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Food Safety and Quality  
“One Health”**

9-12 November 2022

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# 4<sup>th</sup> International Congress on Food Safety and Quality ONE HEALTH

November 9<sup>th</sup>-12<sup>th</sup>, 2022 | Dubrovnik, Croatia

## 4<sup>th</sup> International Congress on Food Safety and Quality “One Health”

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Dear colleagues, dear friends,

It is my great honour and pleasure to invite you on behalf of the Organising and Scientific Committee to participate in the 4<sup>th</sup> International Congress on Food Safety and Quality under the slogan “One Health”, which will be held in Dubrovnik, from 9 to 12 November 2022.

The International Congress on Food Safety and Quality will again be held under the auspices of the competent authorities and institutions and is organised by the Andrija Štampar Teaching Institute of Public Health, the Croatian Metrology Society, and the Southeast European Food Quality and Safety Control Network (SEEN-FSQC). The co-organisers are the Faculty of Agriculture and the Faculty of Food Technology and Biotechnology, the University of Zagreb, the Institute for Health and Food Safety Zenica, and the Institute of Public Health of Vojvodina, in cooperation with other eminent institutions from the country and abroad.

Thanks to the extremely important topics and the esteemed experts who shared their knowledge and experiences with us at the first three Congresses on Food Safety and Quality, we were all happy to see

truly great interest: the Congresses New Achievements and Future Challenges in 2017, Food Life Cycle in 2018, and Food, Health and Climate Change in 2020 were attended by nearly 1,000 participants, while numerous guest speakers from the country and the world presented their colourful presentations in the form of exceptional plenary lectures, excellent oral presentations, satellite symposia, workshops, and posters. On the wings of these successes, our goal is to organise an even more significant, distinctive, and educational 4<sup>th</sup> International Congress on Food Safety and Quality.

We will present the significant experiences of domestic and foreign experts in the field of food counterfeiting, protection of the origin and geographical origin of food, and new, exact analytical achievements in this field.

Food safety and quality, determination of food origin and geographical origin, adulteration of food and its impact on consumer health, as well as the impact of climate change on farming and quality are all topics of crucial significance and impose a shared responsibility on all stakeholders in the food production chain. These are primarily countries, producers, distributors, consumers, but also the media, which are responsible for accurately reporting current topics to the public, including this very relevant issue.

Given that our common task is not only to protect, but also to improve the health of each of our residents, by organising the 4<sup>th</sup> International Congress “One Health” we also want to continue to raise awareness of this important topic in the profession.

Thank you for your response.

Sincerely,  
Prof Branko Kolarić, MD, PhD  
Congress President



**ABSTRACTS**

**INVITED SPEAKERS**

## National quality system for agricultural and food products “Dokazana kvaliteta”

Z. Barać\*

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The national quality system for agricultural and food products “Dokazana kvaliteta” is a voluntary system intended for the labelling of agricultural and food products with special characteristics from different food production sectors (e.g. milk and dairy products, meat production, fruit and vegetables production, egg production, etc.) produced in accordance with the approved product specification. The procedure of verification of product conformity with the specification shall be carried out by the authorised control body. Every producer or processor of an agricultural and food product that wants to use the label “Dokazana kvaliteta” shall initiate a procedure verify product conformity with the specification. The authorised control body shall carry out the procedure of verification of product conformity with the specification in accordance with the official Control Plan for the product in question. The label “Dokazana kvaliteta” with an indication of the country of origin can be applied only on agricultural and food products for which a Certificate of Conformity has been issued, and for which the country indicated on the label is the country where the product was produced and/or processed and which is also the country of origin of the main ingredient. The label “Dokazana kvaliteta” without an indication of the country of origin can be applied only on agricultural and food products for which a Certificate of Conformity has been issued, and for which the country of origin of the main ingredient is not the same country where the product was produced and/or processed.

KEY WORDS: food quality; labelling; specification; verification; voluntary system

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## FDA’s food safety priorities and progress: protecting consumers and the food supply

R. Nalubola\*\*

*U.S. Food and Drug Administration, USA*

In the United States, about 15 percent of the food supply is imported from more than 200 countries or territories, including 32 percent of fresh vegetables, 55 percent of fresh fruit, and at least 94 percent of seafood that Americans eat each year. This increasingly globalized and complex marketplace has also placed new challenges on the U.S. food safety system. In 2011, Congress passed the FDA Food Safety Modernization Act (FSMA), shifting the focus of federal regulators from responding to contamination to preventing it. Over the last several years, FDA has developed prevention-based standards applicable to foreign and domestic food growers, manufacturers, processors, packers, and holders. In addition, under the New Era of Smarter Food Safety initiative that builds on our FSMA achievements, FDA is taking a new approach to food safety, leveraging technology and other tools and approaches to create a safer and more digital, traceable food system. This presentation will provide updates on FDA’s food safety and nutrition priorities to protect consumers and the food supply.

KEY WORDS: federal regulators; Food Safety Modernization Act; nutrition priorities; traceable food system

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## Skin barrier and food allergy

S. Kezic\*

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Food allergies are on the rise and affect up to 6 percent of children in Western societies. For a long time it was believed that children become sensitized to food allergens by exposure through the gut. However, there is increasing and compelling evidence from genetic, epidemiological, and murine studies that the penetration of food allergens across the skin can lead to food sensitisation and food allergy, while early oral exposure causes tolerance. It may be assumed therefore that inherited or acquired skin barrier impairment will facilitate percutaneous penetration and increase the risk for food allergy. Skin barrier impairment may be caused and/or perpetuated by genetic factors such as mutations in the filaggrin gene (FLG), presence of atopic dermatitis (AD) or due to exposure to exogenous factors. Neonates are at particular risk as their skin still does not have a fully competent barrier function and is likely more permeable to food allergens. In this presentation, the structure of the skin barrier and factors which might affect its function will be discussed. Furthermore, recent literature on the association between altered skin barrier and food allergy will be reviewed. Next, interventions aimed at skin barrier repair with the goal of preventing food allergy will be addressed.

KEY WORDS: atopic dermatitis; children; filaggrin; food sensitisation; percutaneous penetration

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## Enhancing food safety and quality through stable isotope techniques

N. Ogrinc\*\*

*Jožef Stefan Institute, Ljubljana, Slovenia*

Proof of provenance has become an important topic in the context of food safety, food quality, and consumer protection in accordance with national legislation and international standards and guidelines. Isotopic and elemental fingerprinting provides a robust analytical tool to determine the origin of food. These techniques provide an independent verification of food traceability systems, thereby helping to protect human health and facilitate international trade worldwide. This presentation deals with a concept of the use of stable isotopes in combination with elemental analysis and statistical evaluation to determine the authenticity and origin of food. The classical approaches investigating bulk hydrogen, carbon, nitrogen, oxygen, and sulfur isotopes and strategies including compound-specific analysis will also be reviewed. Specifically, the presentation will focus on recent progresses in the development of new analytical methods of compound-specific analysis including fatty acids, volatile organic compounds, and aminoacids. These analysis can be used to determine the authenticity of aroma compounds, quality of meat, and organic foods such as wine. Stable isotope composition of nitrogen in amino acids can also be used to discriminate between wild and aquaculture fish. Finally, the limitations and future research directions of isotope analysis in food authenticity and traceability will be presented.

KEY WORDS: consumer protection; geographical origin; light elements; statistical evaluation; traceability

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## Development of DNA-based methods to identify species and test food authenticity in seafood products – the experience gained in Chile

M. A. Larraín\*

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Seafood trade is an important economic activity worldwide as well as in Chile. To assure maximum sustainability in fishery production while protecting the marine ecosystem, governments establish fisheries management measures. However, the attractive potential of large profits encourages illicit activities such as illegal, unreported, and unregulated (IUU) fishing and food fraud. These are topics of growing concern among consumers, food business operators, and regulators, giving rise to seafood traceability and labelling requirements. Specifically, the EU regulation on the common organization of markets in fishery and aquaculture products (EU N° 1379/2013) includes the obligation to declare the commercial and scientific name of the species in foodstuffs. In this scenario, for species identification, DNA analysis is the gold standard, especially useful for processed products, where the identification based on morphological traits is no longer possible. However, to enable an effective enforcement of the regulation, there are several matters to take into consideration, such as the cost of analysis, time to obtain results, possibility of routine and *in situ* application, and that it delivers validated results that can be used as evidence in court. Also, in each organism or group of organisms, the species delimitation criteria, target genes, and algorithms used to obtain the result should be considered. The challenges and experience that have been accumulated developing SI methods to support the Chilean seafood sector will be discussed.

KEY WORDS: DNA analysis; food quality; labelling requirements; species identification; traceability

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## Systematic approach for emerging food systems risk identification

Á. Bernard Józwiak\*\*

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Early identification of emerging food systems risks helps protect human, animal, and plant health and also contributes to strategic planning and analysis, decision-making processes, surveillance planning, thereby serving as input for risk management, mitigation, and prevention measures. However, there are several factors that make this process a complex, interdisciplinary task. Risks may arise from different emerging hazards; however, complex, driver-induced early signals and the increase of exposure for known hazards also must be taken into account in an extremely noisy environment. The timescale of the risks occurring may also vary and is often hard to estimate. A systematic approach that handles these difficulties regarding emerging risk identification has been elaborated and used in practice. Data and information on possible emerging issues are gathered by data mining algorithms and expert knowledge. Then, a multi-step selection procedure with a scoring system is conducted by an expert group in order to select the relevant issues from the large amount of data collected. At the end of the multi-step procedure, emerging risks that need further measures are selected and forwarded to the relevant stakeholders (e.g., authorities, industry, consumers, academia). The elaborated identification system with the supporting data mining algorithms is applicable for the early identification of food systems-related emerging risks and also incorporates a follow-up system for a later tracking of the issues.

KEY WORDS: emerging hazards; expert group; known hazards; risk management; scoring system

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## Achievements and challenges encountered during the implementation of new and upcoming EU regulations in the area of mycotoxins and plant toxins

P. Mulder\*, H. Mol, and M. de Nijs

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The past two years has seen the introduction of a number of new EU regulations, particularly in the area of plant toxins. Six regulations were published that have come into effect during 2022 and more legislation is underway. Examples are regulation (EU) 2020/2040 describing maximum levels of 35 pyrrolizidine alkaloids and regulation (EU) 2021/1408 describing maximum levels (MLs) for tropane alkaloids, both in a wide variety of foodstuffs. In the mycotoxin field, ergot alkaloids are now regulated in (EU) 2021/2001 for cereal milling products including rye, wheat gluten, and processed cereal based foods for infants and young children. Last, but not least, the current regulation on sampling of mycotoxins (EC) 401/2006 is under revision and a similar regulation is being developed for the sampling related to plant toxins. The establishment of MLs for this wide variety of substances and matrices presents a considerable challenge for European Union Reference Laboratories (EURL), National Reference Laboratories (NRLs), and Official Laboratories (OLs). Methods need to be developed and/or validated, accredited, and implemented in laboratories for Official Control. To facilitate this process, continuous efforts are made by the EURL-mycotoxins and plant toxins (MP) to develop methods for the various toxin groups that are robust and easy to apply. These methods are made available via the EURL-MP website and when the required trainings are organised. The capabilities of NRLs and their progress in implementing methods for Official Control are monitored via the organisation of proficiency tests (PTs). The results of these PTs will be discussed. Work in progress on toxin groups that may become relevant in the near future will be discussed as well.

KEY WORDS: EURL on mycotoxins and plant toxins; food and feed safety; legislation; official control; proficiency tests

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## The project “Food Safety and Quality Control Center” KK.01.1.1.02.0004; where we are now and what is next

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The implementation period of the project “Food Safety and Quality Control Center” is from 1 October 2018 to 31 December 2022 and its beneficiary is the Andrija Štampar Teaching Institute of Public Health and partner Faculty of Agriculture, Zagreb. Since the Food Safety and Quality Control Center was established, it has worked in the field of food counterfeiting, protection of the origin and geographical origin of food, ecological production of food, organoleptic investigations, and new analytical achievements. It is also a good platform for the scientific collaboration of experts in Croatia and elsewhere and an improved base for teaching and research experts in the field. We have built new laboratories, reconstructed an old building, equipped laboratories with the highest standards in research and analytical equipment, as well as acquired ICT equipment and vehicles for the implementation of research activities. We have learned to work with analytical equipment and new technologies in times of a pandemic and earthquakes in the Zagreb area. We have developed and are still developing new methods in order to carry out scientific research activities. Samples from all over the Republic of Croatia and abroad (honey, olive oil, meat, cheese, soil, and water) have been collected from 2019 to 2022 with the purpose of designing an isotope map and information database of Croatian indigenous and organic food products. This will lead to their recognizability in the domestic and foreign markets. The project will continue through new ones that are already being implemented, as well as those that are being applied for.

Acknowledgement: This work was carried out within the project “Food Safety and Quality Center” (KK.01.1.1.02.0004). The project is co-financed by the European Union from the European Regional Development Fund.

KEY WORDS: geographical origin; isotope ratio; organic food; public health; samples

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## New trends and an individual approach to pesticide residue risk assessments with the aim of consumer protection

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The improper use of pesticides is the most common cause of the presence of residues greater than those allowed by Regulation (EC) No 396/2005, which prescribes maximum residue level (MRL) values for about 1,100 pesticides. The term “pesticide residues” refers to residues either in or on products of plant or animal origin and in animal feed that are the result of use of plant protection products, biocides and agents used in veterinary medicine. Risk assessment is a complex process based on science-based facts and new knowledge and information and includes four steps: 1. hazard identification; 2. hazard assessment; 3. exposure assessment; and 4. risk characterization. In order to determine whether there is an acute risk to the health of consumers by consuming products with determined pesticide residues above the MRL, a detected value of pesticide residue concentration, dietary models that include consumption data of a certain type of food and dietary habits of a certain population group, as well as toxicological value Acute Reference Dose (ARfD) are needed. If the estimated intake of residues is less than the acceptable dose, the risk is acceptable. From the point of view of food safety, a certain type of food is considered safe for a consumer if the estimated intake of the harmful substance does not exceed the ARfD value. Several food products have recently been withdrawn and recalled from the market due to the use of unapproved pesticides such as ethylene oxide and chlorpyrifos. Such cases, in which it is not possible to conduct an adequate risk assessment for consumers with a sufficient level of safety and precision of the obtained results due to a lack of data, necessitate a different and individual risk assessment approach to ensure a maximum level of consumer protection.

KEY WORDS: Acute Reference Dose; food safety; hazard identification; improper use; risk characterization

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## One Agri-Food Health Approach in the Meat Safety Assurance System

N. Zdolec\*\*

*Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

In our country, the One Health approach has been in place for decades, involving both veterinary and human health professionals to educate the public and eliminate public health threats based on existing risks. When it comes to food safety, especially meat, the roles and responsibilities of the different players at the pre-harvest, harvest, and post-harvest levels are obvious due to the complex interaction of many risk factors in the food chain. In recent years, new and certain traditional biological hazards have (re)emerged in meat production, so a targeted risk-based approach along the food chain is sought. The main biological hazards that need to be addressed in the meat chain of different animal species at the pre-harvest (farm) and harvest (abattoir) levels are *Campylobacter* spp., *Salmonella enterica*, human pathogenic *Escherichia coli*, *Yersinia enterocolitica*, *Trichinella* spp., *Toxoplasma gondii* and ESBL-AmpC gene-carrying bacteria. For some hazards, such as *Campylobacter* spp., *T. gondii*, or *Y. enterocolitica*, better integration of on-farm control data and abattoir-level control measures/interventions should be introduced as part of the Meat Safety Assurance System (MSAS). The risk assessment of public health hazards to be covered by veterinary meat inspection was carried out about a decade ago by the European Food Safety Authority (EFSA) panels that proposed the Harmonized Epidemiological Indicators (HEIs) applied at specific points in the farm-to-abattoir continuum. The regulatory framework has been changed accordingly towards a risk-based approach, bringing all types of inspection services under the umbrella of one regulation to follow the One Agri-Food Health Approach in their work and cooperation.

