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3rd International Congress on Food Safety and Quality "Food, Health and Climate Changes"

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Distinguished colleagues, dear friends,

It is my great honour and pleasure to welcome you on behalf of the Organising and Scientific Committee of the 3rd International Congress on Food Safety and Quality – FOOD, HEALTH AND CLIMATE CHANGE, held virtually, without the physical presence of participants and lecturers.

New circumstances bring new challenges and, in order not to fall behind when it comes to education and exchange of experience, we have had to adapt to this new way of 'socializing'. The great interest for the past two congresses on Food Safety and Quality with International Participation was met, inspiring us to organise an even better and more educational Congress this year, with the aim of fostering an exchange of experiences between Croatian and international experts, as well as raising awareness on the topic of food health and safety.

The organisers and co-organisers of the 3rd International Congress on Food Quality and Safety are the Andrija Štampar Teaching Institute of Public Health, the Croatian Metrology Society, the SEEN-FSQC South-East European Network for Food Safety and Quality Control, the Faculty of Agriculture and the Faculty of Food Technology and Biotechnology of the University of Zagreb, the Institute of Health and Food Safety Zenica, and the Institute of Public Health of Vojvodina, Novi Sad, in cooperation with other distinguished institutions from the country and abroad.

The third Congress will include current topics in the field of food safety and quality, with an emphasis on effects and interaction between food, health, and climate changes. Food safety and quality, determining the origin, geographical origin, and food adulteration and its impact on consumer health, as well as the impact of climate change on cultivation and quality – these are the topics of shared responsibility among everyone involved in the production chain: the country, producers, distributors, professionals, and consumers, but also the media, which hold responsibility for informing the public on current topics. We will hold discussions on new technologies and the development of analytical methods,

organic food production, mycotoxins and plant toxins, pesticides, metals and other contaminants, allergies and food, management systems, testing, certification and accreditation. In addition, there will also be discussions on the microbiological safety of food, the safety of materials coming into contact with food, prevention of diseases and food borne diseases, trends in food supplements, news in the field of proper nutrition in retirement homes and many other important and related topics.

Focus will also be given to how accreditation improves food safety, supporting the confidence of consumers, suppliers, purchasers, regulators, and specifiers in the quality and safety of food. Encouraged by the overwhelming interest generated by the first two Congresses on Food Safety and Quality, attended by more than 700 participants, it is our great desire for the third time to gather prominent local and foreign speakers and many participants, who in their daily work deal with the influence of food on human health and, despite the current epidemiological situation, to continue educating and spreading knowledge in virtual form.

The programme will include workshops, plenary lectures, oral presentations, a round table, satellite symposia, and e-posters online, through the Zoom virtual platform and Project presentation: 2nd International Conference Food Safety and Quality Centre.

The World Health Organization supports the organisation of the round table on "The impact of climate change on food production and its accessibility to the general population", while the European Regional Development Fund funds the "Food Safety and Quality Centre" International Conference.

This Book of Abstracts is a supplement to the journal "Arhiv za higijenu rada i toksikologiju - Archives of Industrial Hygiene and Toxicology", published by the Institute for Medical Research and Occupational Health, Zagreb. We are thankful to the Archives' editor-in-chief and editorial board for accepting the publication of the lecturers' presentation abstracts, as well as other oral and poster presentations from the Congress.

On this occasion, I would also like to extend my gratitude to the Mayor of the City of Zagreb Milan Bandić, for having accepted the Congress' organisation under his high auspices, and the Ministry of Science and Education, the Ministry of Health, the Ministry of Agriculture, the Ministry of Economy and Sustainable Development, the Ministry of Tourism, the Croatian Accreditation Agency, the State Office for Metrology, the University of Zagreb, and the Croatian Healthcare Employers' Association.

We also thank the World Health Organization for their support and active participation in the realisation of this congress.

We sincerely thank the sponsors who, with their support, contributed to the organisation of this Congress.

Finally, once again, we thank you for joining us on the web platform, and with your participation contributing to our mutual task – the protection and promotion of human health.

Yours sincerely,

Zvonimir Šostar, MD Congress President

ABSTRACTS

INVITED SPEAKERS

Improving the evaluation of target and non-target contaminants in food by a new LC-HRMS platform

A. R. Fernández-Alba*

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Modern high-resolution mass spectrometry (HRMS) can be considered an interesting alternative to triple quadrupoles in the field of food safety. New HRMS instruments can compete with triple quads in target analysis, but at the same time, they offer unquestionable advantages of non-target acquisition. Orbitrap is one of the two most common high-resolution instruments applied in the fields of food safety. In the past, Orbitrap was considered a relatively slow mass analyzer. However, that problem has been overcome with the introduction of the so-called high-field Orbitrap, which is almost two times faster than the standard model. The increased acquisition rate helps to develop acquisition methods that resolve typical problems and also expand the multitasking of the system. The non-target acquisition brings very important advantages, the number of analysed compounds is unlimited, and the method development is fast and easy. Unfortunately, around 10-15 % of pesticides experience problems in non-target MS² (all ion fragmentation, AIF). It is mainly related to low sensitivity and/or presence of isobaric interferences that occur especially in difficult matrices. That issue was solved by combining AIF with simultaneous target MS². The target MS² was applied only for the compounds that did not work well in non-target mode. Additionally, the acquisition method included a list of about 1500 other potential contaminants (pesticides, pharmaceuticals, veterinary drugs, etc.). The retention times of those contaminants were not known. However, in the case of the detection of any precursor ion from the list, the instrument automatically triggered a data-dependent MS² scan to obtain a high-quality spectrum for identification purposes, which means that the instrument carried out four different types of data acquisition simultaneously. The cycle time was maintained below 0.8 s and the number of data points per chromatographic peak was enough for a good quantitation.

KEY WORDS: all ion fragmentation; data acquisition; food safety, multitasking; Orbitrap

What has been done within the project "Food Safety and Quality Control Center" KK.01.1.1.02.0004

A. Krivohlavek^{1**}, S. Šikić¹, Z. Grgić², and Z. Šostar¹

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The implementation period of the project "Food Safety and Quality Control Center" is from October 1 2018 to October 1 2022, so what have we done so far? The Food Safety and Quality Control Center has been established in order to control and protect the general and geographical origin of food, ecological production of food, and to develop new analytical procedures in food analyses. New laboratories have been built and equipped according to the highest standards. New research equipment as well as ICT equipment have been purchased. All equipment will be used for the development of new analytical methods for analyses of samples that have been collected so far. The partners in this project, the Andrija Stampar Teaching Institute of Public Health and Faculty of Agriculture, have been collecting samples of food (honey, olive oil, meet, cheese), water, and soil in three years period from all over the Republic of Croatia. The main goal of the project is to establish an isotope map and information database of Croatian indigenous and organic food products, which is vital for their recognizability in the domestic and foreign markets. In addition, all activities which will be carried out within the project should benefit student education and further professional development of research experts in this field.

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

Acknowledgement: The activity is carried out within the "Food Safety and Quality Center" (KK.01.1.1.02.0004) project funded by the European Regional Development Fund

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Evaluation of the metabolism of fluorine in animal tissues introduced in the form of fluorine-containing compounds

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Interest in the determination of fluorine has increased over the years, which is due to its significant role in biological systems. Fluorine compounds are widely used as an additive to foodstuffs, toothpaste or drugs and may thus interact with biological systems in the form of fluorine-containing compounds. The small covalent radius of fluorine can facilitate docking with the target during metabolic reactions or spontaneously if the drug molecule is sufficiently electrophilic to be able to react directly with nucleophilic groups present in proteins, such as the amino group in lysine and the hydroxyl group in serine. Although several analytical techniques are known for their ability to detect fluorine and its compounds, the investigation of its biotransformation in living organisms is not a trivial task. Therefore, much attention is given to the development of new analytical strategies that can trace the metabolism of fluorine-containing compounds. The aim of this work was to design an analytical protocol to conduct a comprehensive study on the investigation of fluorine and fluorine-containing compounds in the tissues of model organisms, including the determination of the total content of fluorine, the identification and quantitative determination of fluorine-compounds towards the distribution of fluorine compounds, and an evaluation of their interaction with proteins using a proteomic approach. The determination of total content of fluorine in tissues was done via measurements of molecular absorption of gallium monofluoride (GaF) at 211-248 nm using a high-resolution continuum source graphite furnace absorption spectrometry (HR-CS GF MAS). Then the determination of fluorine compounds extracted from tissues was performed by a triple quadrupole mass spectrometer coupled to high performance liquid chromatography (HPLC-ESI-MS/MS). Finally, the extracted proteins were digested in-solution with trypsin and the formed peptides were investigated with the use of an ultra-high performance liquid chromatography coupled to an electrospray mass spectrometer equipped with a high resolution Orbitrap mass analyser (nano-UHPLC-ESI-ORBITRAP-MS/MS). Based on registered data, using the appropriate proteomic software (Mascot, MaxQuant) with access to the SwissProt protein database, a qualitative analysis of proteins was achieved.

