis Bull. ex Fries, Boletus aestivalis Paulet ex Fries, Tricholoma portentosum (Fr.) Quelet, Tricholoma terreum (Schff. ex Fr.) Kummer). The analyses of heavy metals in mushrooms and soil substrate were carried out by inductively coupled plasma-optical emission spectrometry (ICP-OES). The highest concentrations of Ni (1.84 mg kg<sup>-1</sup>), Cr (1.32 mg kg<sup>-1</sup>), and Pb (1.38 mg kg<sup>-1</sup>) were determined in *Macrolepiota procera*. The highest concentration of Cd (1.47 mg kg<sup>-1</sup>) was determined in *Agaricus campestris*, while the highest Hg concentration (1.59 mg kg<sup>-1</sup>) was determined in *Boletus edulis*. The concentration of Ni, Cr, Pb, and Cd significantly differed (p<0.001) between the examined saprophytic and ectomycorrhizal mushrooms, while Hg content was not significantly different with respect to the nutrients absorbed by mushrooms. In terms of the anatomical part of the fruiting body (cap-stipe), a considerably greater concentration of the analysed elements was found in the cap for analysed mushroom species. According to the calculated bioconcentration factors, all of the examined species were found to be bioexclusors of Ni, Cr, and Pb and bioaccumulators of Cd and Hg. According to regulations on the maximum levels of certain contaminants in foodstuffs (Official Gazette 154/2008) and the determined concentrations of the analysed heavy metals, it can be concluded that the consumption of the tested mushrooms cannot be considered a toxicological risk for humans. However, to evaluate the possible danger of heavy metals on human health, their level should be analysed more often in wild edible mushrooms.

KEY WORDS: food safety; fungi; ICP-OES; toxicological risk

### Heavy metal analysis of waste sludge: possible use in agriculture?

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The increase in population, as well as daily water needs, has highlighted the problem of disposing waste from wastewater treatments. The purpose of this study was to establish the concentration of heavy metals in various substrates of waste sludge and control substrate: samples of fresh waste sludge (FS), elder waste sludge (ES), combined fresh waste sludge with control substrate (50 %CS+50 %KS), and control substrate (KS). The control substrate was untreated soil. ICP-MS was used to measure the concentrations of Cr, Co, Ni, Cu, Zn, Mo, Cd, and Pb, while AAS hydride technique was used to determine the Hg concentration. The highest concentration of Pb (103.58 mg kg<sup>-1</sup>) was measured in the ES substrate, while the lowest concentration of Pb was measured in the KS substrate (7.26 mg kg<sup>-1</sup>). Very high concentrations of zinc were recorded in all four substrates; in FS substrate (751.46 mg kg<sup>-1</sup>), in ES substrate (640.76 mg kg<sup>-1</sup>), in (50 %CS+50 %KS) substrate (623.9 mg kg<sup>-1</sup>), and in KS substrate (35.42 mg kg<sup>-1</sup>). The Cd concentrations ranged from 0.17 mg kg<sup>-1</sup> in the KS substrate to 1.95 mg kg<sup>-1</sup> in the ES substrate, while the Hg concentrations ranged from 0.19 mg kg<sup>-1</sup> in the KS substrate to the allowed maximum levels for waste sludge used in agriculture (OG 38/08), all of the concentrations were lower. The results indicate the possibility of using sludge from wastewater treatment as compost for food production and suggest the need for a comprehensive study and further research of risks to human health, for example in experimental fields.

KEY WORDS: environmental pollution; food production; ICP-MS; wastewater

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## Practical procedures for the prevention of bee community diseases with the purpose of maintaining the quality of honey

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American foulbrood is a contagious, severe disease of honey bee brood caused by the spore forming bacterium Paenibacillus larvae. The spores present the main problem in suppressing the disease - they are resistant to external influences and remain viable in wax, honey or combs for over 50 years. The aim of this paper was to present basic data on the spread of this disease in the Varaždin County, related legal frameworks, and practical procedures used to detect, suppress, and prevent the spread of this disease. The disease is reported to a veterinary service that clinically inspects bee-keeping communities and takes samples of suspect communities. Upon laboratory confirmation of the disease a veterinary inspector goes out into the field and sets out measures to control the disease. The area where bee communities must be clinically inspected is set within a 3 km radius of the focal point. In each suspect community, a comb sample is taken and inspected. The hive custodian has an important role of locating all the beehives within the infected area. The first case of the disease was reported by beekeepers and confirmed by a laboratory in the fall of 2015 in Donja Voća. From April to June 2016, eleven outbreaks of the disease were detected, with one to twenty infected hives per apiary. The disease focal points were also discovered in Lipovnik, Slivarsko, Klenovnik, Žarovnica, Višnjica, Ivanec, and Goranec. The infected comb, bees, and honey in the apiaries were destroyed and buried; the movement of bees, honey and tools was banned; disinfection measures were taken. There is no authorized treatment for American foulbrood and treatment with antibiotics is forbidden since they would be found in honey sooner or later. Disease control is possible but only with the effort of all interested parties (beekeepers, beekeeping association, and veterinarians).