KEY WORDS: agri-food chain; epidemiological indicators; food safety; hazards; risk

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## Innovative technology in “Paški sir” cheese production

N. Mikulec\*, F. Oštarić, J. Špoljarić, Š. Zamberlin, I. Horvat Kesić, I. Dolenčić Špehar, and N. Antunac

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First records about “Paški sir” cheese production date back to 1912. During the next decades, the production procedure was modernized but traditional principles of curd processing and maturation had been kept the same. The aim of the project “Potential of microencapsulation in cheese production” is the revitalization of production technology for the rennet used in cheese production by incorporating a modern method for microencapsulation of indigenous rennet and dairy cultures. The cheeses matured for 60 days were divided into three groups: (1) cheeses produced with natural commercial rennet and commercial dairy cultures (2) cheeses produced with lyophilized indigenous lamb rennet and microencapsulated dairy cultures (3) cheeses produced with innovative microcapsules containing indigenous lamb rennet and dairy cultures. Sensory evaluation of produced cheeses was carried out. A systematic scoring of evaluated parameters was used with maximum points for exterior appearance, texture, cross-section, color, aroma, and flavour. The results showed that innovative microcapsules containing indigenous rennet and dairy cultures were suitable for “Paški sir” cheese. The application of microencapsulated rennet has no geographical limitations and can be used regionally and globally. This approach allows producers the use pasteurization and production of cheese with the same traits as the one made from raw milk, but with no risk of microbial contamination. This is of great importance for cheeses with protected designations of origin (PDO) in the territory of the EU. The application of this innovative approach in indigenous rennet and dairy cultures preparation for use in traditional cheese production can significantly maximize their recognition in the global market.

KEY WORDS: dairy cultures; microencapsulation; protected designations of origin; rennet; sensory evaluation

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## Challenges in placing upcycled foods on the market

J. Ranilović\*\* and T. Cvetković

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Today’s consumers are aware of sustainability issues and are therefore ready to pay more for a product that meets their subjective standards and attitudes. By-products that arise during the fruit and vegetable processing process represent waste and cost for food producers. The development of new products from or with by-products are upcycled foods, which lead to challenges in the developing phase (technological, safety, hygienic, regulatory) and possibly even more so in the marketing-sales phase. During the EU co-funded research project, several new and innovative upcycled products were developed for human consumption (pepper seed oil, pepper seed flour, vegetable sauces, and spreads with oil or pepper seed flour). Highlighting the claims on the label of upcycled foods pose an important next step in communication towards sustainability-conscious consumers. However, sustainability claims are numerous, voluntary, and not yet regulated, therefore it is questionable whether they would influence consumers’ will to buy. Consequently, consumer attitudes and knowledge related to upcycled foods need to be evaluated on a country-by-country basis to achieve the best outcomes for food producers.

KEY WORDS: attitudes; by-products; consumer; sustainability; upcycled foods

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## The role of testing in fighting food fraud

L. Martin\*

*EUROLAB, Brussels, Belgium*

As the European Federation of National Associations of Measurement, Testing and Analytical Laboratories, EUROLAB gathers 25 national associations all over Europe and beyond. One of its main goals is to cooperate with European and international stakeholders to represent the interests of the Members and raise visibility within laboratory communities, as well as to the external public on the added value and importance of testing for their safety. The first part of the presentation will be an introduction of EUROLAB, its scope and added value for the members, including publications and technical reports, as the Cook Books, webinars, and events on topics of interest for the laboratory community. In particular, insights will be given regarding cooperation activities and strategies with EU institutions and international stakeholders, focusing on key organisations such as TIC Council, EURAMET, IMEKO, and Eurachem, including the new partners My Green Lab and the European Chemicals Agency (ECHA). The second part will provide an overview on food fraud as a growing issue worldwide and the role of the Testing, Inspection and Certification (TIC) sector in fighting this phenomenon. Food authenticity testing aims to prove and document that a food or food ingredient is what it is claimed to be and to identify whether it has been unintentionally – or in the case of food fraud, purposefully – adulterated. The presentation will lay out key data about food counterfeits, characteristics, and types of food fraud and final remarks on the importance of accurate testing and analysis to minimise the risk of food fraud and help identify the fraudulent food products.

KEY WORDS: EUROLAB; food certification; food safety; fraudulent food products

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## European Health Union and the One Health approach

B. Borzan\*\*

*European Parliament, Brussels, Belgium*

Medical, veterinary, food, and environmental sciences are separate fields of expertise at the institutional level in European countries, often located in different institutions and funded by different ministries, resulting in inefficient exploitation of new techniques, redundant or overlapping research activities, and suboptimal systems for risk assessment and management of emerging threats, possibly missing important opportunities for solutions. This fragmented landscape, in a timeframe of new scientific developments and reduced research budgets will benefit from the implementation the Health Union and the One Health approach in the EU. The coronavirus has highlighted that the European Union does not have tools strong enough to deal with an emergency such as the spread of a novel infectious disease, which by its nature knows no borders. While the EU has significant competence in public health, healthcare systems remain the responsibility of Member States, with minimal cooperation at EU level. But the pandemic has changed that, the Union and its Member States cooperated at an unprecedented level healthcare-wise to decrease the infection rates, but most of the responses were improvised, having no practical or legal precedent. If some of these decisions and reforms had been made earlier, Europe would have fared far better during the pandemic. But now we must look to the future. The EU and its Member States are now doing things in healthcare that they would not have even discussed a couple of years before, like implementing the Health Union and the One Health approach, and it is the EU citizens who will profit from that.

KEY WORDS: coronavirus; healthcare; health management; EU Member States

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SPONSORED LECTURE

## Shimadzu analytical toolbox for food applications

M. Potkrajčić\*

*Shimadzu d.o.o.*

The provision of safe, nutritious, high quality, and affordable food to Europe's consumers is the central objective of EU policy, which covers all stages of the EU food supply chain, "from farm to fork". Its standards and requirements aim to ensure a high level of food safety and nutrition within an efficient, competitive, sustainable, and innovative global market. In order to fulfill these requirements, Shimadzu offers a wide variety of instruments which enable sensitive and precise analysis of components in food samples. As an alternative to standard liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of pesticides in fruits and vegetables, Shimadzu developed an improved supercritical fluid chromatography (SFC)-LC-MS/MS method in order to obtain higher sensitivity and selectivity with a reduced matrix effect. Per- and polyfluoroalkyl substances (PFAS) are currently of great concern to public health and the environment. Shimadzu developed state-of-the-art analytical methods for monitoring PFAS in environmental and food samples using the LC-MS/MS technique. For metal monitoring in food samples, there are fully developed inductively coupled plasma mass spectrometry (ICP-MS) methods that are highly accurate and robust. For mineral oil saturated hydrocarbons/mineral oil aromatic hydrocarbons (MOSH/MOAH) analysis in food and food packaging, Shimadzu developed an LC-gas chromatography (GC)-online system according to the European Norm proposal. Furthermore, cannabis testing is also currently a vital topic. To ensure that products based on cannabis fulfil all the required criteria, Shimadzu offers a complete solution for cannabis testing which includes testing cannabinoids, terpenes, pesticides, and heavy metals.

KEY WORDS: cannabis; heavy metals; MOSH/MOAH; pesticides; PFAS

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SPONSORED LECTURE

## Applications for vibrational spectroscopy in food analysis

M. Ries\*\*

*Thermo Fisher Scientific GmbH*

Vibrational spectroscopy is a set of powerful, non-destructive optical tools that can play an important role in analysis at each step of the food chain. It can be applied in the analysis of raw materials, as an *in-situ* technique during food processing, by controlling the quality at certain steps of the production chain or after processing – and even in the analysis of contaminants due to production, packaging, or environmental conditions. Thermo Fisher Scientific offers a broad palette of different spectroscopic instruments monitoring vibrational molecular information, ranging from near-infrared (NIR), mid/far-infrared, to Raman spectroscopy, and from microscopy to *in-situ* spectroscopy options. A user-friendly and powerful software package supports spectral interpretation, literature search with multiple databases, creation of calibration methods, discriminant analysis, microscopic investigation, and chemical imaging. A variety of application examples for different methods is presented: calibration with NIR for quality analysis of wheat flour, contaminant analysis with IR- and Raman-microscopy, as well as in-line NIR- and Raman-analysis during the extrusion of meat analogs.

KEY WORDS: FTIR; NIR; Raman; quality

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## SPONSORED LECTURE

**Is rapid analytics reliable enough?**

S. Rumpl Tunjić\*

*Labena d.o.o.*

The speed of everyday work includes an increasing number of incoming samples and a stagnating number of employees in the laboratory, which is often a considerable problem for food control laboratories. Fast Near Infrared (NIR) and Fourier Transform Infrared (FTIR) analytical methods are nowadays more common solutions to problems in control laboratories and a replacement for classic chemistry analysis. Their simplicity, speed, and reproducibility are unquestionable, and the question arises whether they can be a new standard for reference analysis. Our experience based on the collaboration between Foss, Labena, and a reference laboratory will attempt to answer all potential questions regarding reliability.

KEY WORDS: fast analytical method; food control; food industry; new standard; NIR analytical solution; reference analysis

## SPONSORED LECTURE

**From field to fork – solutions for mycotoxins and alkaloids detection**

C. Rathmann\*\*

*Food & Environmental Market Development Manager CE, Waters GmbH*

Waters and VICAM together offer complete field to lab solutions to address the needs for early detection and finished products verification. Our rapid, antibody-based strip tests provide a fully streamlined approach to preventive monitoring that allows quantitative results for up to 6 mycotoxins in less than 10 minutes. Immunoaffinity columns offer multiple functions: they can be tested with a field-based fluorometer or coupled with high performance liquid chromatography (HPLC) and ultra performance liquid chromatography (UPLC) for confirmatory methods of single or multiple mycotoxins. The high selectivity of Immunoaffinity columns also makes them an ideal clean-up in use with liquid chromatography-mass spectrometry (LC-MS). In a broader approach, the well-known sample prep tools of the Oasis family provide superior performance in sample clean-up that can be applicable to multi-toxin and multi-residue methods. And if you require high through-put, a dilute and shoot method using a high-end mass spec is the answer.

KEY WORDS: antibody-based strip tests; HPLC; LC-MS; Immunoaffinity columns; UPLC

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## **ORAL PRESENTATIONS**

## Dietary exposure and influence of climate on the occurrence of T-2 and HT-2 toxins in Croatia

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Within the context of the One Health Approach, the Croatian Agency for Agriculture and Food conducted a three-year (2017-2019) study on T-2 and HT-2 toxin occurrence in cereals produced in Croatia in order to assess dietary exposure of Croatian adult consumers and influence of climate. Cereal grains and soybeans samples were collected and analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Consumption data were obtained from the National Survey on Food Consumption of the Adult Population in Croatia, conducted in 2011-2012. The average dietary exposure of adult Croatian consumers did not exceed the Tolerable Daily Intake value within the Lower Bound, Middle Bound, and Upper Bound approach. Impact of weather conditions on the occurrence of T-2 and HT-2 toxins was assessed by Spearman test to determine the correlation between weather conditions and occurrence of the sum of T-2 and HT-2 toxins in total in all cereals. The test showed that there was no unambiguous correlation between these conditions ( $p=0.09-0.82$ ). There was a correlation between the amount of precipitation in May/June and the occurrence of the sum of T-2 and HT-2 toxins in wheat ( $p=0.029$ ) and an almost significant correlation for the same conditions in maize ( $p=0.057$ ). These results indicated that it is possible to use weather data during cereal growth as a variable to predict the occurrence of mycotoxins, and thus help farmers decide whether to use fungicides or appropriate methods of storage and processing in order to reduce the risk of harmful effects of mycotoxins on humans and animals.

KEY WORDS: cereals; LC-MS/MS; mycotoxins; One Health approach; weather conditions

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## *Staphylococcus aureus* and its enterotoxins in domestic cheese

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Epidemiological and scientific data show that *Staphylococcus aureus* is often found in raw milk cheeses. Certain strains of *S. aureus* produce enterotoxins during their growth in favorable conditions in food, which are causative agents of staphylococcal food poisoning. Compliance with the food safety criteria for staphylococcal enterotoxins in cheese has to be investigated whenever levels of coagulase-positive staphylococci exceed  $10^5$  cfu/g. The aim of this study was to investigate the occurrence of *S. aureus* and staphylococcal enterotoxins in domestic cheeses produced in the Dubrovnik area, as well as the *in vitro* enterotoxin production ability of *S. aureus* isolates. Out of 30 analysed cheese samples, 18 samples (60 %) were highly contaminated (average  $6.9 \times 10^5$  cfu/g) with *S. aureus* strains. In 17 cheese samples (56.7 %), the *S. aureus* number was above legal safety criteria. However, staphylococcal enterotoxins were not detected in any of the cheese samples. A total of 175 *S. aureus* isolates were collected from 18 cheese samples and confirmed by a latex agglutination test. Enterotoxin production *in vitro* was detected in 37 isolates (21.1 %) by the ELISA method (VIDAS SET2). In 34 out of 37 isolates, the reversed passive latex agglutination (RPLA) method detected the production of staphylococcal enterotoxin C. Enterotoxigenic strains originated from 8 cheese samples of two cheese manufacturers. Although highly contaminated, even with enterotoxigenic strains, staphylococcal enterotoxins were not detected, indicating that the intrinsic characteristics of cheese play an important role in enterotoxin production. However, isolated strains possess enterotoxigenic potential so enterotoxin production in cheeses cannot be ruled out.

KEY WORDS: ELISA; RPLA; staphylococcal enterotoxins

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## Hydroanthracene derivatives in the light of new regulations

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A sedentary lifestyle has led to the development of health problems with digestion in many individuals. Because the self-medication trend is so widespread, consumers are buying and consuming herbal supplements to help themselves. Numerous plant species used as ingredients in food supplements or herbal medicines contain hydroxyanthracene compounds/derivatives that have a laxative effect. According to the Scientific Opinion of the European Food Safety Agency (EFSA) from 2013, it was concluded that hydroxyanthracene compounds have a laxative effect, but the problem lies in the length of application. Long-term use and consumption in high doses can lead to the risk of electrolyte imbalance, impaired bowel function and dependence on laxatives. In view of this opinion and concerns expressed by EU member states during discussions about the health impact and possible harm, the European Commission, after numerous scientific opinions and safety assessments, issued Commission Regulation (EU) 2021/468 amending Annex III. Regulation (EC) no. 1925/2006 of the European Parliament and of the Council regarding botanical species containing hydroxyanthracene derivatives. The review of the composition based on the declarations so far in 53 samples found compliance with the Regulation, while 12 samples were not yet adapted to the new Regulation due to the transitional period until its entry into force. The aim of the work is to determine and develop analytical high performance liquid chromatographic (HPLC) methods for the determination of hydroxyanthracene compounds – sennosides A and B, frangulins A and B, glucofrangulins A and B, aloin, aloe-emodin, rhein, chrysophanol, and total flavonoids essential for product safety. Based on the results and evaluation of the label, the compliance and reformulation of food supplements with the new Regulation will be checked.