KEY WORDS: additive of fluorine-compounds; comprehensive analytical scenario; HPLC-ESI-MS/MS; metabolic pathway of fluorine; Orbitrap

Acknowledgement: The study was carried out at the Biological and Chemical Research Centre, University of Warsaw established within the project co-financed by European Union from the European Regional Development Fund under the Operational Programme Innovative Economy 2007–2013

Climate change as a driver of emerging risks for food and feed safety, plant, animal health, and nutritional quality

A. Maggiore**, A. Afonso, F. Barrucci, G. De Sanctis, and A. Lewandowska

European Food Safety Authority, Parma, Italy

Climate change is a relevant driver of emerging risks. While a broad range of forward-looking studies and reports examine the impact of climate change on food security, future challenges for food and feed safety, plant and animal health and nutritional quality are usually not investigated in depth. EFSA recently completed the CLEFSA (CLimate change and Emerging risks for Food Safety), which aimed to: a) use the specific driver, climate change, for long-term anticipation of multiple emerging risks, using scenarios of climate change; b) use crowdsourcing and text mining to collect a broad range of signals from a variety of information sources; c) use a knowledge network of experts from international organisations; d) design a Multi-Criteria tool for characterising signals through a participatory process, in which expert knowledge is used to identify relevant issues from the vast and often incomplete information; e) developing methodologies and indicators for the analysis of the information available, addressing uncertainty. CLEFSA has identified numerous issues that are driven by climate change and that may affect food safety in Europe, which are either new hazards or known hazards with increased impact or likelihood of occurrence. However, the effects of climate change are characterised by a multidisciplinary nature (human-plant-animal health and environmental sciences) and go beyond the recognition of specific emerging risks. Given the complexity and interdisciplinarity of the subject, engagement and cooperation with a wide variety of stakeholders is needed. A report on the project was published on 25 June 2020.

KEY WORDS: CLEFSA; crowdsourcing; hazard; Multi-Criteria tool; text mining

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Impact of climate change on food availability and population health

A. Kaić-Rak^{1*}, J. Pucarin-Cvetković^{2,3}, and I. Heim⁴

It is estimated that the world's population will reach nine billion by 2050. Our capacities to maintain food security for an increasingly large and expectant population presents a significant global challenge. The Food and Agriculture Organization (FAO) defines food security as "Food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life". Presently, estimates indicate that 1 of 7 people are undernourished, while as much as 1/3 of the food production is wasted. According to modeling-based estimates, an approximately threefold increase in food demand is expected over the coming decades. The anticipated prospect of climatic variability with increased uncertainties about the extent of how climate change will affect the food security and safety and thus directly affect the human health and wellbeing is in the focus of policy makers. The food system is vulnerable to climate change, impacting many steps from production to consumption. The complex interactions between food availability, access to food (e.g. affordability) and utilization of food has to be addressed. Climate change and sustainable food production is challenged by maximizing production, introduction and incorporation of new technologies as well as limitation to the climate footprint of food production. National strategies need to address sustainable food security with equitable access and availability of food for their population. Furthermore, countries are not only confronted with finding new ways to improve the resilience of food production in relation to adverse impact of climate change, but also with the need to protect sufficient quantities of safe fresh water resources.

KEY WORDS: environmental changes; food; fresh water; risk; security

Role of data science in food chain safety decision making: Current status and future trends

A. Bernard Jóźwiak**

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The food chain is becoming more and more complex, and a huge set of various drivers influences it on a daily basis. Such a complexity needs also complex solutions in decision making. With the arrival of large amount of available data and advanced data analysis methodologies and tools, there is an increasing promise of achieving a deeper understanding of the food chain processes like never before. However, the breakthrough solutions are still missing. Where are we in this process? What to expect and what not? The experience from the European Food Safety Authority (EFSA) Advisory Forum Task Force on Data Collection and Modelling is shared, as well as some data analysis and visualisation proceedings of the last few years, showing examples on how various data could be used and re-used in the decision-making process. The data analysis examples focus on emerging risk identification, where we use automated data retrieval, analysis and visualisation solutions to discover hidden patterns and upcoming trends. These tools help us in capturing valuable information out of a very noisy and biased environment, leading to better preparedness and decision making.

KEY WORDS: advanced data analysis; artificial intelligence; data collection; emerging risks; modelling

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The toxicology of opium poppy's main alkaloids

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Poppy seeds from opium poppy (Papaver somniferum L.) are used for food as decoration or cake filling (like in traditional Croatian cakes "makovnjača" and "međimurska gibanica") as well as for edible oil production. The plant latex (milky sap), the primary opium extracted by scoring unripe capsules, contains up to 80 different alkaloids, and some of them are used in the pharmaceutical industry for the production of medicines (analgesics, sedatives, and antitussives), and unfortunately in drug abuse. Seeds of opium poppy plants, used in the pharmaceutical industry, serve as a by-product for food purposes. Mature poppy seeds do not contain latex but may be contaminated with alkaloids by pests and during harvest. Cases of alkaloid intoxication after consumption of poppy seeds in food are described in the scientific literature, but maximum levels have not been established in the EU by any Regulation. The European Commission published recommendations on good practice to prevent and reduce the presence of poppy seed's alkaloids. Opium alkaloids are categorized into two main groups: phenanthrenes (morphine, codeine, thebaine, and oripavine) and benzylisoquinolines (papaverine and noscapine) acting via at least three types of receptors. The μ (mu) receptor plays a crucial role not only in opioid-induced analgesia but also in the unwanted actions of opioids. Toxicokinetic characteristics (ADMET) of main alkaloids will be presented and their side effects discussed with implications of gene polymorphisms of metabolized enzymes, and drug-drug interactions on toxicity outcomes. Thus, monitoring opioid alkaloids concentrations in poppy seeds is of great importance in public health protection.

KEY WORDS: food; opioid alkaloids; poppy seed; side effects; toxicology

Eurachem – supporting reliable measurements to ensure food safety, quality and authenticity

V. Barwick**

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Reliable measurement and test results are essential to ensuring food safety, quality, and authenticity. In increasingly complex production and supply chains, measurement results need to be comparable across space and time. This is achieved by ensuring that results are (metrologically) traceable to the same stated references. However, the cycle of measurement and testing goes beyond the preparation and analysis of samples in the laboratory. To achieve meaningful results, attention needs to be paid to all steps that contribute to the final interpretation of the result. It is essential that measurement procedures used in the laboratory have been demonstrated to be fit for purpose through appropriate validation studies. The estimation of measurement uncertainty arising from both the primary sampling and the chemical analysis, is also required as it will impact compliance assessments. For over 30 years, Eurachem has provided a forum for analytical laboratories and other interested parties to discuss issues relating to quality of measurement. Its main objectives are establishing a system for the international traceability of chemical measurement results and the promotion of good quality practices. Through its active network of member counties, Eurachem produces authoritative guidance addressing quality issues at all stages of the measurement cycle, from sampling through to interpreting results against limits. Eurachem also supports international initiatives on food safety and quality. These include METROFOOD-RI, an EU research infrastructure for promoting metrology in food and nutrition, and contributing, as an observer, to the work of the FAO/WHO Codex Committee on Methods of Analysis and Sampling.

KEY WORDS: guidance; measurement uncertainty; method validation; metrological traceability; sampling

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Presentation of the project "Potential of microencapsulation in cheese production" (KK.01.1.1.04.0058)

N. Mikulec^{1*}, F. Oštarić¹, M. Vinceković², V. Čubrić Čurik³, N. Zdolec⁴, and S. Kazazić⁵

This oral presentation will aim to present the project investigating the development of innovative technologies in cheese production and the conservation of biodiversity. Scientists from the Department of Dairy Science, Department of Chemistry, and Department of Animal Science from the Faculty of Agriculture, Department of Hygiene, Technology and Food Safety from the Faculty of Veterinary Medicine and Department of Physical Chemistry from the Ruder Bošković Institute form a project team with an interdisciplinary approach and a rounded synergy in research. The main goal is to extract rennet from suckling lamb's abomasum and prepare it for cheese production in a microencapsulated form, which is innovative in cheese production. The hypothesis is that this procedure will protect enzyme activity, which will have a significant effect on cheese quality and yield in production and will be successfully used as part of the production technology of all types of cheeses but mainly traditional and "boutique" varieties. The rennet efficiency will be tested for coagulation properties of raw and pasteurized cow, goat, and sheep milk used in the production of various types of cheese by determination of its rheological features and coagulum microstructures, evaluation of sensory properties, and determination of aromatic compounds in produced samples. If this project proves rennet efficiency, it could become a new standard for modern cheese production and enable the application of innovative technologies in the production of high-quality safe food with an added value. The project's implementation will increase the research team's abilities to conduct top research in the field of biotechnical, biomedical, and natural sciences, while the obtained results will be crucial for the development of applied solutions in the industry.