KEY WORDS: American foulbrood; disease prevention; food safety; quality control

### Promotion of proper nutrition for pupils in elementary schools – health workshops "I embrace healthy habits"

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Embracing healthy eating habits is the main topic of the healthcare workshops held within the program "I embrace healthy habits". These workshops are aimed at elementary school pupils with the goal of gaining healthy lifestyle habits already at early age, improving health, preventing the emergence of risk factors, and preventing diseases. The long-term goal of the program is to reduce the incidence of chronic non-communicable diseases in adulthood. Health promotion measures are implemented through interactive educational workshops in the schools of the City of Zagreb (first to fourth grade) called "Proper balanced nutrition", "Healthy eating plate", and "Food is your friend", all of which rely on work in small groups and methodologically designed games. Healthy workshops are also enriched with educational materials, such as "Healthy Board Cards", "Children's Pyramids and Health Plates", games "Dump the Intruder" and "Balanced Foods Puzzles". In the area of the City of Zagreb, 87 health workshops on healthy lifestyles "I embrace healthy habits" with 2511 students were held in elementary schools. About 60 % of the population of Croatia is obese or overweight, which represents an increasing public health problem. According to 2015 figures, about 15 % of children in fifth grade are overweight (15.9 % boys and 15.0 % girls), whereas 13.6 % boys and 10.4 % girls are obese. In the first grade of high school, 11.9 % of boys and 14.2 % of girls are overweight, while 10.9 % of boys and 12.9 % of girls are obese. The workshops have an interdisciplinary approach and are conducted by experts from the Public Health Institute who strive to protect and improve health with the active participation of pupils. Proper nutrition is an integral part of a healthy lifestyle, so it is important that children embrace and apply healthy eating habits at the earliest age to ensure the optimal content of all nutrients necessary for their growth and development. Promoting proper nutrition requires continuous work and a joint multidisciplinar

KEY WORDS: children's obesity; healthy eating habits; obesity prevention programmes; public health

### Risk of methylmercury exposure through consumption of river Jadro fish

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Methylmercury (MeHg) exposure is one of the most significant health risks associated with fish consumption. In this study, a calculation of average weekly exposure was used in Hg exposure assessment, i.e. a calculation of the percentage of the tolerable weekly intake percentage. The provisional tolerable weekly intake (PTWI) of MeHg recommended by the European Food Safety Authority (EFSA) is 1.3  $\mu$ g kg<sup>-1</sup> b.m. The EFSA also determined 70 kg of b.m. as the European adult population default value in weekly exposure assessments. Hg level was determined by cold vapour atomic absorption spectrometry after decomposition under pressure using a LECO analyser. The risk assessment was conducted by comparing calculated median values (x) of MeHg and the values recommended by the EFSA. Hg levels were determined in 60 samples of trout in total, 20 of each of the three trout species inhabiting the Jadro River. The values for total Hg levels were determined and converted into MeHg level by applying the conversion factor 1.0. Median (x) value of the MeHg level (0.0901 mg kg<sup>-1</sup> in softmouth trout, 0.1908 mg kg<sup>-1</sup> MeHg in rainbow trout, and 0.1940 mg kg<sup>-1</sup> in brown trout). With an assumed trout consumption of 30 g per week, the consumer intakes 0.385  $\mu$ g kg<sup>-1</sup> of b.m. of Hg per week by consuming the softmouth trout, i.e. 29.7 % PTWI; 0.82  $\mu$ g kg<sup>-1</sup> of b.m. of Hg per week by consuming the softmouth trout, i.e. 29.7 % PTWI; 0.82  $\mu$ g kg<sup>-1</sup> of b.m. of Hg per week by consuming the softmouth trout, i.e. 29.7 to 64 % PTWI. With the assumed consumption of one trout per week, the MeHg exposure varies from 0.385 to 0.83  $\mu$ g kg<sup>-1</sup> of b.m. of MeHg depending on the species consumed, i.e. 29.7 to 64 % PTWI. The assessed MeHg exposure is lower than the PTWI default value, which leads to the conclusion that the risk for consumer's health is negligible with the assumed consumption of one trout per week. In case of consuming two rainbow trout or brown trout per week (600 g), the MeHg level exceeds the recommended value of 1.3  $\mu$ g kg

KEY WORDS: EFSA recommendations; environmental exposure; food safety; human health risks; trout