KEY WORDS: food supplements; laxative effect; new EU Regulation; quality; safety

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## Dietary exposure assessment of Croatian consumers to ochratoxin A from coffee

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Coffee is one of the most popular beverages in Croatia. Coffee trees are planted in tropical and semi-tropical areas, but coffees are roasted worldwide. Coffee is prone to attacks by various fungi genus *Aspergillus* and *Penicillium* whose species synthesize ochratoxin A (OTA). Contamination of coffee beans with OTA depends on various factors such as fungus species and strain, water activity, processing conditions, storage, and transportation. The most significant impact is related to environmental and climate conditions. OTA can be present in coffee at a certain level, even when processors follow good agricultural practices (GAP). Therefore, GAP guidelines should be updated according to One Health principles. In order to assess the chronic dietary exposure of adult Croatian consumers to OTA in coffee beverages, occurrence data (n=55) derived from national monitoring 2015-2020 and consumption data (n=2002 respondents) from the National Survey on Food Consumption of the Adult Population in Croatia 2011-2012 were used. A total of 1649 consumers reported daily consumption, some even several times per day. For dietary exposure, an assessment a margin of exposure (MOE) approach was applied by use Benchmark Dose Level (BMDL<sub>10</sub>) as referent point for neoplastic (14.5 µg/kg bw per day) and non-neoplastic (4.73 µg/kg bw per day) effects. Average dietary exposure of adult consumers does not exceed values within the Lower Bound (LB), Middle Bound (MB), and Upper Bound (UB) approach for both BMDL<sub>10</sub>. The calculated MOE results for non-neoplastic effects (LB 24481.45; MB 15958.54; UB 11850.06) and for neoplastic effects (LB 75048.84; MB 48921.52; UB 36326.83) represents a low health concern.

KEY WORDS: climate; environment; food consumption; margin of exposure (MOE); One Health

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## Elemental composition and health risk of Fruška Gora wines

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The elements in wine could influence wine quality and safety – are there potential health risks for the consumers of Fruška gora wines? The elemental composition (23 elements) of 131 bottled dry wines, 113 (red, white, and rose) produced in 30 Fruška Gora wineries and 18 (red) from several European countries was obtained by inductively coupled plasma mass spectrometry (ICP-MS) analysis. The exposure of adult men and women was estimated taking into account wine consumption on population average, regular drinkers only, chronic heavy drinkers and level recommended by the Wine in moderation (WIM) for red wine. Related to Fruška gora wines, not one of the investigated elements/wines showed potential for non-carcinogenic risk [hazard quotients and index 10 for men and women, except at P95 in “WIM” scenario (Margin of exposure (MOE) >1, negligible risk)]. With respect to arsenic, evaluation of carcinogenic risk revealed a disagreement between MOE (mean and P95 MOE >10, no risk) and lifetime cancer risk approach (tolerable risk of 1 extra lifetime cancer case per 100,000 persons was exceeded across the exposure scenarios at P90, P75, P75, and P25 for males, and P75 and P50 in “heavy” and “WIM” scenarios for females). Comparison of red wines, Fruška gora vs. international, revealed no significant differences in estimated risk of arsenic, whereas Fruška Gora wines showed a statistically lower MOEs to lead. Apart from the risk assessment, the obtained elemental profiles could be a valuable resource in distinguishing wine geographical origin.

KEY WORDS: hazard index; food safety; ICP-MS; Lifetime Cancer Risk; margin of exposure

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## Monitoring of pesticide residues in honey, olive oil, and soil samples collected as part of project “Center for Food Safety” – preliminary results

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During the 4 years of the project, samples were collected in different parts of Croatia. In the project, samples of honey, olive oil, meat, cheese, soil, and water were collected, while samples of honey, olive oil, meat, cheese and soil were analysed for pesticide residues. For the preparation of samples, the QuEChERS method was utilized for the honey and olive oil samples and a modified version for the soil samples. A gas chromatograph-tandem mass spectrometer (GC-MS/MS) Shimadzu TQ-8050NX and a liquid chromatograph tandem mass spectrometer (LC-MS/MS) Agilent 1260 Infinity II were used to conduct analyses of pesticide residue. Over 600 active chemicals were examined in a combined 150 honey samples, 200 olive oil samples, and 200 soil samples. It was discovered that certain honey samples contain the unapproved chemical coumaphos while pesticide residues were being monitored. Coumaphos tends to gather in wax, where it stays for a while and then gradually transitions into honey. The oil samples contained a variety of pesticide residues, some of which are illegal to use in the EU (such as chlorpyrifos) and others whose concentrations are over the Maximum Residue Level (MRL). Farmers' continued usage of outdated supplies is tied to the use of prohibited plant protection products. Farmers are able to buy and use pesticides that have not been approved in Croatia's neighbouring countries. Pesticide residual levels in all soil samples were below the quantifiable limit (LOQ). The results of this research point to the need for increased monitoring and control of food products for pesticide residues.

Acknowledgements: This work was carried out within the project “Food Safety and Quality Center” (KK.01.1.1.02.0004). The project is co-financed by the European Union from the European Regional Development Fund.

KEY WORDS: chlorpyrifos; coumaphos; GC-MS/MS; LC-MS/MS; QuEChERS

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## Coumarin – how safe are products with cinnamon on the Croatian market

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Many food products contain cinnamon as a spice and it is also used as dietary supplement. Two types of cinnamon are dominant on the market: Ceylon (true) cinnamon and Chinese (cassia) cinnamon. The basic and most important difference between them is that real Ceylon cinnamon is more expensive and does not contain coumarin, while Chinese cinnamon is cheaper and contains the hepatotoxic coumarin. Today, consumers are already better informed about the differences between these two types of cinnamon, but the problem is that the type of cinnamon is not indicated on the declaration of finished products. The legal framework for the amount of coumarin in certain types of food is Regulation (EC) no. 1334/2008 on flavours and some food ingredients with flavoring properties for use in and on food. The aim of the work is to determine the compliance of the analysed products with the highest permitted levels prescribed by the Regulation and to raise awareness about the safety of cinnamon products. For the purposes of this work, samples from four categories of food described by the Regulation and certain types of food in which the highest level is not defined by the regulation, but containing cinnamon and dietary supplements containing cinnamon, were examined. The samples were analysed by a validated high performance liquid chromatography (HPLC) method, and the results were compared with the highest permitted levels according to the Regulation. Elevated levels of coumarin were found in three products. It is safe to conclude that manufacturers are largely aware of coumarin restrictions, but the issue of unregulated food categories remains.

KEY WORDS: analyses; food and dietary supplements; hepatotoxicity; HPLC DAD

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## Declaration of Compliance and conditions for placing on the market products that come into contact with food

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Materials and articles intended to come into contact with food (FCM, Food Contact Materials) must meet certain compliance requirements prescribed by European and national legal regulations. Compliance of the final product can only be ensured if there is an exchange of appropriate data between business entities in the supply chain. In the EU, certain FCM categories must have a written document on compliance, the so-called EU Declaration of Compliance (DoC), for health protection of consumers and to uniform criteria for checking compliance. Regulation EU 10/2011 on plastic materials is one of the best harmonized EU legal frameworks, as a reference for creating compliance requirements for other types of materials where this has not yet been fully defined. According to the requirements of Regulation 10/2011, the DoC should be submitted for finished plastic materials and objects, but also for the products from the intermediate stages of their production, as well as for substances intended for the production of these materials and objects. Our goal is to point out the non-conformities that can occur if the testing conditions under which the DoC was created are not aligned with the requirements of the technological process applied by the food business operator. It is also important to know the legal regulations according to which the compliance of the FCM is assessed in order to determine whether the DoC has been updated with changes in legislation and new scientific knowledge related to restricted and prohibited substances in the composition of the product. In our laboratory routine checks, the most common non-compliance are related to confirming the stability of the product with regard to the test conditions (temperature, time, pH value of food, and storage conditions). Material degradation and increased values of global and specific migrations often occur. Increased specific migrations also occur with FCM when testing is not aligned with the new legal requirements for restricted and prohibited substances. Therefore, it is extremely important to guide business entities about the possible shortcomings of DoCs and the importance of cooperation with authorized laboratories in proving data traceability for their products.

KEY WORDS: food business operator; Food Contact Materials; global/specific migrations; plastic; safety

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## Compliance of paper and board in contact with food-national and EU legislation

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Paper and board are the second most used type of Food Contact Materials (FCM) in Europe, superseded only by plastics. The Commission's Plastics Strategy and the recent decision to ban certain single use plastics has led to increased production and use of various paper products, including those obtained from recycling. Paper and cardboard produced from recycled fibers can be used as FCM if they come from recycled paper and cardboard of special qualities that have been subjected to appropriate processing and cleaning. The final product must be accompanied by a Declaration of Conformity (DOC) and all raw materials, including printing inks and adhesives, must have certificates confirming their suitability for use in contact with food. The substances of high concern that can be found in paper and board are not indicated as an exclusive list, which could be relevant to investigate both from a usage and a safety perspective. In the EU, the legislative framework for paper and cardboard in contact with food is not unique, which is a challenge for business operators and regulatory bodies. In this presentation, current European legislation is compared with the requirements of National Croatian legislation and The Technical Guide On Paper and Board Used in Food Contact Materials and articles (1<sup>st</sup> edition, 2021) EDQM. In summary, there is a need for comprehensive, harmonized regulation on paper and board based on a precautionary approach, from public regulators to the industry, better enforcement ,and improved transparency for consumers.

KEY WORDS: EDQM; Food Contact Materials; paper packaging; recycled paper

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## Impact of the COVID-19 pandemic on the microbiological quality of ice in the Dubrovnik area

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The study aimed to evaluate the impact of the COVID-19 pandemic on the microbiological quality of ice in the Dubrovnik area. Analyses were conducted according to Croatian National Guidelines regarding microbiological criteria for foodstuffs, by using ISO standard methods for aerobic plate count, *Escherichia coli*, *Enterococcus* spp., *Pseudomonas aeruginosa*, sulphite-reducing clostridia. We compared the results for 2019 and 2021 and found that in 2019, 152 ice machines with five elementary units (760 samples) and 168 ice machines with one elementary unit (168 samples) were tested, which is a total of 928 ice samples from 320 ice machines. The test results showed that 301 samples (32.4 %) of ice from 97 ice machines (30.3 %) were unsatisfactory due to high levels of aerobic plate count (205 samples; 22.1 %) and the presence of *P. aeruginosa* (201 samples; 21.7 %). *Enterococcus* was detected in 21 samples (2.3 %) and *E. coli* in 10 samples (1.1 %). In 2021, 113 ice machines with five elementary units (565 samples) and 167 ice machines with one elementary unit (167 samples) were tested, which is a total of 732 ice samples from 280 ice machines. The test results show that 84 samples (10.4 %) of the ice samples from 29 ice machines (11.5 %) were unsatisfactory due to high levels of aerobic plate count (72 samples; 9.8 %) and the presence of *P. aeruginosa* (57 samples; 7.8 %). *Enterococcus* and *E. coli* were detected in 10 samples (1.4 %) and *E. coli* in two samples (0.3 %), respectively. The probable reason for this improvement was the increased disinfection and improved hygiene in all facilities.

**KEY WORDS:** aerobic plate count; *Enterococcus* spp.; *Escherichia coli*; ice machines; *Pseudomonas aeruginosa*

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## The importance of the “food enjoyment” domain in the self-assessment of the quality of life of residents in nursing homes

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The aim of this study was to analyse the self-assessed domains of the quality of life of residents in county-owned nursing homes in the City of Zagreb who are provided with social services from levels 1-3. This analytical cross-sectional study was conducted successively (from 2017 to 2019) in five county-owned nursing homes in Zagreb. The Quality of Life Scales for Nursing Home Residents 2001 test was used to examine the self-assessed quality of life in 248 residents  $\geq 65$  years old, who were provided with social services from 1.-3. level. The self-assessment of the quality of life of residents (N=248) was analysed according to the examined domains: comfort; functional competence; privacy; dignity; meaningful activities; interpersonal relationships; autonomy; food enjoyment; spiritual well-being; safety; and individuality. In this study, nursing home residents (N=248) self-assessed their quality of life using a questionnaire. Among the tested domains, the highest level (point values) was determined in the area of individuality (Me=22.80; IQR=19.00-22.80), while the lowest level was determined in the domain of food enjoyment, where the mean value was 10.60 (IQR=9.00-11.60). The study results pointed to the necessary application of an individual gerontological approach to the elderly, as well as targeted gerontological interventions in relation to the examined domains of the self-assessed quality of life in nursing home residents, such as enjoyment of food, where the lowest point values were achieved, and at the same time suggest the need for a change in standards, norms, and new perspective on the categorisation of nursing homes.

KEY WORDS: categorisation; gerontology; nutrition; nursing home residents; quality of life

## Seroprevalence of anisakiasis in fish processing workers in Croatia

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Fish is an important part of the daily diet, as it is an excellent source of protein, omega 3 fatty acids, vitamins, and minerals. Consumption of mainly raw or inadequately processed fish is a potential health risk. The World Health Organization (WHO) reported 56 million cases of human infestation by parasites as a result of fish and fishery product consumption. The *Anisakis* spp that causes anisakiasis in humans is the tenth-ranked major parasite in Europe and also a significant hidden allergen in marine food. Visible larvae of the parasite undermine the trust of customers in the quality of fishery products, causing economic losses in the fish processing industry. In most countries, anisakiasis is a mainly unrecognized public health issue. Only two cases of anisakiasis have been described in Croatia so far. A study by Mladineo et al. conducted on 500 healthy Dalmatian inhabitants showed a total seroprevalence of anisakiasis by 2 % (3.5 % on islands). The aim of the conducted research was to determine the seroprevalence of anisakiasis in occupationally exposed populations. In total, 600 workers (78 % of total labor force in the fish industry) from 10 factories were included in the survey. All respondents filled out a specifically designed questionnaire (dietary habits, workplace safety, medical record, etc.). For qualitative determination of specific anti-*Anisakis* IgE antibodies, sera from all subjects were tested (ELISA Trisakis-170 kit) in a laboratory in Spain. The observed seroprevalence was 1.8 %. The major risk associated with sensitization of the fish workers to the parasite was having fishing as a hobby, rather than occupational exposure in the workplace.

KEY WORDS: *Anisakis* spp; anisakiasis; fish, fish workers; seroprevalence

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## National nutriviigilance system – health without side effects

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By definition, nutriviigilance is a set of activities and actions related to the detection, definition, and assessment of side effects that occur when consuming food and nutritional supplements. Nutriviigilance is not a legally mandatory system, but in order to monitor side effects and categorize them as dangerous to an individual's health, some EU countries have introduced a system following their own initiative. Croatia joined the countries with a National System for Monitoring Adverse Reactions Related to Food and Dietary Supplements two years ago with a public survey containing carefully selected questions to collect data on side effects and raise awareness of potential risks as another public health policy. After two years of functioning and an assessment of the adequacy and functionality of the system, we concluded that there was a sufficient number of reported side effects for the amount of dietary supplements used. In the future, it is necessary to carry out public health campaigns to raise awareness among the citizens of the Republic of Croatia about the proper way to purchase and use nutritional supplements and to quickly and adequately register them in the existing system in order to avoid adverse effects on human health when using them. The goal of this work is a statistical presentation of the reported side effects in the existing system and a presentation of the level of threat of reported events. The results of the work show that in 80 % of cases the application to the system was made by persons of the female sex, with a higher level of education. All of the reported cases were assessed as mild side effects, and 60 % of them were modelled by consuming products purchased over the internet with unrealistic promises about the product's effectiveness.