KEY WORDS: biodiversity; innovative; microencapsulated form; modern cheese production; rennet

Stable isotopes as a guide to authenticity and origin

N. Ogrinc**

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This presentation aims to provide an overview of the use of stable isotopes in combination with elemental analysis for determining the authenticity and geographical origin of food. First, the theoretical background will be presented and will highlight state-of-the-art analytical techniques and data interpretation tools. It will also stress the need for standardised protocols so that data can be compared with existing data. Second, selected applications will be given, including major food groups and how these techniques can be used to detect fraud. For example, the discrimination of olive oil according to the country of origin was much improved by including compound-specific (13C) isotope analysis of fatty acids. The isotope approach can be used to differentiate aroma compounds of natural versus synthetic origin of fruits and vanilla. Indeed, cheap, synthetic, petroleum-derived compounds have different isotope values than their costly natural counterparts. Amino acids can also be used to discriminate between conventionally and organically grown plants, such as wheat and similar commodities. All these examples will be put in the context of relevant projects and infrastructure, including ISO-FOOD, REALMed, METROFOOD-RI, and FNS-Cloud.

KEY WORDS: authenticity; food; health; origin; stable isotopes

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Food allergy, the pitfalls of allergen analysis, and the way forward

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IgE-mediated food allergy is a significant public health issue. Quantitative food allergen risk assessment (QRA) is gaining increasing traction and recent developments will be described. One output of allergen QRA can be a critical allergen protein concentration, [AP]crit used in risk management. Analysis against [AP]crit can influence whether a food product is safe, precautionary allergen labelling (PAL), e.g. "may contain..." should be applied or if a product recall may be necessary. The proliferation of PAL is widely regarded as sub-optimal for people with allergies and food manufacturers alike. Applications of allergen analysis also include food chain safety surveillance and validation of cleaning protocols. However owing to the nature of allergens as large protein molecules analysis is challenging and may be sub-optimal, examples of which will be described. The promise of QRA may be lost if allergen quantitation cannot be improved. Many have called for the normalisation of allergen analytical data through reference materials. The production, characterisation and applications of a multi-allergen reference material kit will be described briefly and the future steps required to secure international agreement on allergen analysis will be discussed.

KEY WORDS: allergen; analysis; quantitative; reference material

The agrifood sector: challenges, opportunities, and role of research infrastructures

C. Zoani**

ENEA – Italian National Agency for New Technologies, Energy and Sustainable Economic Development – Department for Sustainability – Biotechnology and Agroindustry Division (SSPT-BIOAG), Rome, Italy

The agrifood sector is of essential importance in Europe, representing a key sector for the economy and trade, for the health and safety of consumers, and for the sustainability of production. Food Quality and Safety are concepts strictly interconnected to one another and with that of health, integrating into the broader concept of "food integrity" to indicate healthy, nutritious, safe, tasty, authentic, traceable foods, produced in a sustainable, ethical, and environmentally friendly way. The current COVID-19 health emergency suggests the need to apply an increasingly holistic and interdisciplinary approach, placing growing focus on the sustainability of agrifood systems and the application of an integrated supply chain approach, and also increasingly on the application of the "one health" concept, taking into account the indissoluble link between human health, animal health and environmental health and considering the impacts and close relations with agrifood systems, food production and consumption, and the environment. The COVID-19 pandemic is affecting food systems and all dimensions of food security globally: access to markets and the possibilities to obtain nutritious and quality food have been in some case limited, and any interruptions in the supply chain can lead to food losses and an increase in waste. Research infrastructures can provide a valuable contribution to secure food safety and human health, providing knowledge for expanding the urban agriculture's potential and food production systems and for driving changes towards a sustainable food system, as well as promoting awareness and supporting facilities and activities related to sustainable production and consumption, as well as to circular economy and industrial symbiosis. The METROFOOD-RI project, providing high-quality metrology services in food and nutrition, aims at promoting scientific excellence in the field of food quality and safety comprising an important cross-section of highly interdisciplinary and interconnected fields throughout the food value chain, including agrifood, sustainable development, food safety, quality, traceability and authenticity, environmental safety, and human health.

KEY WORDS: food integrity; one health; sustainability

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Food safety implications of climate change – the role of the World Health Organization (WHO)

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Climate change, including more frequent and extreme weather events, affects food security and increases foodborne and zoonotic disease risks. Changes in temperature, rainfall and sea levels affect local agriculture and food production, and impact the persistence and occurrence of bacteria, viruses, parasites, harmful algae, fungi and their vectors. Climate change constitutes a relevant driver of emerging risks to food and feed safety, plant and animal health and nutrition. As such, climate change increases the risk of foodborne diseases and food poisoning and consequently places public health at risk. No one sector, country or group of stakeholders alone can address the impact of climate change on food safety. Food and feed safety risk management requires a holistic and multidisciplinary 'One Health' approach where multiple sectors communicate and work together to achieve better public health outcomes. WHO, together with partners in the agriculture, veterinary, environment and other relevant sectors, supports Member States to prepare for and respond to effects of climate change. This includes technical support to enhance emergency preparedness and response to better manage the threat of increasing foodborne risks associated with climate change. WHO also provides science-based risk assessments and relevant information underpinning evidence on emerging food safety risks as the basis for countries to develop and implement comprehensive and effective food safety policies, standards and other interventions using the One Health approach.

KEY WORDS: foodborne disease; One Health; public health; risk assessment

Deep tech in health care; biohacking, and transhumanism

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In today's world, technology plays an important role in every industry as well as in health care. Out of all of the industries that technology plays a crucial role in, healthcare is definitely one of the most important. This merger is responsible for improving and saving countless lives all around the world. Advancements in medical technology have allowed physicians to better diagnose and treat their patients since the beginning of the professional practice of medicine. Thanks to the continuous development of technology in the medical field, countless lives have been saved and the overall quality of life continues to improve over time. Medical technology is a broad field where innovation plays a crucial role in sustaining health. Areas like biotechnology, pharmaceuticals, information technology, the development of medical devices and equipment, and more have all made significant contributions to improving the health of people all around the world. Mind-reading exoskeletons, digital tattoos, 3D printed drugs, RFID implants for recreational purposes are mind blowing innovations that are coming to medicine and healthcare almost every single day. Deep tech refers to companies which by funding a scientific discovery or meaningful engineering innovation solve problems through meaningful scientific or technological developments. Transhumanism is a philosophical movement that advocates for the transformation of the human condition by developing and making widely available sophisticated technologies to greatly enhance human intellect and physiology. Biohacking is a fairly new practice that could lead to major changes in our lives. Biohacking is an attempt to manipulate the brain and body in order to optimize performance outside the realm of traditional medicine. Biohacking is the practice of putting RFID chip implants, sensors, magnets, and other tech implants on or under the skin to enhance the performance of a human body and the quality of life. In the healthcare industry, the dependence on medical technology cannot be overstated, and as a result of the development of these brilliant innovations, healthcare practitioners can continue to find ways to improve their practice from better diagnosis and surgical procedures to improved patient care.

KEY WORDS: foodborne disease; one health; public health; risk assessment

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Testing in the digital world

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The recent evolution of the world has caused an extraordinary acceleration of the digital transformation process, for which no one was truly prepared. This new reality, which we call the "new normal" is here to stay and forced society, companies, and governance to rethink the model of economic activities in a new context. In this transition process, concepts that were preparing to enter daily life, became immediate, and the impact of changes in work, commercial relations and communication, added to the ongoing revolution focused on the implementation of Industry 4.0, of the EC guidelines for the *European Green Deal* plan and the *European Digital Single Market*, created a huge challenge for the conformity assessment activity, the core business of the TIC Sector (Testing, Inspection, and Certification). The challenging digital future can be observed under different perspectives: Emerging Technologies & New Materials; Communication & Data Science; Digital Transformation Policy; Remote Activities & Work Relations; Global Market vs. Local Markets; and Social Relations. For the TIC Sector, a strategy needs to be established to face the new type of demands and to be able to provide confidence and safety fulfilling today's expectations. In the "new thinking" concept, the role of laboratory key elements for the future must be evaluated; namely, the need for physical infrastructures, new skills for jobs, and the impact of AI on the organizations. In the new normal, however, traditional testing will remain having impact in economy as high values such as wooden toys, food products and others using traditional manufacturing processes, but laboratories will also need to adapt to new products, processes, and characteristics (e.g., nanotechnology and additive manufacturing) and testing will need to be applied to algorithms and other features of data science. Today, laboratories are getting ready to take the next digital step.