### Review of comparative studies conducted on organic and conventional food

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The aim of this paper was to estimate differences in key areas, food safety, sensory and nutritional ingredients, and impact on human health. An interpretation of previously published results was conducted by comparisons or meta-analyses of organically and conventionally produced food. Smith-Spangler et al. analysed the results of 17 studies conducted on humans and 223 studies on comparing the nutrient content and the presence of harmful substances in organically or conventionally grown foods. They concluded that the risk of pesticide intake was 30 % lower for organic products. Barański et al. carried out a statistical analysis of 343 studies and found that there was up to four times the amount of pesticides and a higher amount of Cd in conventional food. Mukherjee et al. carried out a comprehensive study of 600 different samples analysed for the presence of *Escherichia coli* and *Salmonella* bacteria, comparing the microbiological accuracy of organic and conventional products. *E. coli* was found in 9.7 % of organic samples and 1.6 % of conventional samples. Smith-Spangler et al. argued that the likelihood of finding bacteria in chicken and pig meat is the same for organic and conventionally grown meat. The Danish study states the presence of Campylobacter spp. in 100 % of the 22 organic samples compared to 36.7 % of conventional samples. A number of reviewed studies, meta-analyses, and published data show that there are significant differences in concentrations of nutritive compounds. Organic products have higher antioxidant activity and higher concentration ranges of individual antioxidants, but the nutritional benefits of organic food over conventional are almost insignificant. Scientific research proves that consumption of organic food decreases the intake of pesticides, although the level of pesticides in conventional food rarely exceeds the limits considered safe. We can conclude that there may be nutritionally relevant differences in the composition between organic and conventional food. There remains great uncertainty and controversy as to what extent these differences in contamination and composition affect people's health.

KEY WORDS: bacterial activity; food health and safety; meta-analysis; nutritive value

### Foodborne nematode *Anisakis pegreffii* represents a professional risk in the Croatian fishery industry

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The Croatian fish processing industry is an important sector encompassing almost 800 employees mainly situated in the Adriatic coastal area, with a tendency for growth since Croatia's entrance into the EU market. We determined the degree of sensitization to *Anisakis* sp. antigens in the population of fishery industry professionals and the phenotype frequency of *Anisakis*-seropositive individuals through a study funded by the Croatian Science Foundation (grant no. 5576). IgE seroprevalence to *Anisakis* sp. Ani s l and Ani s 7 allergens was measured by indirect ELISA in the sera of fishery industry professionals employed at ten facilities in the Split-Dalmatia, Zadar, and Istria Counties. In total, 1008 individuals of which 448 represented the non-professional population (negative control), consented to blood drawing and filled the accompanying questionnaire. Furthermore, *Anisakis* IgE positive subjects and their negative controls inhabiting the same region were typed for human leukocyte antigens (HLA) Class II DRB1, DQA1, and DQB1 using the PCR-SSO and PCR-SSP method. Total anti-*Anisakis* seroprevalence reached 0.99 % (1.09 % including one marginal sample), 0.39 % of both allergens, and 0.69 % of only Ani s 7 allergen, suggesting the latter is an immunodominant allergen. HLA typing revealed the presence of DRB1\*1502-DQB1\*0601 haplotype, previously observed in higher frequency in *Anisakis* hypersensitive patients. Although professionals in Croatian fishery industry have shown a lower presence of anti-*Anisakis* IgE circulating antibodies compared to the previously reported healthy population of the Split-Dalmatia County (N=500, 1.5 % anti-*Anisakis* IgE seroprevalence), we have shown that *A. pegreffii* represents a professional risk that requires careful future monitoring. The Ani s 7 allergen with a shorter life span than Ani s 1 has been previously reported also as immunodominant allergen, mainly in patients with chronic urticaria.

KEY WORDS: ELISA; HLA; PCR-SSO; PCR-SSP; occupational health; seroprevalence

#### Food defence principles in food industry

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Food safety is one of the main goals in the food industry but current, among others, EU regulation still relies on the HACCP to effectively prevent accidental contamination. Yet, the present HACCP cannot be applied to intentional contamination. Intentional contamination is becoming increasingly significant and the main initiators for the implementation of the food defence system come from retail chains supported by the GFSI Initiative. Food defence is the missing link in the traditional Food Safety Management System. Food defence effectively manages intentional contamination, but for effective implementation, it is necessary to include all of the stakeholders. It is important to raise awareness of food defence throughout the entire food chain and the overall community at national and international level. The establishment of an educational program is essential for raising the awareness of all of the stakeholders. This also includes educational institutions, which should incorporate food defence in their curricula. Along with raising the awareness of the entire community, it is necessary to incorporate food defence into legal frameworks. Unfortunately, an insufficient number of countries have incorporated food defence into their regulations. The implementation of food defence in the food industry is based on risk assessments that detect critical areas in the food supply chain (vulnerability assessments). Each stakeholder develops his vulnerability assessment methodology or uses the prepared software. Raising awareness, effective education program through the Food Defence Plan that defines the principles and the implementation of the requirements contributes to the mitigation of intentional contamination. Food defence in the supply chain will gain greater importance with increasing the risk of intentional contamination along with potential motives like national, political, economic, business, personal, etc. For all these reasons, it is also necessary to incorporate food defence into legislation.

KEY WORDS: consumer education; food safety; intentional contamination; vulnerability assessment

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#### When does the HACCP system fail to provide food safety?