KEY WORDS: food supplements; national monitoring system; public health policy

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## Interdisciplinary approach through nutrition-gerontological norms in homes for the elderly and gerontological centers

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In the progressive aging of the population of the Republic of Croatia, it is almost necessary to integrate monitoring of gerontological public health indicators of health protection. The aging process dictates the daily energy needs of the elderly, which depend on basic physiological needs, physical activity, individual needs of the elderly, and many other factors. Within the framework of the Social Care Strategy for the Elderly in the Republic of Croatia for the period from 2017 to 2020, the manual "Nutrition-gerontological norms/menus in homes for the elderly and gerontological centers" was created. For the assessment and outcomes of positive health behavior related to proper nutrition for the elderly, gerontoanthropometric characteristics of nutritional assessment, assessment of nutritional status via the NRS 2002 web service, an assessment of energy needs of the elderly in relation to age and physical activity was used, as well as morbidity, ratio of macronutrients and intake of micronutrients and adherence to eight dietary rules for the elderly. The interdisciplinary approach through nutritional and gerontological norms is consolidated in the Manual, where there is a detailed presentation of all areas related to nutrition in the elderly with a practical presentation of menu examples based on the Guidelines for creating menus suitable for the elderly in institutional and non-institutional gerontological care. Adequate nutritional care depends on the quality of the menus and the knowledge of the staff involved in their creation. Monitoring the components of positive health behavior, which include proper nutrition and the analysis and evaluation of gerontological determinants, is essential for the process of active healthy aging and achieving the energy and nutritional needs of the elderly. Such an interdisciplinary approach mainly contributes to the preservation of functional ability, disease prevention, and improvement of health and quality of life in old age.

KEY WORDS: elderly people; interdisciplinary approach; nutrition-gerontological norms

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## Application of geronto-nutritional norms – a quality indicator in nursing homes

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The aim of this study was to investigate whether there was a difference in the application of geronto-nutritional norms in nursing homes in Croatia in 2020 versus 2018. The Department of Public Health Gerontology, Andrija Štampar Teaching Institute of Public Health, has regularly submitted the Questionnaire for Monitoring Quality Indicators in Nursing Homes in 2018 and 2020 and collected responses from authorized persons from nursing homes. In the framework of the aforementioned Questionnaire, which is used to analyse quality indicators, there is a question regarding the application of geronto-nutritional norms. A gerontological-public health analysis of data from nursing homes who forwarded their answers (n=54; 2020, Croatia) found that 78 % of nursing homes applied geronto-nutritional norms, while the norms in question were not applied in 4 %, and partial application of geronto-nutritional norms was recorded in 16 % of nursing homes (for 2 % the answer is unknown). In 2018, geronto-nutritional norms were applied in 70 % of nursing homes (n=64; Croatia) from which responses to the sent Questionnaire were received, the stated norms were not applied in 5 %, and they were partially applied in 24 % of nursing homes (for 1 % the answer was unknown). Research on the application of geronto-nutritional norms through the Questionnaire for monitoring quality indicators in nursing homes in 2018 directed the education of experts of various professional profiles in nursing homes and at the same time encouraged the creation of a manual entitled “Nutritional-gerontological norms/Menus in nursing homes and gerontological centers” published in 2020.

KEY WORDS: gerontology; geronto-nutritional norms; nursing homes; quality indicators; questionnaire

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## Stress situations as a factor of nutritional disorder in young student females

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Young female students, after the transition from high school to college, encounter new stressful factors that can affect eating disorders. The aim of this paper is to indicate the connection between the level of depression, anxiety, and stress with the possibility of eating disorders in female students aged 19 to 21 years. Epidemiological observational analytical research included a sample of 408 healthy female students aged 19 to 22 years. For testing, we used a socio-demographic questionnaire, body mass index (BMI), and standardized tests (EAT-26 to assess the risk of eating disorders and a short version of the DASS 21 test to assess behavioural changes). The sample consisted of female students with an average age of 20.5 years, a height of 168.7 cm, and a body mass of 63 kg (BMI 22.1±3.2 kg/m<sup>2</sup>). The final score of the EAT-26 test showed that 16.7 % of girls were at risk of eating disorders, while DASS 21 indicated the highest percentage of serious and extreme behavior in the anxiety category at 23.5%, than in the stress variable at 18.6 %, and the smallest was in the depression category 10.5 %. The category of moderate stress (3.5 X), the category of extreme depression (3.48 X), and the category of extreme anxiety (3.2 X) had the highest chance of belonging to the risk group of eating disorders. Research has shown that high levels of depression, anxiety, and stress have a greater association with the onset of eating disorders. Knowledge of risk factors is necessary for early detection and implementation of preventive programs.

KEY WORDS: anxiety; depression; eating disorder; prevention

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## Changes in agroclimatic conditions in Croatia over the last 60 years

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The rapid increase in the atmospheric content of carbon dioxide since the beginning of the industrial revolution is considered as one of the main drivers of the recent climate changes on Earth. The negative impact of climate change has been recorded in almost all sectors of human activity, especially in the very vulnerable sector of agriculture. Food production is an extremely complex process that depends on numerous factors and represents a great challenge. Climate change combined with soil and water pollution poses a major threat to food production and quality. Numerous studies carried out in different countries indicated reductions in the yield of wheat, maize, and other crops due to extreme heat and drought events. By some estimates, in the absence of effective adaptation, global yields could decline by up to 25 percent by 2050. This work examined the extent to which some of the most important agroclimatic elements have changed over the last 60 years in Croatia. Anomalies of growing degree days indicate a significantly larger amount of accumulated heat during the recent climatological period (1991-2020) compared to the previous one (1961-1990). In addition, a prolongation of periods with maximum air temperature above 32 °C has been recorded. Analysis of precipitation indicated a more frequent occurrence of dry periods during the recent climatological period. Furthermore, a decrease in the value of the aridity index during the recent climatological period indicates that the climate in Croatia is becoming more arid.

KEY WORDS: agriculture; aridity indeks; climate change, growing degree days; heat and drought stress

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## Isotopic analysis of native cheese samples collected in the Republic of Croatia during 2021-2022

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Seventy-seven cheese samples were collected from the entire territory of the Republic of Croatia. The samples were tested for the basic quality parameters of fat, protein, dry matter, salt, and pH. Their DELTA C ( $\delta^{13}\text{C}$ ) and DELTA N ( $\delta^{15}\text{N}$ ) values were determined and their mutual ratio was used to determine the geographical origin of the cheeses. Different types of cow's, sheep's, goat's cheese, and their mixtures were tested over two years in order to determine whether the climatic conditions have had an influence on the ratio of isotopes, the quality of the cheese, and, ultimately, whether the isotope map is a good basis for examining the geographical origin of unknown cheese samples. Isotope-ratio mass spectrometric (IRMS) measurements were performed by IRMS Delta V Plus (Thermo Fisher Scientific, Bremen, Germany) coupled to Elemental Analyser FlashEA. Instruments were controlled by the Isodat 3.0 software (Thermo Fisher Scientific, Bremen, Germany). The isotopic ratios are reported in the delta notation as part per thousand (‰). Samples were analysed against in-house reference material calibrated by the certified reference materials VSLAP2 and VSMOW2 obtained from the International Atomic Agency, Vienna, Austria. All analyses were normalised against the following reference materials: IVA-CASEIN ( $\delta^{13}\text{C}=-26.980\pm 0.05$  ‰,  $\delta^{15}\text{N}=5.830\pm 0.0$  ‰) and Urea ( $\delta^{13}\text{C}=-36.540\pm 0.18$  ‰,  $\delta^{15}\text{N}=-2.350\pm 0.09$  ‰). Mean values were calculated from triplicate analyses and were ( $\delta^{13}\text{C}=-22.85\pm 3.52$  ‰,  $\delta^{15}\text{N}=4.80\pm 1.01$  ‰). Results showed that this method could be used for determination of quality and origin of cheeses in order to protect consumers and producers that follow good production practices.

Acknowledgement: This work was carried out within the project "Food Safety and Quality Center" (KK.01.1.1.02.0004). The project is co-financed by the European Union from the European Regional Development Fund.

KEY WORDS: geographical origin; quality control; isotopic ratios; climatic conditions

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## Sustainable food system from the soil protection perspective: beneficial land disposal of biosolids

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The capability of land to produce food originates from several attributes, such as inherent soil properties, agricultural practice, climate and topography, water availability and quality, soil fertility, and many others. In the coastal Mediterranean region, characterized by a semi-arid climate, the progressive depletion of soil organic matter is the major threat to the sustainability of the agricultural production system. Soil organic matter (SOM) comprises a wide range of pools and indices of the quality and potential turnover rates of SOM pools add great value to the sole knowledge of the SOM content of a soil. The use of municipal sewage sludge as a source for compost production would be of great value from both an agricultural and environmental point of view. In this study, we evaluated the potential of agricultural land in the coastal Adriatic Karst region for biosolids application by integrating spatial data from different sources: digital maps and remote sensing, parcel identification system, GIS field observations, and measurements focusing on specific land and soil properties. The evaluation revealed that agricultural land in the study area accounts for only 10 % of the total area, but only a quarter of the existing land is suitable for biosolids application. Currently, the end use of biosolids is limited and is determined by the low quality of the product and the available options for beneficial use or disposal. While biosolids can be used as a valuable resource for organic matter, nutrients, and trace elements, land application appears to be a preferred management option compared to conventional disposal, which is now limited.

KEY WORDS: agricultural productivity; climate change; land management practice; soil organic matter; soil quality

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## The project “Investigation of the impact of climate change on the development of moulds, mycotoxins, and grain quality with proposed measures” – preliminary results of mycotoxin analysis and quality parameters

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Cereals are essential as an important source of energy and nutrients for a healthy diet, but at the same time represent a food at high risk for mycotoxins. Mold growth and mycotoxin formation are strongly influenced by factors such as temperature, relative humidity, carbon dioxide concentration, type of substrate, and insect infestation. The intensity of changes in these factors can lead to disruptions of the known pattern of mycotoxin synthesis, affecting the entire food safety system. During the two-year period of our research project, six samplings were conducted on three cereal varieties (maize, barley, and wheat) from three different Croatian regions: northern, eastern, and central Croatia. A total of 450 samples were taken during growth in the field, immediately after harvest, and after storage in warehouses and households. All samples were analysed for the presence and characterization of molds and mycotoxins and for selected quality parameters of cereal grains. For identification and quantification of mycotoxins, aflatoxins B1, B2, G1, and G2, ochratoxin A, zearalenone, deoxynivalenol, fumonisins B1 and B2; T-2 and HT-2 toxins, citrinin, and patulin, liquid chromatography-tandem mass spectrometry (LC-MS/MS) and high performance liquid chromatography (HPLC) techniques were used. Different techniques such as gas chromatography-flame ionization detection (GC-FID), HPLC, inductively coupled plasma-mass spectrometry (ICP-MS), etc. were used to determine the nutritional parameters (energy value, water, ash, fats, saturated fatty acids, carbohydrates, sugars, crude fibers, proteins, salt), selected minerals (magnesium, zinc, iron, phosphorus), and vitamins (E, B3, B5). The preliminary results indicate that molds are present in all cereals regardless of the area and time of sampling, as well as yeast. Regarding mycotoxins, citrinin and patulin were not quantified in any sample. Ochratoxin A was quantified in 1.33 % of samples, Aflatoxins in 3.11 %, T2 and HT2 toxins in 10.2 %, zearalenone in 15.1 %, fumonisins FB1 and FB2 in 17.3 %, and deoxynivalenol in 26.9 %. The highest value of 13 mg/kg was found in the maize sample, during the harvesting in the first year of the project.

Acknowledgements: This work was funded through the project “Investigation of the impact of climate change on the development of molds, mycotoxins, and grain quality with proposed measures” from the European Regional Development Fund and the Croatian Fund for Environmental Protection and Energy Efficiency (K.K.05.1.1.02.0023).

KEY WORDS: contaminants in cereals; food safety; food quality; mycotoxin synthesis; weather changes

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## Effects of pomegranate peel extract on inflammation and oxidative stress parameters in type 2 diabetes: an open labelled study

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Pomegranate peel extract (PoPEX) is a rich source of various bioactive polyphenols assumed to have positive effects on cardio-metabolic disturbances. The aim of the study was to evaluate the anti-inflammatory and antioxidant effects of 8-week PoPEX consumption in overweight/obese diabetes mellitus type 2 patients (DMT2) (n=20) and in healthy controls (n=10). Both groups received capsules containing 250 mg of PoPEX, twice a day for the period of eight weeks. Anthropometric characteristics, blood pressure, lipid profile, and plasma concentration of inflammatory factors as well as oxidative stress biomarkers were measured at baseline and at the end of the study. Participants were asked not to change their dietary habits, physical activities, and medication regimens during the study period. The eight-week treatment with PoPEX resulted in a significant reduction of inflammatory factors and oxidative stress biomarkers only in the diabetic group. Moreover, a significant improvement in lipid profile and systolic and diastolic blood pressure was observed in patients with DMT2. Furthermore, PoPEX consumption significantly lowered both systolic and diastolic blood pressure in healthy individuals. To conclude, PoPEX reduces inflammation and oxidative stress in diabetic patients but with no effects in healthy individuals.

KEY WORDS: diabetes mellitus; hyperlipoproteinemias; polyphenols

## Functional and nutritional properties, packaging possibilities and potential use of stinging nettle-case study

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Demands for healthy, nutrient-rich foods that are minimally processed are on the rise and consumers are paying more attention to the origin of the foods and their functional properties. New agricultural practices are based on providing safe raw materials with uniform chemical composition. This is important for the stinging nettle, which is mostly collected from the wild where it tends to accumulate excessive amounts of nitrates and heavy metals. Previous studies have shown that nettle contains a significant amount of phenols, vitamins, pigments, minerals, proteins, fats, fatty acids, sterols, volatile compounds, tannins, etc. Moreover, numerous pharmacological studies have demonstrated the antioxidant, antimicrobial, anti-inflammatory, antitumor, antidiabetic, antiulcerogenic, hepatoprotective, hypoglycemic, and other properties of nettle. Thanks to its high content of bioactive compounds and proven nutritional and health effects, nettle can be considered an important functional foodstuff so there is a justified reason for placing nettle on the market as a new packaged food product. In accordance with sustainable production and circular economy, it is recommended to use modern food processing (minimal, non-thermal technologies), packaging methods (modified and controlled atmosphere), and materials (such as biodegradable). Appropriate packaging methods and materials can ensure the safety and longer shelf-life of products and help to preserve compounds responsible for biological effects. The aim of this study was to summarize the data from previous research on the nutritional, functional, and pharmacological properties and to discuss the further potential of using, processing, and packaging nettle leaves as a green leafy vegetable in order to popularize this new food product.