KEY WORDS: data science; economic activity; laboratory; TIC sector

Short supply chain and food safety

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The EU's rural development regulation (1305/2013) defines a "short supply chain" as a supply chain involving a limited number of economic operators, committed to cooperation, local economic development, and close geographical and social relations between food producers, processors, and consumers. Consumers continuously increase their demand on food safety and its functionality, product diversity, packaging quality, and the quality of services and products. Additionally, the issue of environmental protection and the economy of sustainable development has become imperative. The advantages of a short supply chain are numerous in the context of food quality and food safety: food comes to the customer fresh with better organoleptic properties; no need for long storage; transport to the customer is no longer than a few tens of kilometres; food does not need to be further treated in order to retain its characteristics by longer storage; the possibility of spoilage of food is reduced, and consequently the reduction of food waste is achieved; the loss of content of certain micronutrients is largely prevented (e.g., vitamin C and chlorophyll); food traceability is fully transparent and accessible to the consumers. This and many more advantages build customer confidence because the consumers recognize foods of higher quality and they recognize it as "healthy" food or domestic product. In addition to this perception, there is a sense of belonging to the local community or region.

KEY WORDS: domestic product, food quality; packaging quality; product diversity; traceability

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SPONSORED LECTURE

OrbitrapTM ExplorisTM – the next generation of Thermo ScientificTM Hybrid OrbitrapTM high resolution mass spectrometers for food and feed analysis

L. Milivojevic*

Thermo Fisher Scientific, Waltham, USA

Short introduction of the new Thermo Scientific™ hybrid high resolution mass spectrometers, changes, new features compared to the Exactive™ platform and their use for the analysis of food and feed. With high resolution mass spectrometry we get the power to see more, get results faster, and expand the scope of the analysis easier. Consequently we have higher confidence in the results which is a base for ensuring that the products reaching consumers are safe and of specified quality. Examples from the multiresidue pesticide analysis of olive oil and large panel mycotoxin analysis of corn feed show excellent quantitation results and high quality data that allows for easy compound confirmation from the existing compounds and custom library building for potential new compounds. Samples acquired with the DIA (Data Independent Acquisition) mode retain the qualitative information of the analysis which besides confirmation also allow retrospective analysis at any time and easy addition of the new compounds of interest to the existing methods. High quality high resolution mass spectrometry as such can offer a new level of confidence in day to day analysis for various compound groups in different matrices and can simplify otherwise laborious method development or expansion of methods.

KEY WORDS: food quality; HRMS; LC-MS; mass spectrometry; qual/quan workflow

SPONSORED LECTURE

Precise testing of pesticides in food using the SCIEX Triple Quad 7500 LC-MS/MS system – QTRAP® Ready, highly sensitive analysis of multi-compound panels in various matrices for food regulations

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The intensive use of pesticides in agriculture has led to the need for rigorous and extensive use of analytical technologies to ensure that there is no impact on human populations. Depending on the class of compounds, this has been primarily achieved through liquid chromatography-tandem mass spectrometry (LC-MS/MS). Maximum residue limits (MRL) are set for regulated residues that define the highest level of a pesticide residue that is legally tolerated in food such that it is safe for consumers. Often these MRLs are set very low for some pesticides, to ensure highest safety, requiring very sensitive instrumentation to accurately quantify these compounds down to their MRL. Therefore LC-MS/MS solutions must be robust and sensitive to meet the needs of food testing labs. In this method, over 700 compounds have been analysed, covering required testing for numerous regions including Europe, North America, and various areas within Asia. This breadth of coverage of pesticides has been achieved in part due to the increased sensitivity observed when utilizing the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready relative to previous generation instruments. Improvements in both the generation of ions and the sampling of ions has resulted in significant sensitivity gains. For residue testing in food testing, this means that more analytes can be combined into a single analysis, complex matrices can be further diluted to reduce matrix effects, while still being able to achieve or exceed the necessary levels of sensitivity.

KEY WORDS: liquid chromatography; QuEChERS; ultra fast mass spectrometry

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SPONSORED LECTURE

How to analyse microplastics?

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Microplastics are any synthetic solid particle or polymeric matrix of plastic origin, with regular or irregular shape and with size ranging from 1 μ m to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water. Demand for global production of plastics remains high, 322 million tons of plastics are produced every year, a number that is expected to double over the next 20 years. Despite growing public and scientific attention, the true risk of microplastics to the environment and human health remains unclear. With the quantity of microplastics in the environment set to increase in the future, it is crucial to urgently put efforts toward an ecotoxicological risk assessment of microplastics using clear, scientific methods to obtain a unambiguous idea of the threat they may pose to humans. Standardization in microplastics testing methods will pave the way for concrete regulatory actions to follow. Agilent's innovative approach to developing new spectroscopy and chromatography solutions for microplastics testing has earned them multiple awards. Both techniques provide specific important information about a sample. For example, gas chromatography combined with mass spectrometry can provide information about the concentration of microplastics, whereas infrared spectroscopy can provide complementary information regarding parameters like the number of particles, size, shape and surface area. Generating both sets of information is important to generate a robust picture about the sample being tested.

KEY WORDS: chromatography; innovative approach; risk; solutions; spectroscopy

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New chocolate and vanilla-flavoured waffle products – sensory consumer tests

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A survey of consumer preferences and acceptance of new premium chocolate and vanilla-flavoured waffle products was performed on a group of 489 persons from four different populations with an even ratio of both genders, three age groups, and four education levels, wherever possible. The following methods were used: survey, a 9-point hedonistic scale, the JAR scale and a preference ranking test. The survey found that 91 % of all subjects, regardless of gender or population, consume waffle products. All four populations listed quality as "exceptionally important" when selecting products (78, 64, 70, and 80 %, respectively). Regardless of gender or population, consumers preferred the new products. For the premium chocolate waffle product produced with coconut oil in comparison to the old product, this difference was statistically significant only for all subjects, all male subjects, and the investigated staff population. For the premium vanilla-flavoured product containing coconut oil in comparison to the same product containing palm fat, consumer preferences also leaned towards the new product. This difference was statistically significant only for all subjects, all subjects grouped by gender, and for all population groups, with the exception of the student population. The average impression for individual sensory properties and overall impression for both new products was high, which should guarantee a strong placement on the market. The premium vanilla-flavoured product achieved higher grades for overall impression and individual impressions for sensory properties than the premium chocolate product. Female subjects also gave higher grades for average impression than male subjects.

KEY WORDS: consumer acceptance; consumer preference; premium chocolate product; premium vanilla-flavoured product; survey

Extra virgin olive oil's phenolic profile changes during a one-month simulated consumption

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Preserving extra virgin olive oil's (EVOO) chemical composition is essential to maintaining its beneficial health effects, which are mostly attributed to the abundant phenolic content. Indeed, the EFSA authorizes a health claim for olive oils that contain a minimum of 5 mg of phenols (hydroxytyrosol and its derivatives) per 20 g of oil, claiming them beneficial for the protection of blood lipids from oxidative stress. This study aimed to investigate the applicability of this claim during EVOO domestic consumption. For that purpose, three dark glass bottles of Buža cultivar EVOO were stored for one month at 21 ± 3 °C. During this period, 20 mL of EVOO was withdrawn daily from each bottle, simulating domestic consumption. The phenolic content in oils was analysed after 0, 7, 21, and 28 days of storage using the high performance liquid chromatography with diode array detection (HPLC-DAD) system. The simple phenols content remained unchanged during this period. However, the secoiridoid, phenolic acid and lignan content decreased already after the first week, but after which the concentrations remained unchanged for the next three weeks. The same outcome was observed for the total identified phenolic content. Although the secoiridoids content decreased from day 0 to 28 by 21.05 %, it remained sufficient to satisfy the health claim. The obtained results indicated that Buža EVOO consumed within one month under optimal domestic conditions preserved its beneficial phenolic content.