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The aim of this paper was to provide support to food business operators by presenting an objective evaluation of the functionality of the HACCP system, which could be different from the usual opinions and beliefs of those using HACCP as their own everyday work tool. For this purpose, descriptive statements based on the facts established by the HACCP food safety verification process were used. This paper shows the most common reasons for the failure of the HACCP system, despite good intentions for the system to be properly developed and set up. There are a number of possible faults, and their interdependence causes a number of reasons for the non-functioning of the food safety system. The HACCP system is not functional 1) if the management or the owner's lack of motivation (in reality) does not support the development and maintenance of the HACCP system; 2) when members of the HACCP Team are not well selected and motivated; 3) if not all of the materials used (e.g., packaging) are included in the food description; 4) when the flow diagram of production does not correspond to actual production; 5) when hazard and risk analysis does not include all food ingredients; 6) if the critical limits are inadequately determined or misplaced during the supervision; 7) if the Critical Control Points (CCPs) are not adequately placed within the production flow; 8) if the corrective measure is of the non-functional degree and fails to prevent the risk to the consumer; 9) if verification procedures do not include pre-requisite HACCP systems; 10) if the HACCP system is not regularly validated; 11) if the inspection of the competent bodies is too lenient or absent; 12) when the food declaration is incomplete. HACCP is an excellent tool for quality and food safety. It is based on a simple idea of preventive action in food business, but its action is not necessarily ultimately linked to food safety.

KEY WORDS: consumer health and safety; food industry; safety standards

### In silico prediction of the toxic profile of antimicrobial sulphonamides relevant to ecotoxicity

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In recent years, pharmaceuticals have been detected in diverse environments, including groundwater, river water, and municipal wastewater. Antimicrobial sulphonamides are regarded as emerging contaminants introduced into the environment because they are widely used in human and veterinary medicine and as feed additives to promote growth in concentrated animal feeding operations. They are found as water pharmaceutical contaminants due to their high mobility, lack of strong sediment properties, and slow degradation process. Different antimicrobial agents including sulphonamides have been analysed as water pharmaceutical contaminants in samples collected along Croatian rivers and sulfamethoxazole, sulfapyridine, sulfadiazine, and sulfamethazine were detected. The aim of this study was to predict the toxic potential of the most used sulphonamides (n=15) and their impact on the environment and health. ADMET-related descriptors (ADMET Predictor 8.1, SimulationsPlus, USA) relevant to environmental toxicity were computed using four models: the fathead minnow acute toxicity model based on lethal effects on *Pimephales promelas* (Minnow  $LC_{50}$ , TOX FHM), the concentration needed to inhibit 50 % of growth in the protozoan species *Tetrahymena pyriformis* (Th pyr pIGC $_{50}$ ), the lethal concentration that results in the death of 50 % of *Daphnia magna* (water fleas) (Daphnia  $LC_{50}$ , TOX DM), and the bioconcentration factor (BCF). The results of this study revealed that the investigated sulphonamides are non-biodegradable molecules. The highest score for BCF was computed for sulfachloropyridazine and the lowest for sulfadiazine (BCF 8.354 and 4.188, respectively). Sulfamethisole was predicted as the most toxic against fathead minnow (TOX FHM 311.556 mg/L) and *Daphnia magna* (TOX DM 0.180 mg/L). Sulfomonomethoxine and sulfamethazine have been predicted with the TOX FHM as 1731.641 mg  $L^{-1}$  and TOX DM as 49.005 mg  $L^{-1}$ , respectively. Sulfadoxine has been predicted as the most and o.990 mmol  $L^{-1}$ , respectiv

KEY WORDS: environmental pollution; environmental toxicity; pharmaceutical contaminants

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# The analysis of the connection between quality of diet and other risk factors for colorectal cancer in persons tested positive on the faecal occult blood test within the implementation of the national colorectal cancer screening program in the City of Zagreb

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The aim of this study was to present the correlation of diet and other risk factors of colorectal cancer in persons who have tested positive on the faecal occult blood test (FOBT), during the 2<sup>nd</sup> cycle of the national colorectal cancer screening program. The analysis was performed on the date obtained through a questionnaire given as part of the screening program. Differences among respondents who have been tested positive were tested using chi-squared test. As many as 40,114 tests were processed and in 1,103 persons (2.7%) a positive outcome was recorded, of which 54.3 % were male and 45.7 % female. Regarding the correlation of diet and its importance in the occurrence of colorectal cancer, the following results were determined: data on the quality of diet were available for 686 persons, of which 6.1 % answered they had a "very healthy" diet, 79.9 % a "healthy" diet and 14.0 % answered their diet was "fat". The average BMI value of the entire group equalled 26.86±3.8. A statistically significant difference was determined in female respondents and their quality of diet (women more frequently had a very healthy or healthy diet in comparison to men, P=0.001), educational level, and the quality of diet (persons of a higher level of education more frequently to had a very healthy diet, P=0.002). No statistically significant difference was observed regarding the connection between diet and marital status and physical activity. Our results show a statistically significant difference in self-reported diet quality in women and in all respondents of a higher level of education. They indicate a need to implement healthier lifestyles in the population. In order to decrease the risk of getting colorectal cancer and improve health, education regarding diets is essential.