KEY WORDS: functional food; modified atmosphere; packaging materials; specialized metabolites, stinging nettle leaves



## EFSA and HAPIH in the “EU Choose Safe Food” campaign

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The European Food Safety Authority's (EFSA) core activity is the scientific assessment of risks arising from food and feed. In order to bridge the gap between science and consumers, another important role of EFSA is the independent and timely communication of risks in food and feed with partners, stakeholders, and the general public. As a result of the growing interest of the general public in the topic of health and food, communicating the science has become important and challenging. In 2021, EFSA launched a multi-year communication campaign “EU Choose Safe Food”. The national holder of the campaign is the Croatian Agency for Agriculture and Food (HAPIH) as EFSA's national contact point. Partners from other relevant institutions in the food safety system are also involved in the campaign. The campaign aims to encourage citizens to think critically when choosing food, as well as to raise citizens' awareness of the science behind the high standards set for food safety in the European and Croatian markets. The campaign focuses on health, cooking, and an active lifestyle. The target population is from 25 to 45 years old, with an emphasis on women and young parents. This campaign aims to utilize the latest insights from the field of communicating food safety to consumers and put a friendly face on science and scientific facts. Therefore, it aims to reach the target population in an informative, simple, and easy-to-understand way. The main communication channels include digital and social media, with influencers as campaign ambassadors. The campaign covers various food safety topics from food waste and food contaminants to food hygiene, foodborne disease, and animal welfare.

KEY WORDS: communication; food safety; science

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**POSTER PRESENTATIONS**

## Application of high pressure to preserve quality and safety during cheese ripening

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Among the many ways of using non-thermal preservation methods in the food industry, application of high-pressure treatments in cheese production is showing satisfactory results. An advantage of using high pressure during cheese ripening can be seen in the reduction and inactivation of unwanted microbial populations without affecting its physical and chemical characteristics. Consequently, such conditions ensure the extension of the products shelf life, nutrient retention (e.g., vitamins), acceptable sensory qualities (e.g., reducing bitterness, colour, early or late blowing), and are considered safe overall. By applying high pressure, it is possible to speed up or to arrest the ripening time of the cheese. Pressure-treated cheeses show greater syneresis and enhanced primary and secondary proteolysis, but on the other hand, excessive pressure levels can inactivate enzymatic activities and inhibit the biochemical reactions involved in cheese ripening. Despite the studies conducted, further research is needed to define the optimal effects of high-pressure treatments on cheese to achieve the right balance of positive effects. This paper conducted a detailed analysis of the impact that high-pressure application during ripening has on the quality and safety of cheese. A critical evaluation of the advantages and disadvantages of high-pressure treatment in the context of its application in cheese production is presented as well.

KEY WORDS: microbial inactivation; non-thermal preservation; nutrient retention; sensory quality; shelf life

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## Detection of mycotoxins in food and animal feed in the Zenica-Doboj Canton, Bosnia and Herzegovina

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Food for human consumption and animal feed can be contaminated with mycotoxigenic fungi during cultivation, harvesting, and storage causing spoilage and mycotoxin production. The aim of this study was to identify and quantify aflatoxins in food and feed. A total of 33 samples (11 food, 6 feed, and 16 raw milk) were analysed on moulds according to ISO 21527-1:2008. Mycotoxins were detected and quantified in 87 samples (11 food, 30 feed, and 46 dairy products) using enzyme-linked immunosorbent assay (ELISA) specific kits (Eurofins Technologies, Budapest, Hungary). Quality control materials with the standard concentration were used. Among 17 food and feed samples, moulds were present in all of the samples at the quantity range of 1100-15000 cfu/g. Among 30 feed samples, aflatoxin B1 (AFLB1) was detected in six samples within the range of 1-20.3 µg/kg. Seven samples were contaminated over the EU regulatory limits (15.5 and 20.3 µg/kg; reference value <5 µg/kg). Deoxynivalenol (DON) and zearalenone (ZEA) were found in three and two samples, with range 105-237 and 16.0-23.7 µg/kg, respectively. All food samples were contaminated with ochratoxin A (Ochr A) with a median value of 5.8 µg/kg (range 2.8-36.0 µg/kg). Eight food samples were contaminated with more than one mycotoxin. Two samples were contaminated with the four mycotoxins: AFLB1, DON, ZEA, and Ochr A. Aflatoxin M1 were detected in 32 samples within the range of 0.006-0.27 (three samples were contaminated over the EU regulative limits). These results emphasize the urgency for establishing regular and obligatory monitoring programs for mycotoxins in staple grains in Bosnia and Herzegovina.

KEY WORDS: aflatoxin B1; aflatoxin M1; deoxynivalenol; EU regulatory limits; zearalenone

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## Migration of bisphenol A from new unused plastic packaging and various household products to food simulants

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Bisphenol A (BPA) is a plasticizer used in the production of polycarbonate plastics and epoxy resins, which are widely used in the production of household goods. Free BPA is known to migrate from food packaging to food resulting in exposure of consumers to BPA. Exposure to BPA is associated with cardiovascular disorders, diabetes, hepatotoxicity, and disruption of endocrine activity. Therefore, plastic substances are subject to limitation due to the EU and consequently HR norms. The objective of this study was to assess possible BPA leaching from different plastic packaging and household products used for food consumption and food preparation to aqueous food simulants placed on the Croatian market, but prior to first use. Levels of BPA in food simulants (aqueous 3 % acetic acid) and water at different temperatures and migration times were evaluated in 62 samples collected during 2012-2022 according to the directive 10/2011 for plastic materials and products in contact with foodstuff. The results showed that the levels of BPA that migrated to food simulants for all items were below the limit of quantification (LOQ) and specific migration limit to food of 0.05 mg/kg according to EU legislation. Consequently, none of the analysed items were considered to present health hazard for consumers regarding BPA exposure.

KEY WORDS: health; food packaging; food safety; specific migration limit

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## Defining key physicochemical parameters affecting milk coagulation properties

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This research aimed to determine the chemical and physical properties, hygienic quality, calcium concentration, and coagulation properties from different types of milk (bovine, ovine, and caprine). A specific aim was to compare three different types of milk and to define key parameters affecting their coagulation properties. A total of 33 milk samples were collected from March to December 2021 from three different regions in the Republic of Croatia. Chemical composition was determined with Milkoscan FT3 (Foss, Denmark) and physical properties were determined with a Seven Multi pH-meter (Mettler Toledo, Switzerland) and Cryostar 1 (Funke Gerber, Germany). Hygienic quality was determined with Bactoscan FT 50 (Foss, Denmark) and Fossomatic Minor (Foss, Denmark). Calcium concentration was determined by inductively coupled plasma-mass spectrometry, ICP-MS (Shimadzu, Japan). Milk coagulation properties were determined with a lactodynamograph (MA.PE SYSTEM, Italy). The highest average casein content (g/100 g) was determined in ovine (4.76), bovine (2.77), and caprine milk (2.42). The average ionometric acidity (pH) of the bovine milk was higher (6.55) than caprine (6.40) and ovine milk (6.42). Different calcium concentrations (mg/kg) in bovine (1501.11), caprine (1387.65), and ovine milk (2271.43) were determined. The conclusion based on the results was that calcium concentration, casein content, and ionometric acidity had a significant effect ( $P < 0.05$ ) on milk coagulation properties.

KEY WORDS: calcium; casein; cheese production; ionometric milk acidity; milk coagulation

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## Parvalbumin as a genetic marker for fish DNA identification

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A worldwide debated topic in the field of food analysis is its adulteration. Most of the population encounters adulterated food often without even knowing it, while fish and fish products are among the most frequently adulterated foods. Since in many cases it is almost impossible for the consumer to identify individual types of fish, there is a possibility for the producer to deceive consumers for the sake of financial profit. Correct labeling is also important in terms of health impact; some people may only eat certain fish due to allergies. The most common determination of fish species is based on analysis of morphological features; unfortunately, this approach cannot be applied to processed fish meat and/or complex dishes. Related to this is the need to develop methods for identifying fish and detecting their adulteration. The correct determination of the fish species is important due to their high allergenicity. Parvalbumin is considered the main fish allergen, the gene encoding this protein can be used as a molecular marker for species identification of fish. Currently, the polymerase chain reaction (PCR) is most often used for these purposes, but other identification methods such as the loop-mediated isothermal amplification (LAMP) are also emerging. In our work, the parvalbumin-analysing PCR protocols have been developed for the differentiation of black seabream (*Spondyliosoma cantharus*), mackerel (*Scomber scombrus*), and angler (*Lophius piscatorius*). The specificity of all used protocols was experimentally verified with a panel of fish and fish products samples.

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KEY WORDS: adulteration PCR; black seabream; mackerel; angler

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## LAMP – a promising method for fish species identification?

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Fish and fish products are one of the most expensive and most traded commodities, which predetermines them to be adulterated. Replacing high-quality or rare fish with a less valuable species is common. However, such replacements can endanger consumers because fish is one of the main sources of allergens, whose content differs among the species. Therefore, a reliable, robust, and fast control system for fish species identification is necessary. The Loop-Mediated Isothermal Amplification (LAMP) is a promising tool for fish authentication that combines speed, high specificity, and simplicity. Moreover, it does not require expensive laboratory equipment, thus, it can make a routine analysis easier. Over the last few decades, LAMP has been commonly used for the detection of microorganisms, including a fish virus. In this work, we present the capability of the LAMP method to differentiate between the fish species themselves. We tested the LAMP method for the detection of pike (*Esox lucius*) and mackerel (*Scomber scombrus*), confirming a very high level of specificity for the tested species, and then compared the sensitivity with the polymerase chain reaction, the most often used molecular-biological method for the detection and identification of species.

KEY WORDS: adulteration; DNA analysis; fish authentication; food fraud; food quality

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## Authentication of opium poppy (*Papaver somniferum* L.) using DNA analysis

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Opium poppy (*Papaver somniferum* L.) is an important oilseed used in food and the pharmaceutical industry. Poppy varieties grown for food purposes have good nutritional and sensory quality and low levels of opiates. Therefore, they are important ingredients in food items such as cakes or buns in many countries of central-eastern Europe. However, there have been several cases of poppy seed adulteration when food-grade poppy seeds were mixed with cheaper seeds from pharmaceutical varieties that contain a higher amount of opium alkaloids and have poorer sensory properties. In this work, we tested the possibility to differentiate *P. somniferum* L. species and varieties with low- and high-alkaloid content by DNA analysis. For this purpose, we analysed the genes important for the biosynthetic pathway of opium alkaloids, such as COR or REPI, commonly used for DNA barcoding, and microsatellite loci. The selected sequence of the COR gene and barcoding markers allow one to reliably distinguish *P. somniferum* L. from other tested poppy species, whereas microsatellite loci are suitable for the differentiation of poppy varieties.

KEY WORDS: food fraud; food quality; opium alkaloids; species identification

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## Examination of sprouts for the presence of STEC using the polymerase chain reaction method in real time (real-time PCR)

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Shiga toxin-producing *Escherichia coli* (STEC) belongs to the group of the most important bacteria that can cause severe food poisoning and disorders in humans such as diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome. Cattle are considered a natural reservoir of STEC, and many poisonings have been linked to ground beef, but often raw milk and sprouts were the cause of food poisoning. Some of the sprouts that are most often used in food are vetch, cereals, cabbage, and plants from the onion family. Sprouts are highly valuable food rich in vitamins, proteins, and minerals. They are low in carbohydrates but contain high fiber, so they effectively promote digestion. There is a lot of evidence of the health and nutritional value of sprouts, but also numerous poisonings caused by the consumption of sprouts that were not safe have been recorded. The first warning related to STEC in sprouts was issued by the US Food and Drug Administration back in 1999, and in March 2013, STEC was included in the legal framework of the EU through Commission Regulation (EU) no. 209/2013. The detection method by classical cultivation is carried out according to HRN EN ISO 16654:2003. This method is intended only for serotype O157, while this serotype and all others mentioned in the Regulation (O157, O26, O103, O111, O145, and O104:H4) can be detected by the Real-Time PCR method. This paper presents the results of STEC detection (8 serotypes) in the sprout samples (n=113) during the period from 2017 to 2022.

KEY WORDS: food poisoning; food safety; pathogen; serotype

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## Evaluation of polycyclic aromatic hydrocarbons in fermented dried sausages from the Serbian market

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Polycyclic aromatic hydrocarbons (PAHs) are compounds that widely geographically distributed and remain in the environment for a long time. Meat and meat products may be contaminated by PAHs through the process of food smoking. This study provides original analytical data on the levels of benzo[a]pyrene and sum of PAH4 compounds [benzo[a]pyrene (BaP), benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), and chrysene (CHR)] in different types of fermented dried sausages commonly consumed in Serbia. A total of 228 fermented dried sausages, collected from 2020 to 2021, were classified in 4 groups (kulen, cajna, sremska, and budimska sausage). Homogenized samples were extracted by acetonitrile. The purification procedure of the extract was based on the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) approach. Levels of estimated PAH compounds were determined by gas chromatography-tandem mass spectrometry (GC-MS/MS). The following ranges were found ( $\mu\text{g}/\text{kg}$ ): kulen (n=56): BaP <0.2–0.5, PAH4 <0.2–2.5; cajna (n=103): BaP <0.2–1.0, PAH4 <0.2–8.6; sremska (n=37): BaP <0.2–0.4, PAH4 <0.2–3.4; budimska (n=32): BaP <0.2–0.3, PAH4 <0.2–1.4. The maximum residue limits (MRL) for BaP and sum of PAH4 compounds, which were defined both by the legislation of Serbia and EU regulation, were not exceeded in the analysed samples and hence pose no risk to consumers.

KEY WORDS: budimska sausage; cajna sausage; kulen; PAH; sremska sausage

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## Elemental composition and health risk of hemp foods

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Bioaccumulation of harmful toxic elements is the intrinsic signature of the *Cannabis* species. When industrial cannabis (hemp) is used for food products, they have no market value if the elemental contamination is above the permissible level. Elemental composition (23 elements) of 46 hemp foods (20 oils, 9 protein, 12 seeds, 3 sweets, and 2 beverages) was obtained by inductively coupled plasma mass spectrometric (ICP-MS) analysis and further propagated into risk assessment for hemp foods consumers. Regarding non-carcinogenic risk, the hazard quotient (HQ) of each individual element in any of the samples was below the limit of 1 when the adult population was considered. However, in case of older and younger adolescents, the HQ for Hg in 1 seed sample, and additionally for younger ones, for Mn in 1 seed and 1 protein sample, exceeded the limit. The hazard index, a summary metric for all elements in a sample, exceeded 1 for numerous samples: 1 oil, 8 protein, and 6 seed samples in case of adult consumers, an additional 2 seeds for older adolescents, and 1 protein and 5 seeds for younger adolescents. The margin of exposure (MOE) associated with Pb nephrotoxicity was below 10 for the 4 oils and 1 seed sample if consumed by adults, but an additional 1 and 2 seeds for older and younger adolescents, respectively. The carcinogenic risk due to As exposure exceeded the tolerable 1 extra lifetime cancer case per 100,000 persons across the population groups, for 11/13/16 samples, or, when the MOE approach was applied, for 0/2/4 samples. Consumption of hemp foods by children would be even more disputable.