KEY WORDS: domestic consumption; extra virgin olive oil; health claim; HPLC-DAD analysis; phenols

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Validation of a multi-locus PCR HRM method for species identification in *Mytilus* genus with a food traceability purpose

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Reliable species identification methods are important to monitor compliance with labelling regulations. For seafood, Europe demands a label with the commercial designation and scientific name of the species (EU N°1379/2013). The fit for purpose of a 10 SNPs panel for *Mytilus* species identification (*M. chilensis, M. edulis, M. galloprovincialis*, and *M. trossulus*) based on high resolution melting (HRM) analysis was assessed by an internal validation process. The applicability established the scope of the method testing individuals with different processes and three DNA extraction methods. The specificity of primer pairs was evaluated *in silico* and *in vitro* using target and non-target species, determining false positive and false negative rates. The sensitivity was determined by LOD₆ and LOD₉₅ and the robustness evaluated the influence of small variations in the method parameters. Concordance of the results after the transference to a second laboratory was determined using Kappa statistics. The method was applicable to four species, fresh, frozen, and canned with brine and oil packing medium. False-positive and negative rates were zero. LOD₆ was 5 ng/ μ L and LOD₉₅ was between 5 and 1.25 ng/ μ L. The method was robust against small variations in DNA quality, annealing time and temperature, primer concentration, reaction volume and HRM kit. Standardized operating procedures (SOP), 220 samples, and the reference materials were transferred obtaining a high concordance in the inter-lab assay (Cohen's Kappa = 0.925, P <0.001). The method was suitable for the intended use and is ready to be applied in the seafood industry.

KEY WORDS: high resolution melting; identification method; inter-lab assay; sensitivity; standardized operating procedure

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Effect of sex on physical characteristics of wild boar meat

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The aim of this study was to determine the effect of sex on the physical characteristics of wild boar meat. Animals (n=85; 27 male and 56 female) were harvested during collective hunts in the period between November 2018 and January 2019 in the open state hunting ground "Prolom". Muscle samples (m. *psoas major*) were taken *post mortem*, vacuum-packed and stored at minus 20 °C until analyses. Physical characteristics (colour, pH, thawing loss, and cooking loss) of muscle samples were described according to Honikel (1998). Meat colour was measured using Minolta Chromameter CR-450 (Minolta Co. Ltd., Japan) with a 50-mm measurement area after 1 hour of blooming and the pH was measured using pH meter S2 Food Kit (Mettler-Toledo, Columbus, Ohio, USA). Statistical analyses were made using SAS Software V9.4 (Cary, NC, USA). Average values of meat colour parameters (L*, a*, b*) were as follows: 37.97±0.38, 21.47±0.28, 9.33±0.21. Differences in meat colour parameters between males and females were minor (38.32±0.48 vs. 37.78±0.49; 21.16±0.37 vs. 21.69±0.37; 9.39±0.31 vs. 9.38±0.27). Average pH was 6.22±0.32 and was lower in males (p>0.05) compared to females (5.73±0.04 vs. 6.44±0.48). Average thawing loss was 9.58±0.44 % and was lower (p>0.05) in females than in males (9.44±0.52 vs. 10.19±0.77). Average cooking loss was 17.90±0.62 and was higher (p<0.05) in males than in females (19.83±1.06 vs. 17.15±0.73). Sex showed minor effect on the analysed physical characteristics of wild boar meat obtained from collective hunting.

KEY WORDS: colour; cooking loss; pH; Sus scrofa; thawing loss

Food waste management in a sample of Croatian high school dormitories

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With the gradual development of society, from agrarian to industrial, the evolution of technology set in, and with it the ever increasing reliance on natural resources. The world's population keeps growing and natural resources cannot replenish at the same rate at which they are exploited. Over seven billion people inhabit the planet and all, regardless of where they live, use natural resources to fulfill their needs. In doing so, they generate large quantities of waste. A vital component of a well-rounded system of waste management are efforts to reduce the amount of waste originating from food. Man continuously produces food waste, both at home and at the workplace or school and even on the way to work or school. It results practically from all fields of human activity. This study focuses on a target group determined through preliminary research to generate significant amounts of food waste. A questionnaire was used to analyse the habits and attitudes of high school dormitories on food waste and its proper management. It was applied to a sample of nine high school dormitories located in Croatia. Based on the obtained results, we have concluded that the dormitories that have responded to the questionnaire understand the issue of excessive food waste and that they strive to reduce their amounts of food waste as much as possible through education and measures proposed by the HACCP system. The largest limitation of this study was the fact that only 9 of the 58 dormitories to which the questionnaires was sent were returned. This clearly points to the fact that most of the studied dormitories do not realise the importance of reducing waste, sorting waste, and educating their staff and pupils on proper management.

KEY WORDS: eco-awareness; education; HACCP system; questionnaire

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Laboratory tests – scope of accreditation and test reports

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In Croatia, more than 30 laboratories have been accredited for food and animal feed testing. During 2019/2020, most of the laboratories implemented a management system according to the new HRN EN ISO/IEC 17025:2017. For part of the activities in the area of food and animal feed testing, a flexible scope of accreditation is particularly applicable. The final product of the testing laboratory is the test report. The content of the test report has to fulfill requirements of HRN EN ISO/IEC 17025:2017. Laboratories perform testing based on customer requests. Prior to testing, the customer submits a request to the laboratory defining the required scope of testing, need for expression of measurement uncertainty, and statement of conformity according to a defined specification. ILAC G8 gives guidelines on decision rules and statements of conformity. The results from the test report refer only to the sample tested. Testing is an evaluation activity outsourced by certification (control) bodies for certification of products in organic production, accredited according to HRN EN ISO/IEC 17065. Control bodies are responsible for sampling for organic production and delivery of samples to the testing laboratory. For the purpose of making decisions on certification and control activities, the certification (control) body evaluates the results from the performed testing, stated in the test reports. The experience from accreditation assessments triggers discussions on responsibilities for defining the scope of required tests, the content of the test report, the flexible scope of accreditation, and issuing statements of conformity.

KEY WORDS: Croatian Accreditation Agency; flexible scope; HRN EN ISO/IEC 17025; HRN EN ISO/IEC 17065; statement of conformity

Survey of salt content in cheese on the market in the City of Novi Sad, Serbia

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There is strong evidence that an excess of salt intake increases blood pressure and consequently, the risk of cardiovascular diseases. Salt in cheese serves as a preservative and contributes to flavour and quality. The Serbian population traditionally consumes considerable quantities of salted cheese varieties, which significantly contributes to the total daily salt intake. The aim of this study was to investigate the salt content of cheese sold in supermarkets in the city of Novi Sad, Serbia. Sodium-chloride in 54 samples of cheese, sampled in supermarkets in Novi Sad was determined, using a potentiometric method. The mean salt content was 1.84±1.23 g/100 g, ranging from a minimum of 0.18 and a maximum of 6.51/100 g. There was a large variation between different categories of cheese, and within the same type of cheese. Samples were categorised as: hard and half-hard cheeses, processed, "cottage-like" and "soft" cheeses, spread cheeses, "feta-like" cheeses and plant fat analogues. The average determined sodium chloride content per 100 g of controlled cheeses categories was: 2.04, 1.41, 1.90, 1.78, 0.90, 2.26, and 2.01 g, respectively. Calculated on the average portion (30 g), the average salt content contributed with 11 % of the recommended daily salt intake for an adult 5 g/day. Fresh spred cheeses had the lowest average and maximum value of salt content in 100 g (0.9 and 1.11 g/100 g). Hard and half-hard cheeses had the highest average and maximal values (2.04 and 6.51 g/100 g), as well as the largest variety within the category (0.66-6.51 g/100 g). Regarding provisions for food labeling of the Food Standard Agency of the United Kingdom, the average value of salt content in all categories could be characterized as high (≥1.5 %), except for processed and spreadable cheeses which could be categorized as medium (0.3-1.5 %). It is essential to draft a national program and set national goals and recommendations on salt intake reduction. Multisectoral cooperation in achieving these goals is required.

KEY WORDS: cardiovascular diseases; dietary; monitoring; salt intake reduction; sodium

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Food consumption data – importance and possibilities

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National food consumption surveys are important policy instruments that could monitor the food consumption of a certain population. Food consumption data collected on an individual level, based on EU standardised methodology, represent the most possible accurate assessment of consumed food. However, the collection and handling of such detailed information require tremendous efforts. Collected data can be analysed and structured on different formats in order to fit for purposes of various users. Croatia has food consumption data on the adult population, available on the EFSA and EC websites. There are two on-going surveys, on infants and children and on the adolescent, adult and elderly population. Both surveys are headed by the Croatian Agency for Agriculture and Food (HAPIH) with respectable partners such as the Faculty of Food Technology in Osijek, Faculty of Food Technology and Biotechnology in Zagreb, and Croatian Institute of Public Health. The importance and possibilities of these data are recognized on many different levels, like identifying significant relationships between food group consumption and socio-economic, demographic, and lifestyle for risk assessors in order to access exposure to hazards related to food; assessing the impact of energy and nutrient intake distribution of population; food consumption patterns are relevant to disease risk; form the basis for dietary intake recommendations for a specific population; form the basis of market research for food industry; form one of the basis for policy makers for national regulations, taxes on some food categories, etc. In the coming years, many activities and obligations related to the European Green Deal to promote a shift towards to healthy, sustainable diets will be put into motion. Food consumption data originating from national surveys will play many important roles in these future activities.