KEY WORDS: FOBT; healthy lifestyle; questionnaire

### Role of health promotion in quality and food safety education – examples of good practice

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Health promotion focuses on the general, high-risk, and vulnerable populations. Educational activities of health promotion raise the level of awareness on the importance of quality and food safety. As an example of good practice, the workshops "Health Plate" are held in the primary schools of the City of Zagreb. Through these workshops, pupils learn about the importance of a balanced diet and are sensitised toward taking responsibility for their health. They are also informed and educated about the importance of quality and safety of food and its proper use. The online journal "Health for All" covers public health issues from all professional aspects of the Teaching Institute of Public Health and promotes continuous education on the quality and safety of food. The main objective is education about the risk factors for health and the possibilities of reducing them. The project "We eat responsibly" is run by the association "Lijepa Naša" (European Development Education and Awareness Raising, 2014). Nine member states of the Foundation for Environment Education are included. The main objective of the project is to motivate young people to acquire new skills and therefore motivate a globally responsible way of eating food in new EU Member States. The role of the Institute is to make the project more visible and accessible to target groups and provide expert support to the project's implementation. A total of 2511 pupils are included in the workshops, whereas "Health for all" covers the general population (readers). The project comprises 1800 teachers, 65,000 elementary school students, 25,000 high school students, and 100,000 parents. The health promotion methodology, with appropriate coverage, enhances the population's knowledge on the importance of food.

KEY WORDS: balanced diet; children's nutrition; food quality; workshops for children

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## Phytochemical characterization of polyphenols of *Laurus* nobilis L. (Lauraceae) from the northern and central Adriatic region

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The phytochemical characterization of bay laurel leaves (*Laurus nobilis* L., Lauraceae) from the northern and central Adriatic populations (Lovran, Cres, Mali Lošinj, Dugi Otok, Žirje, and Split) was performed as part of the research project TEUCLIC, financed by the Croatian Science Foundation. The presence of polyphenols was proven with general reactions of the formation of coloured products and/or precipitates. Thin layer chromatography revealed the presence of quercetin, rutin, isoquercitrin, and quercitrin in all of the analysed methanolic bay laurel leaf extracts. The quantitative analysis of biologically active phenolic compounds was carried out using three different spectrophotometric methods, following adequate extractions: total polyphenols (TP) and tannins (T) with Folin-Ciocalteu's phenol reagent (at 720 nm); total flavonoids (TF) with AlCl<sub>3</sub> (at 425 nm); and total phenolic acids (TPA) using the nitrite-molybdate reagent of Arnow in a sodium hydroxide medium (at 505 nm). The TP content was the highest in the sample from the island of Žirje (7.45 %), while the lowest overall TP content was 6.07 % (Dugi otok). The highest amount of T was recorded in Split (3.53 %), while the smallest T content was found in the population from Dugi otok (1.77 %). The TF contents ranged from 0.23 % (Split) to 0.72 % (Žirje), while TPA content varied from 2.34 % (Lovran) to 3.08 % (Split). This phytochemical characterization contributes to knowledge on Croatian bay laurel populations with regard to the content of polyphenolic biologically active substances and completes the current understanding of the phytotherapeutic potential of this species.

KEY WORDS: natural remedies; phenolic compounds; phytochemistry; tannins

## The impact of the category protocol of the geriatric healthcare program of four grades on the nutritional status of old people's home users

Lukić M\*\*, Garić S, Deucht A, Šepec S, Šimunec D, Kalauz S, Mićović I, Ćurić S, Bastalić R, Sertić Đurđević M, Županić M, Tomek-Roksandić S, Šostar Z, Šimunec D, Kolarić B, Tomasović Mrčela N, Katić M, Cerovečki Nekić V, Fortuna V, Vrbić Lj, Popek I, Maltarić M, Mravak S, Sajko D, Holjevac J, Lovrić K

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The Republic of Croatia with 17.7 % of the population aged >65 is challenged with appropriately planning healthcare for the elderly. In geriatric healthcare, standards have been adopted to define the domain, tasks, and quality of geriatric healthcare. The determination, monitoring, and evaluation of the Program of Four Grades of Geriatric Healthcare with nursing documentation and category protocol of a four-grade geriatric healthcare model and concept developed by N. Roper, L. Juchli, V. Fiecher, and M. Meier. Out of the 148 category protocols sent to old people's homes in all Croatian Counties and the City of Zagreb, 31 category protocols were received in response. The implementation of the Four Grades of Geriatric Healthcare category protocol in old people's homes in Croatia (4521/100.00 %) and City of Zagreb (1655/36.61 %) in 2016 showed the number of category protocols according to geriatric healthcare grades as follows: 1500 (33.18 %) users in geriatric healthcare grade I; 1003 (22.19 %) users in grade II; 1258 (27.83 %) users in grade III; and 760 (16.81 %) users in grade IV, yielding a total of 4521 users. The nursing documentation on geriatric healthcare kept according to the model and concept developed by N. Roper, L. Juchli, V. Fiecher, and M. Meier, through the use of 19 forms as a standard established by the Croatian Nursing Chamber via GeroS/CEZIH, will rationalize and upgrade the provision of efficient and evaluated geriatric healthcare both institutionally and extra-institutionally. GeroS/CEZIH enables the functioning of the Croatian Fund for Geriatric Healthcare. Through GeroS/CEZIH, the Program of Four Grades of Geriatric Healthcare allows for the monitoring and evaluation of healthcare needs and functional ability of those insured as well as geriatric patients.