KEY WORDS: hazard index; ICP-MS; Lifetime Cancer Risk; margin of exposure; food safety

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## Microbiological gut analysis of two species of freshwater crabs

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Freshwater ecosystems in Croatia are inhabited by seven crayfish species. This study aimed to compare the microbial gut composition of crayfish, using the domestic species *Pontastacus leptodactylus* and the invasive foreign species *Pacifastacus leniusculus*. Considering that these crayfish are used in human nutrition, the presence of lactic acid bacteria (LAB) and microorganisms that cause food contamination (enterobacteria, sulfite-reducing clostridia, and coagulase-positive staphylococci) were examined using the spread plate technique. The obtained results showed that there are no significant differences in the microbial gut composition of these two crayfish and that the concentrations of the tested microorganisms exceed those allowed by national guidelines, which means that such crabs are not safe for consumption. Lactic acid bacteria were found only in the indigenous type of crayfish *P. leptodactylus* in a concentration of  $2 \times 10^1$  CFU/g. Bacterial isolate was identified with analytical profile index (API) biochemical test and the strain was identified as *Lactobacillus fermentum* with 99.8 % of accuracy. Additional research is needed to determine the characteristics of this isolate for further application in freshwater aquacultures.

**KEY WORDS:** food safety; invasive species; lactic acid bacteria; *Lactobacillus*; microorganisms

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## Mycological analysis of coffee beans of different origin

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Coffee is one of the most popular beverages in the world, and due to its chemical composition, is considered a functional drink. The two most important types of coffee on the market are *Coffea arabica* and *Coffea canephora* var. *robusta* grown in tropical areas around the world. Like most other foods, coffee is subject to contamination by molds and bacteria that cause various diseases, so it is essential to control the quality of the product. The aim of this work was to determine the occurrence of mold on the green, roasted, and ground coffee beans of different geographical origins. Molds were isolated by direct inoculation of coffee beans and ground coffee onto nutrient media. The method of three-point puncture on agar and microscopy with a light microscope was used to identify isolated molds. Identification (carried out to the genus level) was made by observing the morphological characteristics of the isolated molds. Isolated and identified molds belonged to *Aspergillus*, *Penicillium*, *Fusarium*, and *Chaetomium* genera. The analysis of the obtained results found that the presence of mold genera depends on the type and form of coffee as well as the geographical location of cultivation. *Aspergillus*, *Penicillium*, and *Fusarium* species were isolated from Arabica coffee beans, while *Aspergillus*, *Penicillium*, and *Chaetomium* species were found on Conilon coffee beans and both originated from Brazil. On Robusta coffee beans, which originated from Guatemala, only *Aspergillus* and *Penicillium* molds were found. The results show that ground coffee is the best in terms of microbiological safety, however during the grinding of roasted coffee beans contamination can still occur.

**KEY WORDS:** *Aspergillus*; *Chaetomium*; *Fusarium*; identification, *Penicillium*

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## Encapsulation of bacterial cultures *Lactiplantibacillus plantarum* in alginate microparticle formulations

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Starter (bacterial) cultures are used in the cheese production for the purpose of acidifying milk and creating desirable sensory properties. One of the most commonly used bacterial species in cheese production is *Lactiplantibacillus plantarum*. In order to increase the stability and survival of bacterial cultures, the process of their encapsulation in alginate microparticle formulations is carried out, which achieves their protection and release in certain time intervals and conditions. The goal of the work was to prove that the encapsulation process of this bacterial species in alginate microparticles will ensure the appropriate number and activity of bacterial cells in the microparticles and thus enable the controlled release of components inside the microparticles during cheese production. The encapsulation process, carried out by the ionic gelation method, of the *Lactiplantibacillus plantarum* strain in alginate microparticles proven to be effective. After the encapsulation process, a satisfactory number and activity of *Lactiplantibacillus plantarum* bacterial cells was achieved in alginate microparticle formulations, therefore the obtained microparticles can be used for the controlled release of the components inside them during cheese production. The physicochemical characterization of alginate microparticles filled with *Lactiplantibacillus plantarum* strain was successfully performed. After the encapsulation process, the vital fraction was  $9.0 \pm 0.1$  log CFU/g, and such a result corresponds to the requirements of international standards for fermented products. The degree of swelling of microspheres with encapsulated cells of *Lactiplantibacillus plantarum* was  $155.4 \pm 10.8$  % and the degree of swelling of ALG/Ca microspheres was  $48.7 \pm 12.21$ , thus confirming the expected assumption that the degree of swelling of microspheres filled with bacterial cells is higher than the degree swelling of empty microspheres. Microscopic examination confirmed the presence of short, gram-positive rods of *Lactiplantibacillus plantarum* within alginate microparticles. The size of the bacterial cells and the size of the prepared microspheres were determined with an optical microscope. The dynamics of the release of *Lactiplantibacillus plantarum* bacterial cells from the microspheres was successfully measured. *Lactobacillus plantarum* strain is released immediately after encapsulation ( $4.82 \pm 0.07$  log CFU/ml). Most of it is released by the 5<sup>th</sup> day ( $7.65 \pm 0.05$  log CFU/ml). After the 20<sup>th</sup> day, the release of the *Lactobacillus plantarum* strain gradually decreases until the 40<sup>th</sup> day. Based on the analysis results, it can be concluded that *Lactiplantibacillus plantarum* bacteria are suitable for use in the technological process of cheese production.

KEY WORDS: autochthonous dairy cultures; cheese; ionic gelation method; microscopy

## Proving internal traceability of ready-made meals in institutional kitchens

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The institution “Dobri dom” of the City of Zagreb, as an institutional form of providing social services to citizens in need of social protection, annually prepares about 900,000 ready-made meals. The institution has implemented the HACCP system since 2007, and this paper will describe the internal traceability system, which is one of the basic requirements of the food safety management system. The purpose of this work is to ensure the possibility of tracing the ready-made meal through all stages, preparation and storage, and to determine the activities and responsibilities for ensuring their traceability. The subject of the research is a ready-made meal, and the methods used were based on proving the traceability of the ingredients that make up the ready-made meal through all the records in the traceability system within the HACCP system. The review of all the ingredients that made up the tested meal determined and described the existence of traceability records in every step of the production process, which is proof of the efficiency of the system and the high level of quality control and management in the Institution. By proving traceability based on a successfully established methodology and quality-maintained records, the traceability of the finished meal can be determined, which is an imperative for obtaining a high-quality and healthy meal for the Institution’s users.

KEY WORDS: food safety; institutional form; quality control; social services

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## Nitrates in vegetables from local markets in Split

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The increased demand for vegetables encourages the development of intensive agriculture, leading to the use of large amounts of fertilizers that are a source of nitrates. High levels of nitrate in the soil are correlated with its higher content in the leaves of green plants. Nitrates are slightly toxic to the human body. However, their metabolites nitrites and nitrosamines can be a health risk. Regulation 1881/2006 prescribes, among other contaminants, the maximum levels of nitrates in vegetables. The Regulation covers only three types of leafy vegetables (spinach, lettuce, and rucola) while some of them like swiss chard, various cabbages, which are very common in the diet of the local population, are not included in the Regulation. Forty-five samples of different vegetables from local markets, produced on local farms, were sampled in the period May-July. Determination of nitrate concentrations was carried out on high performance liquid chromatograph (HPLC) with a diode array detector (DAD). All of the analysed samples contained nitrates. There was no exceedance of the maximum levels set in Regulation 1881/2006. The highest concentrations were found in swiss chard and rucola samples, and the lowest in carrot samples. Comparing the results with the results of other similar researches, higher values were obtained. Therefore, it is planned to continue the research on a larger number of samples and include more plant species. Also, it is planned to carry out sampling in different times of the year in order to determine if there are any differences in nitrate content and how much they differ depending on the season.

KEY WORDS: cabbage plants; HPLC; leafy vegetables; swiss chard

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## Virgin olive oil at local markets in Split

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Fats as the main energy source are an important part of the diet. Extra virgin olive oil (EVOO) has special value among fats thanks to its composition, especially monounsaturated fatty acids and minor components (phenolic compounds, tocopherols, and carotenoids), which are associated with health benefits. Therefore it is a vital part of the Mediterranean diet. EVOO production is demanding, but storage is an even greater challenge. Due to high levels of unsaturated fatty acids, EVOO is prone to lipid oxidation generating lipid peroxides, potentially toxic compounds that can cause cellular damage. The Dalmatian region is the largest olive oil producing area in the Republic of Croatia. Over the years it has become an increasingly important tourist center. Therefore, apart from being an important food product, EVOO becomes an important part of tourism promotion. For this reason, during two tourist seasons (May-September 2021-2022), samples were collected at local markets where the local population, as well as visitors, usually shop. In 37 samples, quality parameters were analysed in accordance with Regulation 2568/91 [peroxide value, free fatty acid (as oleic), absorbance in UV area, ethyl esters, waxes, as well as stigmastadienes]. Analyses were performed using standardized and accredited methods. The results are extremely worrying considering that 73 % of the samples did not meet the requirements of the Regulation. Analysing the results that indicate a high degree of oxidation and adulteration, it is clear that there can be no question regarding the beneficial effect of EVOO; in fact, quite the opposite is true.

KEY WORDS: adulteration; health impact; monounsaturated fatty acids; oxidation; quality parameters

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## MALDI-TOF mass spectrometry for identification of aerobic mesophilic bacteria in raw and preserved milk

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The number of aerobic mesophilic bacteria in milk is one of the indicators of the hygienic quality of milk. A quantity greater than prescribed indicates inadequate hygiene during production and processing and contamination represents a potential health hazard and negatively affects the quality of milk and milk products. The aim of this work was to determine aerobic mesophilic bacteria and their number in raw unpreserved milk and milk preserved with azidiol. In 40 samples, the total number of aerobic mesophilic bacteria was determined using the flow cytometry method and the classic method of counting colonies on a nutrient medium according to the international standard HRN EN ISO 4833-1:2013. The results showed a decreasing trend regarding the number of grown colonies in milk preserved with azidiol. The number of samples was too small to conclude a generally negative influence of azidiol on the total number of aerobic mesophilic bacteria in milk. With matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry, it is possible to quickly and easily identify bacteria at the species and genus level. This technique successfully identified 298 bacterial colonies in raw milk samples and 243 colonies in preserved milk samples. Twenty-nine genera and 45 bacterial species were identified in the raw milk samples, and 25 genera and 36 bacterial species were identified in the preserved samples. This way, a more detailed insight into the hygienic quality of milk and influence of preservatives on the presence of certain species before and after being preserved with azidiol was obtained.

KEY WORDS: azidiol; flow cytometry; hygienic quality

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## Assessment of black locust (*Robinia pseudoacacia* L.) honey

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Black locust (*Robinia pseudoacacia* L.) honey is one of the most appreciated honeys on the Croatian market. This work aims to create scientifically based criteria for assessing the quality of black locust unifloral honey. Honey samples were collected from several regions in the continental part of Croatia with a moderate continental climate during four years as part of the project Center for Food Safety and Quality. The analysis was carried out at the Teaching Institute of Public Health Dr. Andrija Štampar and the Faculty of Agriculture, University of Zagreb. Organoleptic, physicochemical, and melissopalinalogy methods were applied. We focused on samples that best represent the problem of identification of black locust honey. All beekeepers assumed that the collected honey samples were black locust. Samples were analysed and 300 pollen grains were identified and counted with an Olympus BX53 microscope at 400× magnification. According to Croatian legislation, honey is considered black locust if the pollen frequency of black locust is at a minimum of 20 %. Pollen percentage of black locust in the collected samples varies from 13 to 27 % and electrical conductivity from 0.151 to 0.3 mS/cm. The results showed not all samples could be declared as black locust honey. The minimal criteria proposed for assessing black locust honey should be melissopalinalogy analysis, electrical conductivity, and sensory analysis.

KEY WORDS: electrical conductivity; melissopalinalogy; pollen; sensory analysis; unifloral honey

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## Changes in total flavonoid and polyphenol content in Croatian honey depending on storage conditions

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Total flavonoids and polyphenols are the main bioactive substances that we associate with the antioxidant activity of honey. Measuring the total content of flavonoids and polyphenols in honey is important for the characterisation of honey samples. The main objective of this research was to explore changes related to the content of antioxidant properties of honey during storage temperature conditions. Twenty-four Croatian honey samples were collected from different regions corresponding to different botanical species: acacia, multiflower, and forest honey. All samples were stored for 3 days at a temperature of 20, 40, and 60 °C. The samples were analysed and measured with a UV-VIS spectrophotometer using quercetin and aluminum chloride and modified Folin-Ciocalteu method. For the detection of total flavonoids and total polyphenols we used quercetin and gallic acid as standards, where the obtained calibration curve equations were  $y=0.0066x+0.0102$  ( $R^2=0.9992$ ) and  $y=0.0065x+0.148$  ( $R^2=0.9992$ ). The results show the highest concentrations of gallic acid and quercetin in forest honey at a temperature of 20 °C ( $117.28\pm 7.0$  gGAE/100 g), ( $12.10\pm 0.37$  gQE/100 g), while at a temperature of 40 and 60 °C these values were lower than at 20 °C, in the range of 27 to 70 % depending on the type of honey. The methods have been successfully applied in laboratory analyses and can therefore be applied in routine analysis, which will provide us with a better picture regarding the conditions of honey storage, exposure to sunlight, and high temperatures in order to preserve the antioxidant properties of honey.

KEY WORDS: antioxidant activity; temperature; UV-VIS spectrophotometer

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## Thujone – health risk and control in products

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Thujone is a ketone and a monoterpene found in the essential oil of bitter wormwood (*Artemisia absinthium* L.), cypress, juniper, oregano, mint, essential oil of thuja, and essential oil of sage (*Salvia officinalis* L.). Sage is known for its wide range of effects, but it should be used cautiously and in moderation because its ingredient thujone can be very toxic and should not be consumed in combination with alcohol. Thujone is toxic to the brain, kidney, and liver cells and causes convulsions if used in excessive doses, and affects the central nervous system. According to the Directive on substances that can be added to food and used in food production and substances whose use in food is prohibited or restricted (Official Gazette 160/2013), the maximum permitted amount of thujone in food or food supplements has not been specified, only long-term use and consumption of sage. The goal of the research is to determine the amount of thujone in the selected samples of alcoholic beverages and compare it with the highest allowed amounts prescribed by Regulation (EC) 1334/2008, and to raise the awareness of producers, authorities, and the public about the risks associated with exceeding the allowed amount of thujone in products. From the obtained results of analysis, we can see that there is a risk of exceeding the highest permitted amounts of thujone in alcoholic beverages on the market and that it needs to be controlled more systematically.

KEY WORDS: alcoholic beverage; maximum allowed quantity; sage;  $\alpha$ -thujone ; $\beta$ -thujone; bitter wormwood

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## Correlation of pH value and aerobic psychrophilic bacteria in deionized water

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Food safety and quality is monitored by microbiological cultivation methods. Therefore, a laboratory for the preparation of culture media (CM) occupies an important role in the quality assurance of microbiological results in bacteriological diagnosis. For the preparation of culture media, laboratories use purified water (distilled, deionized water, etc.). Microbial contamination according to European standard EN ISO 11133:2014 should preferably be below  $10^2$  cfu/mL. The aim of this study was to determine the difference in values of measured chemical parameters between samples with isolated aerobic psychrophilic bacteria (APB) and those without bacteria. A total of 58 samples taken after the preparation of the water were analysed. The pH was measured using a calibrated pH meter with a combined glass electrode at the point of sampling. Microbial contamination was monitored with incubation at  $22 \pm 1$  °C for  $68 \pm 4$  h using tested CM. Kolmogorov-Smirnov test showed a normal distribution of results, and therefore in further statistical analyses parametric methods were used. Among 58 samples, APB was detected in 17 samples and the median pH value in the samples with APB was 7.51 and 7.32 in the negative samples. T-test was used for a comparison of the pH between the positive and negative samples and the obtained values were  $t = -0.725$  for the pH value. The obtained results showed that there was no statistically significant difference between pH in the samples with and without APB.