KEY WORDS: Croatian dietary survey; European Green Deal; exposure assessment; food consumption survey; sustainable diets

Organic versus functional food

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Organic and functional foods have an established place in the food market. In the European Union, both are regulated by food-related regulations, with foods produced by organic production also regulated by a regulatory framework for organic production. The term functional food first appeared a few decades ago when people began to pay more attention to foods that have a positive impact on human health. Functional foods as a new food category originated in Japan (legal definition in Japan-Foods for Specified Health Use FOSHU), from where the concept spread to Europe and America. The European Commission defines functional food as one that, in addition to having nutritional value, has a positive effect on one or more targeted functions in the body as well as reducing the risk of disease. The group of functional foods includes: 1) foods fortified with specific nutrients (e.g., iron-fortified, calcium-fortified, Omega-fortified, etc.); 2) modified foods promoted for specific health benefit claims (e.g., reduce cholesterol, lower blood pressure, etc.); 3) natural foods with specific nutrient/disease associations (e.g., tomatoes – lycopene, flaxseed – Omega-3, oats – b-glucan fiber, garlic – sulfur, dairy – calcium, fish – Omega-3, beef – conjugated linoleic acid, and so on). While functional foods combine and focus on nutrients, organically produced food's primary targets are the environmental benefits. Consumers of organic products believe that organic food has positive effects on health and do not have a positive attitude about buying functional foods. For both consumer groups, health is important, but the groups differ in how they try to achieve better health and well-being.

KEY WORDS: consumers; differences; human health; organic production; perception

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Contribution of aflatoxin M₁ from cheese to the burden of hepatocellular carcinoma in Vojvodina (Serbia)

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The health risk of dietary exposure to genotoxic carcinogen aflatoxin M₁ (AFM₂) was assessed based on the enzyme-linked immunosorbent assay (ELISA)/high performance liquid chromatography (HPLC) analysis of a total of 56 cheese samples purchased on the market of Novi Sad in 2019-2020. Risk was characterised by means of margin of exposure (MoE), based on benchmark dose for incidence of hepatocellular carcinoma, and carcinogenic risk, considering prevalence of hepatitis B and C viruses. Range of MoE associated to mean exposure of population groups to AFM₁ was 84792-22235, whereas on the 95th percentile MoE ranged from 24098 to 6319 (higher values for adults and lower for preschool children). Preferable consumption of cheeses produced in Serbia would result in a lower MoE (64540-16924 and 15886-4166). MoE values were below the limit of 10000 for preschool children on the 95th percentile of exposure, or in case of exclusive consumers of Serbian cheeses, for preschool children on the 90th percentile and for 7-10 y children on the 95th percentile, thus indicating unacceptable risk. Assessment of carcinogenic risk, taking into account life expectancy of 70 years, revealed up to 0.11 and 0.39 new cases of hepatocellular carcinoma on 100,000 people, related to mean and 95th percentile of exposure, respectively. Exclusive consumption of domestic cheeses would cause a rise of new carcinoma cases up to 0.15 (mean) and 0.59 (95th percentile). Although the estimated carcinogenic risk was below the level of concern of one new cancer case in 100,000 people, it should be emphasized that the study was focused on cheese, whereas other dietary sources of AFM, were not considered.

KEY WORDS: ELISA; HPLC; margin of exposure; public health; risk assessment

The main objectives of Project P-K.K.05.1.1.02.0023: Investigation of the impact of climate change on the development of molds, mycotoxins, and grain quality with proposed measures

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Frequent weather disasters, heavy rainfall, extremely high, and/or low temperatures result in the development of moulds and mycotoxins on various crops that affect the development of diseases in plants, animals and humans, and contribute to the reduction of grain quality. In the Republic of Croatia, there is currently no systematic monitoring of the occurrence of mould and mycotoxins from the field to the final product, so it is extremely important to take measures to protect crops using pre-harvest, harvest, and storage measures. Proper variety selection for individual climatic areas is one of the essential factors for successful yield and safe production. The aim of this project is to isolate and identify all moulds and mycotoxins that will develop on cereals during their growth and harvest or immediately after harvest and in warehouses. Sampling will be carried out in northern, central, and eastern Croatia. According to the obtained data, an algorithm will be established to assess the impact of climate change on the occurrence of molds and mycotoxins, as well as on the nutritional composition of cereals. Models with guidelines for dealing with different climatic situations will benefit the agricultural sector in order to approach the protection of cereals from possible weather conditions in a timely and proper manner. The results of this project will provide insight into the food safety of cereals as the basic food of every food pyramid, in order to protect human health and prevent the development of chronic diseases.

KEY WORDS: cereals; food safety; health; monitoring; safe production

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The regulatory framework for mycotoxins and plant toxins in the service of health protection

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The importance of healthy food in our everyday life is unquestionable. Advances in food technology, globalization of trade but also climate changes and new eating habits provide new opportunities for food production, processing, and distribution. The food we consume can contain potentially harmful substances, be they natural ingredients of food or contaminants. Mycotoxins and plant toxins as natural contaminants, produced by fungi and plants respectively, generally have a negative impact on the quality of food and may represent a risk to human health by reducing people's access to healthy and sufficient food. To protect public health, one of the fundamental demands is to keep these toxins in food at toxicologically acceptable levels. Regulation (EC) No 1881/2006 and its amendments stipulate the legal limits on mycotoxins and plant toxins in food for the EU. Maximum levels (MLs) and/or guidance levels were established for aflatoxins, ochratoxin A, citrinin, patulin, major *Fusarium* toxins (deoxynivalenol, zearalenone, fumonisins, T2 and HT2-toxins), and ergot sclerotia. Discussions on the setting of maximum levels for ergot alkaloids in food have been finalised. Regarding plant toxins, maximum levels for erucic acid, hydrocyanic acid, and tropane alkaloids in certain foodstuffs exist. For pyrrolizidine alkaloids draft legislation is in the final stage of approval. Regulation of acetylated and glucoside forms of deoxynivalenol, Alternaria toxins and opium alkaloids is being considered and the list is not exhaustive. Also, in current legislation, changes are anticipated to MLs for foodstuffs not yet covered as well as a revision of existing MLs.

KEY WORDS: food quality; food safety; legislation; maximum level; natural toxins

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Measurements of ionising and non-ionising electromagnetic radiation generated by a high voltage plasma discharge device

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Electrons in plasma discharge may reach temperatures close to 10⁵ K, but due to their low mass, their kinetic energy is not strong enough to significantly heat neutrals and ions which can be at room temperature. As such, plasma technology is actively researched as an alternative non-thermal food preservation technology. In our experiment, high voltage plasma device and argon gas were used for the generation of plasma in a glass reactor containing distilled water and two stainless steel electrodes in point to plane orientation. Short high voltage pulses (100 ns) at voltage ranging from 30-60 kV were applied to the electrodes, one of which was used for gas injection. Operating at such a high frequency regime (100 ns pulses) and high voltages, we measured both ionising (*bremsstrahlung*) radiation and non-ionising radiation. Measurements presented many challenges. Due to high electromagnetic interference, not one of the electronic dosimeters worked correctly. A decision was made to employ passive dosimeters that were strategically placed at different locations in the reactor's vicinity. Dosimeters were exposed for 6 h and 28 min. No statistically significant increase in dose was measured. Investigation of non-ionising radiation was carried out with Narda SRM 3006 and Narda EPH-50 field strength analysers for measurements of high and low frequency components, respectively. Due to the chaotic nature of the generated electromagnetic interference and sweeping nature of the analysers, non-ionising radiation was measured unsuccessfully. However, future plans and recommendations for measurement of such fields based on obtained experience were given.

KEY WORDS: food preservation; dosimetry; ionising radiation; non-ionising radiation; plasma discharge

Is "5G" a food contaminant? Is electromagnetic contamination of food plausible?

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Modern physics and biogenetics scientific research of radiation covering entire electromagnetic (EM) spectrum frequencies are mainly focused on delivered energy interactions with living matter using animal models and recently, artificial intelligence computational modelling and simulations. Results are not easily transposed to a real absorption human model. One of the physical reasons is the fact that information and communications technology (ICT) EM frequencies lie well below (or above) visible light frequencies natural to humans to which they are constantly exposed, whilst ICT are not. This, additional, unnatural global non-stop energy varying exposure to non-ionizing radiation causes a lot of public controversy. Food is not excluded from that artificial exposure. Microwave, ultraviolet, and infrared irradiation; all of these are parts of the EM spectrum and are used to treat food. But, are these exposures of food a contamination? On the other hand, "food irradiation" for conservation purpose is an ancient idea. The example of fig drying will be discussed and linked to an ICT energy spectrum exposure of food. The absence of chemical compounds and additives was and still is one of the major highlighted advantages of food irradiation technology today. Halfhearted recognition of food irradiation technologies from EU countries health regulators not issuing the relevant permits for their use based on the populistic public concern debates were the major reason inhibiting the technology to be used more broadly. The educational aspect will also be pointed out. Some new "artificial" social concerns based on ignorance, like "5G" social media madness, will be discussed.