KEY WORDS: elderly population; geriatric patients; nursing

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## Implementation of geroprophylactic measures in the prevention of obesity in early old age and malnutrition in deep old age

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Obesity and malnutrition in the elderly (age 65+) are two extremes of the gerontologic public health problem of inappropriate nutritional status. Obesity (ICD 10: E66) with established unfavourable implications for health and functional ability is a significant contributing factor for the development of many chronic non-communicable diseases, in particular of the circulatory system, the leading cause of death in the elderly. Gerontologic public health indicators show that geriatric patients in hospitals or old people's home nursing wings and welfare users are at a higher risk of malnutrition and thus more susceptible to infections, pressure ulcers, falls, and higher mortality. Numerous risk factors and gerontologic public health problems leading to diseased aging, including inappropriate nutritional status in old age can be eliminated by the implementation of the Program of Primary, Secondary, Tertiary, and Quaternary Prevention for the Elderly. This points to the necessity of regular assessment of nutritional status in care for the elderly (age 65+) and use of geroprophylactic measures with the implementation of nutritional gerontologic norms according to the Croatian Nutritional Guidelines for the Elderly. Using the NRS 2002/GeroS/CEZIH web service is of utmost importance for allowing timely gerontologic and/or geriatric intervention, thus preventing mental and physical function impairments in the elderly, reducing disease complications, and rationalizing the growing geriatric consumption due to malnutrition or obesity. A gerontologic public health analysis of defined determinants of monitoring via NRS 2002/GeroS/CEZIH web service was conducted in individuals aged >65, including BMI in particular. The results on 218 geriatric and gerontologic insures for the period from March 1 2015 to August 31 2017 showed the greatest proportion of subjects in the category of obesity (n=36) to be in their early and middle old age (65-84 years; n=33), whereas the greatest proportion of subjects in the category of obesity (n

KEY WORDS: elderly population; geriatric healthcare; NRS 2002/GeroS/CEZIH web service

#### The role of a nurse in the diet of a patient

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Nutritive substances are food compounds used by a human body for its growth, development, and energy supply. Nutritive substances that the human body cannot produce itself must be consumed through food and are considered essential. Proper nutrition and adequate absorption of nutritive substances from the intestinal lumen are crucial for maintaining body integrity, i.e. homeostasis of every energetic and metabolic process. From a technical point of view, a diet can be defined as an entry of nutrition preparations, carbs, fats, proteins, vitamins, electrolytes, and elements in traces, entered via the enteral or parenteral way. Ensuring proper nutrition means improving the nutritional status of a patient. The goal of every program for proper nutritive consumption is entering an optimal amount of energetic and metabolic nutritive substances to prevent starvation and avoid organism overload. If a patient cannot consume a food through the mouth, a parenteral or enteral diet is introduced as a form of nutritive support. The parental diet is a form of nutritive support that provides the organism with water, carbohydrates, and other nutritive substances through the vascular system. Enteral diets, on the other hand, are a form of diet that includes feeding through the catheter or stoma. By ensuring a proper diet, we can prevent illness or, in a case of patients who are already ill, we can significantly affect the quality of life and the illness' progress. The role of a nurse in the diet of a patient requires a radical, systematized, complete, and individualised nursing approach, as well as applying quality and standardized nursing documentation and procedures as well as educating patients and their families.

KEY WORDS: enteral diet; nursing; nutritional needs; parenteral diet

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### Implementation of nutritional status determinants in the CEZIH panel (NRS 2002 web service)

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Ever more public health professionals want to have accurate data available at any time of the day. In the last decade, the computerization of the healthcare system has been introduced at an ever faster pace, resulting in drastic changes in providing healthcare services. Owing to technological advances, data and information are more available than ever before. However, this also implies that personal data confidentiality is even more difficult to protect. This is best exemplified by modern healthcare in gerontology. The geriatric patient/gerontologic insuree to physician relationship has transformed into the geriatric patient/geriatric insuree to gerontologic healthcare/social team relationship. Development of public health information technologies will further increase demand for a greater quantity and quality of data, on treatment outcomes in particular. The aim is to develop a system, i.e. subsystem that will enable interactive digital management of knowledge in the field of gerontology. The aim of managing information systems in gerontology is to support the collection, search, and management of public health data and information for the promotion of elderly healthcare. Professionally and methodologically, the entry and processing of nutritional status (malnutrition/obesity) should be based on the following determinants: monitoring, studying conditions in the elderly according to age groups (early old age, middle old age, and deep old age), sex, occupation, primary diagnosis, secondary diagnosis, functional ability according to physical mobility and mental independence, body mass index, upper arm circumference, screening entity, and features of negative health behaviour (physical inactivity, alcoholism, and smoking habit). Under current circumstances, gerontologic insurees and geriatric patients are not recognized adequately in the healthcare system. A great deal of documentation is kept as classic paperwork. There is no connection between healthcare and social welfare institutions, which is necessary in case of gerontologic insurees and geriatric patients. GeroS is strongly connected with CEZIH in the monitoring and evaluation of healthcare needs and functional ability of gerontologic insurees and geriatric patients. If only one healthcare or social welfare institution enters the required data from their respective field of work via the GeroS/CEZIH web form, all other professionals will see the data for which they are authorized. Currently, healthcare and social welfare systems do not exchange data directly by computer. GeroS/CEZIH provides a link between the two systems, offering the users a uniform insight into the data relevant to the care of the geriatric insuree across three healthcare levels, old people's homes, and foster families within the social welfare system. A gerontologic workshop was held within the frame of GeroS/CEZIH, aimed at the implementation of the NRS 2002 web service – nutritional screening modified according to NRS 2002 tool for gerontologic insurees and geriatric patients. The objective of the web service for nutritional status calculation (by the NRS 2002 method) is monitoring and reporting of nutritional status of gerontologic insurees and geriatric patients because of the high prevalence of malnutrition in the deep old age group (age >85). The NRS 2002 web service will be used by healthcare professionals in hospitals (in particular those for long-term treatment), old people's homes, as well as all physicians, general/family medicine teams, geriatric nurses at old people's homes, and home-visiting nurses in primary healthcare. The healthcare also includes nutritionists additionally educated in the field of gerontology who take part in creating appropriate gerontologic menus in order to provide nutritional support to underweight gerontologic insuree or modify current menus. Therefore, it is necessary to monitor the nutritional status of the elderly (obesity and malnutrition) via the Panel integrated in the existing CEZIH.