KEY WORDS: food safety; food quality; microbial contamination

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## Difference in the content of collagen in meat cuts and minced meat

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The aim of this study was to analyse and evaluate the quality parameter of different meat samples in order to determine the hydroxyproline content and calculate the collagen content. The samples of fresh meat and three types of fresh minced meat will be analysed according to the standardized method. The results of the amount of collagen will be compared with EU legislation. Samples were analysed and divided into four categories, so six samples were processed from each category. After homogenization, the samples were prepared according to the official colorimetric method (990.26) from the AOAC International Journal and recorded on a spectrophotometer at a wavelength of 558 nm on the hydroxyproline content. Twenty-four samples were analysed, of which there were 6 samples of pork legs, 6 samples of minced pork, 6 samples of minced beef, and 6 samples of mixed minced meat. All samples complied with the Regulation (EU) No 1169/2011. On average, pork leg contained 1.31 g, minced pork contained 2.12 g, mixed minced meat contained 2.87 g, while minced beef contained 3.57 g of collagen. We can conclude that pork leg had the lowest collagen values, while minced beef contained the highest values. When the mixed minced meat consists of beef and pork meat, it is to be expected that, depending on the ratio in the amount of certain meat, the values vary. Generally, it is noticeable that higher collagen content is present in mixed meat with a higher beef ratio.

KEY WORDS: beef; colorimetric method; hydroxyproline; pork; UV-VIS spectrophotometer

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## Determination of ethanol concentration in different food categories according to the Halal standard

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According to the EC Regulation on consumer information on food No. 1169/2011, all foodstuffs that contain more than 1.2 % vol. of alcohol must give information about their content. Furthermore, ethanol is forbidden to be processed with or added to food under Halal certification, therefore the control of the presence of alcohol in such types of products is very demanding. The main objective of this research was to determine the presence of alcohol in quantities <1 % vol in products that are certified according to the Halal standard. Standardized method for determining low levels of alcohol in food (AOAC 2017.07 Enzymatic assay for ethanol in foodstuffs and other sample materials) via spectrophotometric technique was applied, with a limit of quantification of the method (LOQ) of 0.02 %. The accuracy of the method and the truthfulness of the results were confirmed by a Reference Material (FAPAS-3117). In total, 32 food samples of different categories (jams, pasteurized vegetables, various snack products, baby food, and honey) were analysed. The analysis revealed that 98 % of the samples were in accordance with EC Regulation No. 1169/2011 and conditions that must be met for a Halal certificate, while 2 % of the analysed samples did not comply with the requirements. The obtained results indicated that the used analytical technique is reliable and accurate for determining low contents of ethanol in food and can be recommended for use during the Halal certification, as well as for label checking for food with very low alcohol content.

KEY WORDS: alcohol; enzymatic assay; label; spectrophotometry

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## The presence of aflatoxins in peanuts and peanut products

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Aflatoxins are mycotoxins produced by two species of *Aspergillus*; *Aspergillus flavus* and *Aspergillus parasiticus* which are found in areas with hot and humid climates. They can enter the food chain as a result of crop infection before or after harvest and are typically found in foods such as cereals, peanuts, dried fruits, nuts, and spices. Aflatoxins include aflatoxins B1, B2, G1, G2, and M1. Aflatoxin B1 is the most common in food and among the most potent genotoxic and carcinogenic aflatoxins. Due to the toxicity of aflatoxins, exposure through food should be kept as low as possible. Temperature and humidity are important parameters for the growth of fungi and because of that climate changes can have a great impact on the presence of mycotoxins in food. The aim of this work was to demonstrate the presence of aflatoxins in peanuts and peanut products during official controls in the period from 2017 to 2022. For the detection of total aflatoxins and aflatoxin B1, the enzyme immunoassay (ELISA) was used. In the selected period, 74 samples of peanuts and peanut products were examined. Of the 74 samples in total, 5 samples were positive for the presence of aflatoxins and not in accordance with Regulation 1881/2006. Considering the high toxicity and high risk for consumer health and the proportion of defective samples and because of climate changes, it is very important to control the presence of aflatoxins in peanuts and other nuts imported from third countries into the EU market.

KEY WORDS: ELISA; mycotoxins; official control; total aflatoxins

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## Risk to public health from tropane alkaloids in commercial maize products

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The maximum level of tropane alkaloids (TA) in certain cereals and cereal-based products was regulated in 2021, following the EU Commission recommendation for the monitoring of the presence of TA in food, which provided the occurrence data needed to assess potential health risks. Based on the atropine and scopolamine content of 103 commercial maize products (33 grits, 39 polenta, 31 semolina; Serbian market, 2021) obtained by LC-MS/MS analysis, the possibility to exceed the acute reference dose (ARfD; 16 ng/kg bw/day for the sum of atropine and scopolamine) was tested on a case-by-case basis, since TAs are not bioaccumulative or genotoxic and do not exhibit chronic toxicity. Overall, across the age classes – children, younger and older adolescents, and adults – the ARfD was exceeded by 11.7, 4.9, 6.8, and 6.8 % of the samples (33.3, 13.9, 19.4, and 19.4 % of positive ones), with maximum exposure reaching as much as 933, 494, 704, and 616 % of the ARfD. Atropine alone was responsible for the risk posed by 6.8, 3.9, 4.9, and 3.9 % of the samples (19.4, 11.1, 13.9, and 11.1 % of positive ones) with maximum exposure at 795, 423, 600, and 525 % of the ARfD. However, as not one of the polenta samples was among those exceeding the ARfD, polenta could be a safe option for children (positive samples 17.9 %, maximum contribution to the ARfD 68.3 %). According to the reported findings, adverse health effects of TAs such as inhibition of muscarinic acetylcholine receptors in the central and the autonomic nervous systems cannot be ruled out.

KEY WORDS: acute reference dose; atropine; food; risk assessment; scopolamine

## Determination of stable isotope ratios of <sup>13</sup>C/<sup>12</sup>C in honey samples by LC/EA-IRMS – preliminary results

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Stable isotope analysis has become indispensable for verifying the authenticity of honey. As part of the project “Center for Safety and Quality of Food”, we analysed the <sup>13</sup>C/<sup>12</sup>C stable isotope ratio ( $\delta^{13}\text{C}$ ) in samples of multifloral honey harvested from small stationary beekeepers. Honey samples were collected in different Croatian regions, including north (Zagorje), east (Slavonia), west (Istria), and south (Dalmatia). To determine the smallest differences between isotopes in compounds produced by physical and biochemical isotope fractionation in nature, we used the technique of liquid chromatography/elemental analysis-isotope ratio mass spectrometry (LC/EA-IRMS). Bulk stable isotope analysis of honey was performed using EA-IRMS. For separation and isotopic analysis of individual sugars in honey, including glucose, fructose, disaccharides, and trisaccharides, LC-IRMS was used. Several physicochemical analyses (according to the Council Directive EC 2001/110/EC on honey) and pollen analyses confirmed that all of the honey samples were of excellent quality within the botanical varieties. No honey sample showed signs of adulteration, as no extraneous sugars were detected. This was also confirmed by LC/EA-IRMS. The preliminary results of  $\delta^{13}\text{C}$  analyses from LC-IRMS and EA-IRMS showed that there were differences between honey samples from the continental and Mediterranean parts of Croatia. These preliminary results indicate good possibilities to determine the authenticity of honey from different parts of Croatia. These results are very important for the creation of a unique database of stable isotopes for Croatian honey. To obtain more accurate results, honey samples from the same area would have to be studied in the coming years to confirm the above stated thesis.

Acknowledgement: This work was carried out within the project “Food Safety and Quality Center” (KK.01.1.1.02.0004). The project is co-financed by the European Union from the European Regional Development Fund.

KEY WORDS: Croatian honey; food fraud; geographical origin; honey authenticity; stable isotope analysis

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## Volatile profile of “paški sir” cheese from the island of Pag

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Cheese is one of the most popular fermented dairy foods, with an incredible diversity of varieties, shapes, textures, and flavors available locally and globally. The goal of this study was to determine the characteristic volatile compounds found in cheese from the Croatian island of Pag with a protected designation of origin (PDO). “Paški sir” cheeses produced for this research (n=18) were divided into three groups: (1) control group, cheeses produced with natural commercial rennet (Bioren, Christian Hansen, Denmark), and commercial dairy cultures (Di Prox, Bioprox, France); (2) group 2, cheeses produced with lyophilized indigenous lamb rennet and microencapsulated dairy cultures; (3) group 3, cheeses produced with innovative microcapsules containing indigenous lamb rennet and dairy cultures. The cheeses were sampled on the 60<sup>th</sup> day of the maturation process and the (n=18) volatile profile was analysed. For the extraction, identification, and quantification of 47 volatile compounds, including 13 acids, 14 esters, 6 ketones, 5 alcohols, 4 aldehydes, and 5 miscellaneous chemicals, a headspace solid phase microextraction (SPME) approach employing polydimethylsiloxane/carboxen/divinylbenzene (PDMS/CAR/DVB) fiber in combination with gas chromatography-mass spectrometry (GC-MS) was applied. The results are processed in SAS 9.4 (Cary NV: SAS Institute Inc.). Results revealed quantitative variations in various volatile components between the cheeses. Group 3 showed significantly higher levels of volatiles compared to group 1 and 2.

KEY WORDS: GC-MS; microcapsules; rennet; SPME; volatiles

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## Research and development of meat products with the aim of reducing added nitrates and nitrites

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A food additive is any substance not consumed as a food ingredient but added to food for a specific purpose such as inhibiting microorganism growth and maintaining sensory quality. Potassium and sodium nitrates and nitrites are among the most commonly used food additives in the meat industry and in cured meat products due to their antimicrobial effect, in particular to prevent the growth of *Clostridium botulinum* and its spores. Additionally, they provide specific sensory attributes, primarily the inherent pink color of meat products. Studies show that excessive intake of nitrates and nitrites may pose a risk to human health. With this in mind, a group of experts from the Croatian Institute of Public Health, in cooperation with a representative from the meat industry, is conducting a research and development project for cured meat products without common additives in the meat processing industry (nitrites, nitrates, antioxidants, phosphates, and polyphosphates). The second part of the project is focused on the influence of added nitrates and nitrites naturally found in vegetables, their reactions with specific microorganisms, and implementing these findings to attain meat products that are equal to traditional products in quality.

KEY WORDS: additives; antimicrobial effect; health risk; natural sources of nitrates

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## How microbial quality of the air impacts longevity of pasta products in a Croatian pasta production factory

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Indoor microbial air quality depends on several factors: human activity, microclimate, hygienic practices, and ventilation types. The air quality in pasta production factories often goes neglected, yet it greatly affects the longevity and quality of the final product. The present study analyses the microbiological quality of air in one pasta production factory in Croatia. The air sampling was done using impaction technique next to the dough mixer, paper wrapping machine, chilling room, and processing room. Outdoor air was also sampled at a distance of 1.5 meters from the factory building. The enumeration of bacterial and mould count was carried out using air sampling (impaction method). Air quality was evaluated by comparing indoor and outdoor air according to the Portuguese standard. The analyses revealed that microbial counts of moulds were significantly higher near the dough mixer followed by the paper wrapping machine, while they were low in the chilling room and processing room in comparison with ambient air. The bacteria count was low in all of the areas. The results on air microbes obtained in the present study by air sampling were not within the Portuguese standard recommendations, which can affect product quality and shorten shelf life. This study on bio-aerosols should help us implement clean room practices for good manufacturing practices in the pasta production industry.

KEY WORDS: air sampling; clean room practices; impaction technique; indoor air; Portuguese standard

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## The quality of nutrition in the wards of two hospitals in the Zenica-Doboj Canton during 2021

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The goal of this study was to evaluate nutrition in the two general hospitals of the Zenica-Doboj Canton (ZDK) and give recommendations for improving them. Data was collected from an existing programme. There were 94 samples taken in two cantonal hospitals from four wards at both institutions. Meal composition was noted, samples were chemically analysed for energy content and for carbohydrate, protein, and fat content. The average daily caloric intake was 2215.32 kcal (Zenica) and 2296.00 kcal (Tešanj). The average percentage of energy needs met was 103.04 % (Zenica) and 101.74 % (Tešanj), ranging from 89.95 % (Obstetrics) to 113 % (Surgery) in Zenica, and 98.2 % (Internal medicine) to 104 % (Surgery) in Tešanj. The average daily energy intake from fats was 470.31 kcal in Zenica and 683.68 kcal in Tešanj. There was a statistically significant difference in energy intake from fats between two hospitals and between hospital wards. Data has shown that evening meals contain most of the energy content and that breakfast met the least amount of energy intake needs. There was no statistically significant difference in daily energy intake distribution between hospitals or wards. Fresh fruit and vegetable content in meals was lacking in both hospitals. Meals in the two general hospitals in ZDK met the basic energy needs but did not contain the necessary fresh produce and dairy. Energy intake from fats is on average within recommendations but varies between hospitals and wards. Meals should contain more fresh produce and dairy products and more care should be put into the nutritional composition of meals and not only into energy content.

KEY WORDS: clinical nutrition; dietetics; hospital nutrition; medical nutrition; nutritive value

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## DehydraTECH-CBD dietary supplement and its effect on hypertension

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Cannabidiol (CBD) is a bioactive cannabinoid with non-psychoactive effects consumed as a food supplement by millions of people. DehydraTECH-CBD is a patented capsule formulation (developed by Lexaria Bioscience Corp.) that increases the bioabsorption of active content due to its enhanced lipophilic composition by bypassing (or reducing) first-pass liver metabolism. It was conducted as a double-blinded, placebo-controlled, cross-over pilot study in which 24 eligible volunteers (12 males and 12 females) were recruited and completed all of the experimental parts. The aim of that study was to extend the findings of DehydraTECH-CBD on the cardiovascular system in humans with mild or moderate untreated hypertension. Each participant received in a randomized and double-blinded order CBD or DehydraTECH-CBD (12 capsules with 300 mg of active substance). Blood pressure and heart rate were measured in triplicate every 10 minutes throughout the study. Three venous blood samples were taken; at baseline and in the 120<sup>th</sup> and 180<sup>th</sup> minute. CBD concentration was determined by a gas chromatography-tandem mass spectrometry (GC-MS/MS) system. Our results have shown that in the subset of volunteers who were Stage 2 hypertensive, peak systolic BP reductions from the baseline were observed as much as approximately 13 mmHg by the 50-minute time point with DehydraTECH-CBD and systolic BP remained depressed throughout almost the entire 3-hour duration of the study. We can conclude that DehydraTECH technology enabled a rapid and sustained drop in blood pressure, especially systolic pressure.