KEY WORDS: electromagnetic radiation; exposure; food irradiation; food preservation; sun-dry foods

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Microbiota of spontaneously fermented game meat sausages as a pool of genes encoding for antibiotic resistance and biogenic amines

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Spontaneously fermented game meat sausages produced by traditional techniques are complex microbial ecosystems that can be sources of beneficial or spoilage and pathogenic microorganisms. This study aimed to investigate the fate of indigenous microbiota of spontaneously fermented game meat sausages in order to estimate microbiological hazards as well as to identify and preserve the microbial pool that could serve as a source of strains relevant for standardization of sausages production. Three wild boar and three dear meat sausages produced by five different manufacturers following traditional techniques were physicochemically and microbiologically investigated at different time points of their production. Special focus was placed on the content of biogenic amines in ripened sausages as well as the presence of genes encoding for biogenic amines and antibiotic resistance among indigenous microbiota. The histamine content was below 5.0 mg/kg for all sausages. The content of tyramine, however, varied between sausages and was between 47.3±6.6 mg/kg and 219.0±13.0 mg/kg. Of all the isolated and identified lactic acid bacteria (LAB), 26.32 % harbored the gene encoding for a tyrosine decarboxylase (tdc) and no other biogenic amines genes were detected. It was also found that 20.80 % of all LAB strains were resistant to at least one antibiotic (kanamycin, gentamicin, erythromycin or clindamycin). Due to the elevated number of presumptive pathogens (*E. coli, Enterobacteriaceae, B. cereus* group, coliforms) in ready-to-eat sausages, 33.33 % of products can be considered as inappropriate for human consumption.

KEY WORDS: indigenous microbiota; lactic acid bacteria; traditional meat production; tyrosine decarboxylase (tdc)

Oral allergy syndrome – rare diagnosis but common illness?

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The oral allergy syndrome (OAS) was first described in 1987 as part of the pollen-food allergy syndrome (PFAS). It is an IgE-mediated hypersensitivity with a typical clinical image of itching and swelling of the lips, palate, and tongue after the consumption of fresh vegetables and fruit. The aim of this article was to review current available literature regarding the etiology, pathogenesis, epidemiology, clinical picture, and possible treatments for oral allergy syndrome. Literature search in PubMed, Google Scholar, and Hrčak databases was conducted using keywords: oral allergy syndrome, OAS, pollen food allergy syndrome and food anaphylaxis. The fact that OAS mostly occurs in patients with a history of seasonal allergic rhinitis led to the assumption that a pollen contamination of food could be the cause. Further research made clear that the real cause is a cross-reactivity between aeroallergens and food allergens. For example, similar antigenic determinants that trigger allergic reactions have been found in birch pollen and apple fruit. Most of the proteins that cause an allergic reaction in consumed food are thermolabile, which explains the majority of symptoms being related to the upper gastrointestinal tract. Other thermostable proteins can cause rare but severe systemic reactions such as anaphylactic shock. Non-awareness of possible OAS can cause confusion in pediatricians and general practitioners leading to unnecessary alergologic testing, resulting in false negative results or avoidance of fruit and vegetable consumption. Thermic processing of food before consumption and antihistamines could be effective in the prevention and treatment of OAS.

KEY WORDS: food allergy; food anaphylaxis; OAS; pollen food allergy syndrome

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How much BPA do we eat and drink?

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Bisphenol A (BPA) is used as an intermediate in the production of polycarbonate plastics and epoxy resins. Although BPA is considered a ubiquitous environmental pollutant, BPA has raised concern regarding adverse human health effects. The aim of this article was to investigate human BPA exposure through food and beverages. A literature search was conducted combining the keyword BPA with keywords food, water, air, soil, health in PubMed, Science Direct, Web of Science, Scopus, and Google Scholar databases, covering both the area of environmental pollutants and influence on health. Food for human consumption is contaminated both during growth and cultivation, through contaminated soil and water as well as processing and packaging due to the materials containing BPA. Most of BPA migrates from packaging into liquid or solid phase showing that migration from PET bottles ranges from 2.89 to 38.9 ng/L, from PVC pipes between 0.01 and 28.83 ng/L, and from can coating between 21.86 and 1858.71 μg/kg. Concentrations of BPA in fruit and vegetables range from 18.3 to 128.9 μg/kg, in honey from <LOQ to 107 ng/g, in meat from not detected (in raw meat) to 21.3 ng/g (for canned meat), in milk from 33.8 to 127.2 μg/kg and in infant formulas between 0.003 and 0.375 μg/g. Current EFSA and EU legislative recommends a tolerable daily intake of 0.004 mg/kg body weight/day and migration limit for food contact materials of 0.4 mg/L. Researchers' awareness of the harmful effects of BPA has led to the production of alternative materials as well as to the gradual reduction and elimination of the use of BPA-containing materials.

KEY WORDS: Bisphenol A; food; human health; legislation; water

Potential implementation of autochtonous yeasts in the bakery industry

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Yeast strains were isolated from several varieties of grapes, with the original intent to evaluate their winemaking fermentative capabilities in tandem with their potential implication in the bakery industry. A total of 30 samples following isolation were detected and each isolate was subjected to microbiological assays, including optimum growth conditions, osmotolerance, fermentative capacity, and growth kinetics. Furthermore, the strains were characterized on a morphological, microscopic, physiological, and biochemical level using tests such as ethanol tolerance, saccharose assimilation, D-trehalose assimilation, xylitol assimilation, and D-lactate assimilation tests among others. The reasults revealed that all 30 strains belong to the genus *Saccharomycetes* and the six strains V134, V130, V120, V113, V90, and V79, isolated from Merlot, Vranec, Smederevka, and Chardonnay grapes showed the best ability as leavening agents in bread-making tests. V90 showed up to 15 % ethanol tolerance, while others tolerated 12 %. The six leading strains demonstrated osmotolerance up to 50 % glucose, as well as outstanding fermentative capabilities. These strains may contribute to the production of geolocal industrial bakery products as well as local homemade bread. This study has shown that autochtonous yeasts isolated from grapes have high potential impact in the bakery industry and that native yeast strains could be used effectively as baker's yeast available for public consumption.

KEY WORDS: autochtonous strains; bread; isolation; microbiological assay; wine yeasts

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Nutritional composition of primary school meals contributes to climate impact

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Schools are known as major consumers of natural resources and generators of greenhouse gas emissions (GHGEs). Overall 1/3 of GHGEs are related to dietary patterns where at the same time food with high climate impact has lower nutritional quality and negative impact on human health. Therefore, the aim of this study was to estimate the climate impact of the nutritional composition of school meals. The nutritional composition of 6859 meals (2379 breakfasts, 2377 lunches, and 2103 snacks) was analysed from annual menus of 14 primary schools in Zagreb City. The impact was estimated using Sustainable Nutrient Rich Food Index (SNFR) which was related to GHGEs and $\rm CO_2$ production through the equation GHGEs($\rm log_{10}CO_2eq/100~g)$ =(6.25-SNRF)/2.45. Kruskal-Wallis test was used to investigate differences between schools. SNRF significantly differed across schools, and on average SNRF of breakfasts (p<0.001) ranged from -0.46 to 0.20, of lunches (p<0.001) from -0.04 to 0.37 and of snacks (p<0.001) from -0.20 to 0.20. Accordingly, $\rm CO_2$ production differed between schools in breakfasts (p<0.001), lunches (p<0.001) and snacks (p<0.001) and varied from 302 to 622 $\rm CO_2eq/100~g$, 257 to 380 $\rm CO_2eq/100~g$ and 316 to 724 $\rm CO_2eq/100~g$, respectively. There were 0 to 10 % of meals among schools estimated with SNRF \geq 1, which implies their higher nutriient density and lower $\rm CO_2$ production. Overall, from the results it can be assumed that school meals were lower in nutritive quality, which causes higher $\rm CO_2$ production. New meals should be implemented in schools aimed at improving meal nutritional quality as well as sustainability of school supply chains.

KEY WORDS: greenhouse gas emissions; nutrient density; SNRF index; sustainable diet; sustainability

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Healthy diet and physiotherapy, what do they have in common?