KEY WORDS: elderly obesity; elderly malnutrition; geriatric healthcare

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#### THE OFFICIAL JOURNAL OF

Croatian Medical Association - Croatian Society on Occupational Health Croatian Society of Toxicology Slovenian Society of Toxicology Croatian Radiation Protection Association Croatian Air Pollution Prevention Association

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#### **AUTHOR INDEX**

#### A

Abičić I 39

Adanić Pajić A 33

Agić A 36

Agić D 37

Antolković Ilić V 41

Antunac N 51

Arnuš L 23

#### В

Bakić H 38

Balen B 46, 65

Balog D 55

Baričević L 55

Barišin A 40

Barušić L 55

Bastalić R 73

Benković G 65

Berišić I 35

Bevardi M 57, 58

Biba R 46, 65

Bieńczak K 61

Bijelović S 24, 43

Bilandžija A 46

Bisi A 31

Bjelanović J 24

Blaznik U 23

Bobić S 24

Bojić M 65

Bokulić Petrić A 58

Borzan B 18

Bošnir J 27, 47, 55, 57, 58, 66

Boštjan K 23

Brčić Karačonji I 60

Brera C 18

Brkić D 27, 69

Brok P **64** 

Bubalo D 63

Budeč M 27

Bulaić M 22

Bulska E 19

Buntak K 62

Bušelić Garber I 43, 70

Butković I 45

#### $\mathbf{C}$

Capak K 29, 40, 48

Cerić L 50

Cerovečki Nekić V 73

Cescutti P 25

Cvjetko P 46, 65

#### Č

Čanak I 57

Čečuk Jeličić E 70

Čendak I 37

Čop A 34, 62

Čulig B 27

#### Ć

Ćurić S 73

#### D

Denžić Lugomer M 52

Deucht A 73

Devčić Jeras A 35

Dinić T 42

Domijan A-M 46

Dragić N 43

Držaić V 66

Dunkić V 73

#### Ð

Đugum J 58

Đurković G 56

#### E

Ekroth S 20

Ercegovac L 46

#### F

Fanelli C 25

Feher Turković L 66

Filipčić L 74

Florencio M. Ubeira 70

Fortuna V 73

Franić R 41

Frece J 57

Frelih T 54

Fuart Gatnik M 23

#### G

Galić N 59

Ganjto M 66

Garić S 73

Gladan M 50

Gladovič N 45

Grabež M 22, 50

Grbić M 69

Gregurić-Beljak Ž 61

Gross Bošković A 42

Gruja J 30

Gržinčić B 37

Gusman V 43, 56

# H Haltrich K 44 Hanzer R 31 Hengl B 42 Holjevac J 47, 73, 74, 75 Horvat Kesić I 51 Hrabar J 43, 70 Hrga I 38 Hrg Matušin Ž 63 Hrženjak V 54 I Ibrahimagić A 50 Idaszewska N 61 Idrizović E 50 Isaksson M 20

Ivanoš J 23

Ivezić A 42

Ivešić M 32, 57, 59

## Jadrijević-Mladar Takač M 71 Jakopović Ž 57 Jakovljević J 22 Jaman S 70 Janković M 51 Jelavić M 72 Jeretin B 23 Jergović M 40 Jukić M 38, 65 Jurak G 58 Jurčević Podobnik V 25 Jurica K 60

Jurišić Grubešić R 60, 73

#### V

Juričić Ž 73

Kaić Rak A 47 Kalambura S 28 Kalauz S 73 Karakaš S 23 Karas I 26 Kasap A 66 Katić M 73 Kecerin R 61 Kiš M 52 Knezović Z 53 Kolarić B 40, 47, 72, 73, 74, 75 Kolek Sirovina K 35 Koprivica D 52 Korčok D 36 Kosanović Ličina ML 61 Kosić-Vukšić J 57, 58 Kovaček I 60