KEY WORDS: blood pressure; cannabidiol; food supplements; GC/MS method; liver metabolism

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## Probiotic bacteria isolated from fermented meat displays antioxidant and anti-inflammatory potential

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A high-calorie diet is associated with meta-inflammation, which contributes to many noncommunicable diseases and is a global public health problem. There is clear evidence that diet-induced changes in the gut microbiota have a strong influence on the development of metabolic diseases. One way to address the emerging problems of an unhealthy diet, such as disturbances in the microbiota, inflammation, and oxidative stress, is through the use of probiotics. The discovery and selection of appropriate probiotic bacteria is a difficult process. The aim of this study was to investigate the probiotic potential of *Lactiplantibacillus plantarum* 1K isolated from traditional Croatian fermented meat products. The antioxidant potential was measured using DPPH radical scavenging. In addition, small bacterial metabolites were extracted and their anti-inflammatory activity was tested in a lipopolysaccharide-stimulated (LPS) inflammation model in human peripheral blood mononuclear cells. The safety properties of the metabolites, including cytotoxicity and genotoxicity, were also determined. The studied strain exhibited high antioxidant capacity, and the viable bacterial cells retained high antioxidant activity even after exposure to the simulated gastrointestinal conditions. The extracted probiotic metabolites significantly inhibited TNF- $\alpha$  production in LPS-stimulated immune cells, thus exerting an anti-inflammatory effect. The metabolites alone showed no cytotoxic or genotoxic activity on isolated immune cells. The obtained results enable the possibility of using fermented meat products as carriers for certain probiotics to provide antioxidant and anti-inflammatory benefits to consumers.

KEY WORDS: fermentation; inflammation; probiotics; TNF- $\alpha$ ; genotoxicity

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## Correlation of the recommended quantity of salt, dietary intake, and sensitivity threshold of the population of northern Croatia

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Salt is an essential micronutrient that should be used in small amounts. The recommended daily intake of salt based on Regulation No. 1169/2011 on consumer information on food is 6 g and 5 g according to the World Health Organization. According to the latest research by the Croatian Food Agency, the daily intake is about 11 g, which is worrying since the intake of salt is directly related to cardiovascular diseases and represents a major public health problem. An individual's sensitivity threshold to table salt is an important factor. According to the National salt strategy for reducing the excessive intake of table salt in the Republic of Croatia 2014-2019, and related to the Ordinance on cereals and cereal products (Official Gazette 81/2016), the quantity of salt in ready-to-eat baked bread has been limited to 1.4 % and later to 1.3 % by the new Ordinance (Official Gazette 101/2022). The aim of this research is to check the sensitivity threshold of 150 respondents to table salt of the population of northern Croatia by sensor analysis. Model solutions of sodium chloride (NaCl) in Millipore-filtered deionized water were used. For the Harris-Kalmus sensitivity threshold, the concentration level for NaCl from "not detectable" to "definitely detectable" for most people is  $3.05 \times 10^{-2}$  to  $2.50 \times 10^2$  mmol, so the model solutions are created within that range. The results were presented by statistical analysis of the research and a statistically significant difference was observed within the region, between genders, between age groups, and between smokers and non-smokers.

KEY WORDS: daily intake; legislative framework; National salt strategy; sensor analysis; table salt

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## Gastrointestinal symptoms and intolerance to gluten as new clinical challenges

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Until recently, gluten intolerance was believed to be typical of celiac disease (CD) and wheat allergy (WA). Statistics show that 60 % of the population suffer from intolerances against at least one foodstuff, which may cause clinical symptoms or enhance them. Symptoms are usually confined to the gastrointestinal system such abdominal pain, bloating, and an irritable bowel. The objective of the present review was to provide an overview highlighting the interaction between gastrointestinal symptoms and intolerance to gluten. The study population included 82 patients, 62 of which had gastrointestinal symptoms and 20 had none. For this purpose, tests regarding food intolerance to 44 allergens from serum in patients with gastro-intestinal symptoms were done. The allergen we studied in particular among these 44 was gliadin, a component of gluten. The Nutritional ELISA test kits have been designed for the detection and quantitative determination of specific IgG4 antibodies against food antigens in serum and plasma. The results of gluten intolerance tests showed that most patients with gastrointestinal symptoms (n=61) were intolerant to gluten (gliadin) and had an average concentration of immunoglobulins IgG4 5.51 U/ml. By comparing the results from the intolerance to gluten group with gastrointestinal symptoms with the control group, a statistical difference of  $p=0.029123$  was determined. The results suggest an association between specific antigen gluten with gastrointestinal symptom complaints. These findings may help establish the overall magnitude of gastrointestinal symptoms, separating thereby fact from myth.

KEY WORDS: ELISA; gastrointestinal symptoms; gliadin; IgG4

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## Viability of novel microencapsulated indigenous cultures of *Lactiplantibacillus plantarum* and *Lactococcus lactis* in Pag cheese pilot production – preliminary results

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Within the project “The Potential of Microencapsulation in Cheese Production”, the aim of this study was to characterize the isolates of lactic acid bacteria from the traditional cheese production chain and to select the potentially most suitable starter culture for application in microencapsulated form with rennet in the production of Pag cheese. Based on the established biochemical properties, the *Lactiplantibacillus plantarum* strain from the lamb abomasum and *Lactococcus lactis* strain from sheep milk were selected for microencapsulation in two different formulations: separately (S2) and together with rennet (S3). Both formulations with non-encapsulated commercial starter cultures (S1) as control were used in the traditional production of Pag cheese and therefore six cheeses were produced from each formulation (N=18). Cheese samples were collected and microbiologically analysed every 30 days during 120 days of ripening. To gain insight into the microbial population and the proportion of bacterial species, morphologically distinct colonies were selected in each analysis and identified using matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrophotometry. The average number of bacteria at day 120 was  $6.78 \pm 0.02$  (S1),  $6.49 \pm 0.27$  (S2), and  $6.82 \pm 0.28$  (S3)  $\log_{10}$  CFU/g with uniform variations during the ripening process. While the *Lb. plantarum* strain was isolated at each sampling stage (5-70 %), the proportion of *Lc. lactis* strain decreased at day 30 and was not isolated later. Although additional molecular analysis should be performed to confirm authenticity with the original strains, this result suggests that the microencapsulated form of started cultures and rennet may become a new standard in cheese production while maintaining the traditional values of the product.

Acknowledgement: This research was part of the project: “The Potential of Microencapsulation in Cheese Production”, KK.01.1.1.04.0058 funded by the Operational Program for Competitiveness and Cohesion 2014-2020.

KEY WORDS: cheese production; innovations; lactic acid bacteria; microencapsulation

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## The effect of incubation temperature and duration on the sensory attributes of semi-durable sausages

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The aim of this paper was to determine the change of sensory traits of semi-durable sausages produced under different incubation temperatures and durations. For that purpose, four experimental treatments differed in incubation temperature (30 and 40 °C) and duration (1.5 and 3 hours) with *Staphylococcus carnosus* and nitrates prepared, while the control treatment represented typical production with nitrites. The difference from control test was used and 18 trained assessors participated. Samples were presented in pairs and assessors were instructed to firstly assess the control sample. They were asked to indicate the magnitude of the difference between the two samples and to provide information on which attributes were different in the two samples. Assessors were made aware that the test sample may be the control. The data was analysed by one-way ANOVA to determine if a significant difference between treatments existed. When a significant difference was found, the Dunnett’s multiple comparison test was applied to determine which of the test samples were significantly different from the blind control. It was found that the application of lower incubation temperatures (30 °C) and a shorter duration (1.5 h) did not cause a significant change of sensory attributes, so such incubation conditions could be used in the production of semi-durable sausages with the addition of nitrates and starter cultures. Taste and aroma were most often marked as different in 37 and 24 % of responses, respectively. Appearance and texture were marked in 14 and 16 %, while the odour was marked in only 8 % of responses.

KEY WORDS: assessor; difference from control; nitrates; starter culture; taste

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## Antioxidant profile of willow bark extracts obtained by ultrasound-assisted extraction

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Antioxidant compounds from medicinal plants can promote human wellbeing and reduce the risk of oxidative-stress related diseases. Willow bark (*Salix* spp., Salicaceae) is a herbal remedy used for its anti-inflammatory, analgesic, and antipyretic properties. Beside the salicylic glycoside salicin, phenolic and flavonoid compounds represent some of the major classes of bioactive constituents of willow bark, responsible for its antioxidant activity. This study aimed to compare the antioxidant profile, total phenolic and flavonoid content of bark extracts of five different willow species (*S. alba*, *S. amplexicaulis*, *S. babylonica*, *S. fragilis*, *S. triandra*) obtained by ultrasound-assisted extraction (UAE). UAE was carried out in an ultrasonic bath at 25 °C for 30 min, using water as solvent. The antioxidant activity of extracts was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl (OH) radical scavenging assays. Total phenolic and flavonoid content was determined by spectrophotometric methods. DPPH radical scavenging capacities (IC<sub>50</sub>) ranged from 3.32 to 7.35 µg/mL, while those of the OH radical from 17.06 to 36.29 µg/mL. Extract of *S. alba* exhibited the strongest DPPH and OH scavenging activity. Total phenolic and flavonoid contents were within the range of 4.97-39.27 mg gallic acid equivalents (GAE)/g dry weight (d.w.) and 1.90-12.97 mg quercetin equivalents (QE)/g d.w., respectively. The highest amount of total phenolics and flavonoids was found in the bark extract of *S. amplexicaulis*, while the lowest was in *S. fragilis*. The obtained results indicated that willow bark extracts exert strong antioxidant activity and contain significant amounts of bioactive compounds, suggesting they could be further explored as sources of antioxidants for innovative food and pharmaceutical purposes.

KEY WORDS: antioxidant activity; flavonoids; phenolics; *Salix*

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## Lamiaceae tea extracts as functional food

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Functional food provides health advantages because of its physiologically active substances. The Lamiaceae family represent a rich source of strong components that have a positive impact on the treatment of different diseases. This study aimed to determine the antioxidant potential in samples of selected plant species of Lamiaceae and to compare their activity in traditional and modern extraction (infusions and microwave prepared extracts). Ten of the most frequently used plants (oregano, lavender, basil, savory, garden thyme, wild thyme, sage, rosemary, lemon balm, and mint) from the Institute “Dr Josif Pančić” were examined. Plant material was pulverized and extracts were prepared as infusion according to recommendations and instructions. Antioxidant activity was tested by spectrophotometric method measuring total phenolic and flavonoid content and inhibitory activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. All extracts showed a significant antioxidant activity with an IC<sub>50</sub> value within the range of 3.73-8.03 µg/mL for traditional extracts and 2.63-7.7 µg/mL for modern extracts. The content of total phenols in traditional infusions ranged 91.53-286.07 mg gallic acid equivalents (GAE)/g dry extract, and in microwave extracts 122.44-204.58 mg GAE/g dry extract. The content of total flavonoids in traditional infusions ranged 24.14-166.18 mg KE/g of dry extract, and in microwave extracts 51.12-163.27 mg KE/g of dry extract. Regardless of the choice of method, the selected plants of the Lamiaceae family showed noticeable antioxidant activity, better in microwave extracts, which can be explored in different market applications in the future, especially as a functional food.

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KEY WORDS: antioxidant potential; flavonoids; infusion; microwave extraction; phenolics

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## Ultrasound-assisted extraction of proteins and sugars from *Curcubita moschata* pulp and by-products

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Pumpkin is recognized as a functional food source. Although pumpkin pulp is a nutritionally valuable food source, its chemical properties change over time. The objective of this work was to determine spectrophotometrically the total protein and sugar content of pumpkin pulp dried at an air flow of 1.0 m/s and a temperature of 50 and 60 °C, respectively. Protein and sugar content were also determined in pumpkin peels and seeds to address the global sustainable trend of recycling unused by-products. Proteins and sugars were extracted from all samples using ultrasound-assisted extraction (UAE) at a frequency of 37 kHz, a temperature of 50 °C, and durations of 10, 20, and 40 min. The recovery of both nutrients was also compared with reflux extraction performed during 20 and 40 minutes. The obtained results showed that high contents of proteins (12.83 %) and sugars (48.93 %) were present in the dry samples extracted with UAE. The content of proteins and sugars depended on the drying temperature and extraction time. The contents of proteins and sugars obtained by reflux extraction were also high, 10.66 % (proteins) and 56.14 % (sugars). The raw pulp contained between 0.83 and 1.54 % proteins and 4.64 to 5.71 % sugars. The raw seeds contained 8.99 to 27.55 % proteins and 2.36 to 14.06 % sugars, while the raw peels contained 1.21 to 2.41 % proteins and 1.90 to 2.72 % sugars, depending on the extraction method. Therefore, both by-products are valuable sources of two nutrients and could be used as an alternative sustainable source of food ingredients.

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KEY WORDS: chemical composition; hot air drying; dried pumpkin; pumpkin by-products; pumpkin pulp

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## Development and optimization of a method for determining the aroma profile of strawberries

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Strawberries are commercially produced for immediate consumption and processing as frozen, canned, or juice. Given their perishable nature and low likelihood of mechanical harvesting, this fruit is generally grown near consumption or processing centers where sufficient labour is available. An optimised method for analysing the aroma profile of strawberries grown in the Zagreb area was developed. Whole strawberry fruits (*Fragaria ananassa* × Duch., cv. Albion), their processed forms (i.e. purees and juices), and their by-products were analysed. Gas chromatography-tandem mass spectrometry (GC-MS/MS) in SCAN mode was used to analyse the aroma profile and determine the proportion of individual components. Samples were prepared and analysed by solid phase microextraction (SPME). The aromatic profile of strawberry samples contains predominantly polar compounds, therefore Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB-CAR-PDMS) fiber and a polar GC column (RTX-WAX; 30m/0.25 mm/0.25 µm) were chosen for analysis. The effects of sample mass, ionic strength, and temperature of the agitator and sampling time were examined, i.e. exposure of the fiber to the sample. The experiments showed that higher mass, higher ionic strength, and higher temperature of the agitator contributed to a higher intensity of the received signals, but not to a higher number of total signals. Higher ionic strength was obtained by adding 20 % NaCl solution to the sample. In the analysis of strawberries, 5 mL of 20 % NaCl solution was added to 5 g of weight, and 1 g of pure NaCl to 5 mL of juice. The aromatic profile of strawberries contained mainly furanones, esters, aldehydes, and carboxylic acids. The proportion of each compound was determined by the area normalization method.

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KEY WORDS: Albion; GC-MS/MS; SPME



## Organic UV-filters in cosmetic products: determination and risk assessment

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Usage of sun-protection cosmetic products that contain UV-filters is on the rise due to climate changes and increased summer UV-indices. Therefore, a collection of cosmetic products comprising 79 samples from the Serbian market was subjected to high performance liquid chromatography (HPLC)-UV analysis, using a validated method with seven UV-filters in its scope, a compliance check related to the Cosmetics Regulation (1223/2009), and a risk assessment using a Margin of safety (MOS) approach. The amount of UV-filters was in line with the Regulation. The risk assessment showed that phenylbenzimidazole sulfonic acid, ethylhexyl salicylate, butyl methoxydibenzoylmethane, ethylhexyl methoxycinnamate, and octocrylene in sun-protection products did not pose a risk for consumers. Conversely, benzophenone-3 was a cause of a concern in 2 of the 13 analysed sun-protection products for children (MOS below 100). In case of homosalate, the risk assessment revealed a huge disagreement between the regulated maximum concentration and the resulting MOS. More precisely, the substantially reduced NOAEL, established by the EU Scientific Committee on Consumer Safety in 2021, led to an unacceptable MOS below 10. The MOS for analysed sun-protection products for adults ranged 6-24 (16 out of 31 products), whereas in children the MOS depended on age, with an overall range of 5-12 (4 out of 13 products). Risk from any of the UV-filters in adults' face creams, tinted creams, and liquid powders, as well as lip balms was negligible. However, an aggregate exposure from multiple products, with sun-protection products included, poses a risk from homosalates. Therefore, a recommendation for the reduction of homosalate concentration should be seriously considered by both cosmetics producers and regulators.

KEY WORDS: face cream; homosalate; lip balm; margin of safety; sun-protection products

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