Kovač M 22
Kovač T 22
Kraljević-Pavelić S 64
Kremer D 73
Krga M 51
Krivohlavek A 32, 38, 57, 58, 59, 71
Krističević T 48
Krznarić Ž 26, 47
Kuharić Ž 57
Kukić E 23
Kunović A 26
Kušar A 23

#### L

Ladiš A-M 66

Lakić M 26
Lasić D 27
Leder R 56
Legen S 28
Leitner E 34
Lekić J 56
Levanić D 67
Lončar J 25
Lovrić K 73
Lovrović M 37
Luetić S 53
Lukić D 24, 43
Lukić M 47, 73, 74, 75
Lušić D 37

#### LJ

Ljevaković-Musladin I **26** Ljubas Kelečić D **26** 

#### M

Majetić Germek V 37 Majić Z 53 Majstorović D 72 Maletić M 37 Malmvärn A 20 Maltarić M 47, 73, 74, 75 Mandić-Miladinović M 51 Marić Bajs M 72 Marin M 45 Markov K 57 Martínez-Sernández V 70 Martin L 20 Matijević L 42 Mićović I 73 Mićović V 25 Mikrut Vunjak S 41 Mikulec N 37, 51 Milenković T 44 Miloš S 44 Mioč B **66** 

Mitić B *36*Mladineo I *43, 70*Mlinar Z *60*Mojsović Ćuić A *66*Mravak S *47, 73, 74, 75*Muhić I *54* 

#### N

Nevistić A Nieuwenhuis D Ninčević J Nonković D **43, 70** 

#### 0

Odžak R **59** Orlandini S **19** 

Paklarčić M 23

#### P

Palman Škulj A 54 Pantić-Palibrk V 51, 52 Parroni A 25 Pavić T 27, 47, 55 Pavlek Ž 57 Pavliček D 52 Pavličević N 60 Pavlović G 56 Peharec Štefanić P 46, 65 Peraić A 64 Peršurić Ž 64 Petković V 22, 50 Petric IV 56 Petrović M 57, 58 Pietricola C 25 Pihlström T 20 Pleadin J 45 Pocrnić M 59 Poljak M 40 Poljak V 43, 70 Pollak L 33 Popek I 73 Popijač V 70 Popović M 24, 56 Pravst I 23 Prđun S 63 Prodan Bedalov M 53 Prokurica Pavlinić I 44 Prskalo I 27 Puc M 23

Puhač Bogadi N 70

Puljak A 68, 72

#### R

Radeljević B 51
Radmanić L 45
Radulović N 36
Randić M 73
Reverberi M 25
Ribeiro AS 20
Rimac H 65
Ristić M 52
Rizzo R 25
Roginić J 40
Romić M 38
Rotim C 74
Rudić Grujić V 22, 50
Rydevik A 20

#### S

Sabarić J 55 Saftić L 64 Sajko D 47, 73, 74, 75 Samardžija M 45 Scarpari M 25 Sertić Đurđević M 73 Sever Z 44 Skočibušić M 59 Smolej Narančić N 47 Solina N 44 Srečec S 73 Stavljenić Rukavina A 47 Stefanović M 62 Stojanović D 54 Strelec Dučak A 56 Sudec S 38 Svečnjak L 63 Szymański G 61

#### Š

Šabarić J 38, 46, 65, 66 Šarkanj B 22, 39 Šepec S 73 Šikić S 38, 46, 65, 66 Šimat V 43 Šimleša D 69 Šimon S *56* Šimunec D 73 Širić I 66 Škes I **69, 71** Škes M 68, 69, 72 Šostar Z 40, 47, 73, 74 Španić Ciceli N 34, 62 Španić V 39 Špoljarić J 37, 51 Štefanac M 72

#### $\mathbf{T}$

Takač T 71
Tešić V 72
Tkalec M 46, 65
Tomasović Mrčela N 47, 73, 74
Tomasović-Mrčela N 75
Tomek-Roksandić S 47, 73, 74, 75
Torović Lj 24
Toth I 28
Tripković I 53

#### U

Učakar V *23* Upton J *32* Uršulin-Trstenjak N *67* 

Tršić-Milanović N *36* Trstenjak J *67* 

#### V

Van den Bijgaart 19
Vazdar R 27, 41
Vdović N 30
Velicki R 24
Vešligaj B 30
Vinković Vrček I 38
Vitali Čepo D 38, 60
Vranešić Bender D 26, 47
Vrbatović A 70
Vrbić Lj 73
Vučinić V 26
Vukić Lušić D 37
Vuković Rodríguez J 73
Vuljanić K 27

#### W

Walkowiak-Tomczak D 61

#### $\mathbf{Y}$

Ye J, Reddy S 31

#### $\mathbf{Z}$

Zamberlin S *51* Zdolec N *51* Zjalic S *25* 

#### Ž

Živadinović E 43 Živanović J 24 Žuntar I 38, 65, 71 Župan A 36 Županić M 73 Žure K 59

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