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The principles of physiotherapy are based on movement sciences and aim to restore the function of multiple body systems. To manage a patient as a whole with a holistic approach, it is essential for physiotherapists to possess basic knowledge about the role of healthy diet and nutrition. Many conditions managed and seen by physiotherapists are directly affected by diet and nutrition; for example, a patient's diet has an effect on muscle function which is one of the key concerns in physical therapy practice. Around the world, we find ourselves facing global epidemics of obesity, Type 2 Diabetes and other predominantly diet-related diseases known as public health problems. These conditions can greatly be prevented by merely ingesting food with good nutrition values. Furthermore, it is important to focus on avoiding packaged and processed foods and opting for more fresh ingredients whenever possible. According to the World Health Organization, "Nutrition is the intake of food considered in relation to the body's dietary needs. Good nutrition – an adequate, well-balanced diet combined with regular physical activity – is a cornerstone of good health. Poor nutrition can lead to reduced immunity, increased susceptibility to disease, impaired physical and mental development and reduced productivity".

KEY WORDS: disease; nutrition; physical therapy; public health; well-balanced diet

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Perception of food safety and knowledge about food safety in employees of student canteens in the city of Zagreb

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Employees play a significant role in the occurrence of food-borne illnesses. Proper food handling contributes to food safety and therefore employees must be trained. In addition, the overall organisation should be such that there is a positive atmosphere at all levels of the company with regard to food safety. The basis is an established prerequisite program, good infrastructure, and appropriate (hygienic) condition of the equipment and processing plant. A survey on atmosphere (perception) and knowledge of food handlers about food safety was conducted in 13 student canteens in Zagreb during 2019. Atmosphere and knowledge were assessed by completing a questionnaire according to previously published papers. The questionnaire, which checks the atmosphere, i.e. the employee's opinion on food safety in the facility where she/he is employed, included claims about food safety in the facility that relate to communication, importance of food safety, resources and organisation, risks and documentation, and cleanability of equipment. The questionnaire, which tested employees' knowledge, included questions on cross-contamination, cooling, heat treatment, and cleaning. A total of 371 employees participated. The atmosphere in the facilities was found to be generally positive. Employees were more critical of infrastructure than of organisation. Grinding and cutting devices, deep fryers, ovens were listed by many employees as un-cleanable. Knowledge of food safety was higher compared to the knowledge of employees from Hungary and Portugal. Respondents from Croatia showed greater knowledge of cleaning procedures. The biggest shortcomings in knowledge were related to heat treatment, which is consistent with results of other authors.

KEY WORDS: food-borne illnesses; food handlers; food safety knowledge; food safety perception; questionnaire

Preservation of functional fruit juices using hurdle technology

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Functional fruit juices have become a lucrative commodity on the world's markets due to a number of health benefits. Although juices can be enriched with various functional ingredients, they can also be obtained by blending different fruit juices with higher levels of functional compounds. However, the short shelf-life of these products fostered the developments of innovative non-thermal technologies capable of retaining nutritive and sensory qualities while still preserving food safety. In order to use the benefits of milder processing conditions from the abovementioned technologies, researchers applied the "hurdle" concept. This concept involves using different processing technologies at slighter operating conditions than when each is used alone. The major advantage of this approach is referred to as the synergistic effect of various mechanisms (e.g., cavitation, electroporation) resulting in a high-quality product. For instance, "hurdle" technology for preservation of juices commonly combines pulsed electric field (PEF), high hydrostatic pressure (HPP), and high-power ultrasound (HPU). The additional combining of any of these technologies with thermal processes under lower temperatures (<70 °C) is also used. In contrast to conventional thermal processing, this concept could provide the microbiological safety with minimal nutritional and sensory degradation. However, the operating parameters of each technology need optimization to achieve maximum effectiveness. Finally, it can be concluded that the positive outlook of "hurdle" technology as well as its application in the food industry can be expected to flourish even more in the future.

KEY WORDS: food safety; functional ingredient; high-power ultrasound; high-pressure processing; pulsed electric field

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In silico prediction of ADMET properties and ecotoxic profile of garlic sulfur compounds and their metabolites

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This study aimed to evaluate the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of garlic sulfur molecules by ADMET Predictor™ to elucidate their impact on the environment and health. Garlic has been used safely in cooking as a popular condiment or flavoring and traditionally for medicinal purposes. However, its excessive consumption can also cause problems (breath odor, allergic reactions, stomach disorders, a decrease of serum protein, anemia, bronchial asthma, contact dermatitis, inhibition of spermatogenesis, and damage to the intestinal lining). Also, excretion of drugs and food constituents or their metabolites in wastewater may negatively impact the reproduction and survival of many freshwater organisms and affect the ecosystem through altered fertility and increased genetic defects. In this study, the investigated compounds were revealed as biodegradable molecules with ecotoxic potential. Diallyl trisulfide (DATS) was predicted as the most toxic, and 2-propene-1-sulfonic acid (PSA) the least toxic to the fathead minnow and *Daphnia magna*, whereas the most and the least toxic to *Tettrahymena pyriformis*, were allyl mercaptan (AM) and ajoene, respectively. The highest scores of ADMET_Risk and mutagenicity were computed for allicin and ajoene. (SAMC), whereas carcinogenicity was predicted for almost all molecules, except for alliin and S-(allylmercapto)-L-cysteine (SAMC), whereas carcinogenicity was predicted only for 2-vinyl-4H-1,3-dithiine (2-VDT). The cytochrome (CYP) P450 mediated sulfoxidation and allylic hydroxylation were the main biotransformation pathways. CYP Risks, due to CYP1A2, were predicted for allicin, ajoene, and DATS. The chromosomal aberration for allicin, ajoene, SAMC, diallyl disulfide (DADS), DATS, allyl methyl disulfide (AMDS), and 3-VDT, was predicted, as was the reproductive toxicity for all molecules.

KEY WORDS: bioconcentration; biotransformation; ecotoxicity prediction test tools; safety; toxicity

Antiaggregatory effect of propolis and Ginkgo biloba

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Propolis and *Ginkgo biloba* extracts are often components of dietary supplements due to their beneficial effects on human health. *Ginkgo biloba* leaf extract contains phenolic acids, proanthocyanidins, flavonoids (e.g. isorhamnetin, kaempferol, quercetin), and terpenoids (ginkgolides, bilobalides). These extracts are part of dietary supplements used for enhancement of cognitive functions. Propolis is a resinous mixture and beneficial health effects such as antibacterial, antiviral, antitumoral, antiaggregatory, and hepatoprotective are mainly attributed to its polyphenols – flavonoids and phenolic acids. The objective of this study was to assess the antiaggregatory potential of *Ginkgo biloba* and propolis extract on the platelet aggregation. Impedance aggregometry was performed on the whole blood samples of healthy subjects at the Croatian Institute of Transfusion Medicine. Adenosine diphosphate (ADP) and thrombin receptor activator peptide (TRAP), a hexapeptide surrogate of thrombin, were used as a weak and strong agonist of aggregation. In the ADP induced aggregation assay, propolis showed antiaggregatory effect at a concentration of 10 mol/L. In the same test, the antiplatelet effect of *Ginkgo biloba* extract was absent. In the TRAP aggregation test, propolis showed an antiplatelet effect at a concentration of 20 mol/L as well as *Ginkgo biloba* extract. Combinations of propolis and ginkgo in a ratio of 1:25 and 1:50 showed an antiplatelet effect at 20 mol/L concentrations of extracts. Based on the results obtained, it can be concluded that the combination of propolis and *Ginkgo biloba* extracts in dietary supplements is justified from a pharmacodynamic viewpoint, as propolis and ginkgo have different mechanisms of action because they act on different signaling pathways of aggregation.

KEY WORDS: aggregation; health effects; impedance; platelets; polyphenols

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Variation in biological activities of curcumin and ferrocene-containing curcumin analogue in Hepa 1-6 cell culture

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Malignant transformation of normal hepatocytes into hepatocellular carcinoma occurs through repetitive liver cell injury manifesting as inflammation, oxidative DNA and protein damage, loss of cell cycle control, avoidance of apoptosis, and aberrant proliferation. Curcumin, a natural substance from curcuma displays antioxidant, anti-inflammatory, and anticarcinogenic properties. The aim of the study was to *in vitro* (against mouse hepatoma cells Hepa 1-6) investigate toxicity and antiproliferative effects of curcumin and ferrocene-containing curcumin analogue (FCA) in which one benzylidene group of curcumin is replaced by ferrocene. Curcumin and FCA were tested in the range 1-100 mol/L and cell viability was monitored by two *in vitro* cytotoxicity assays, while intracellular effects were assessed by cytofluorimetric analysis. Concentration-dependent growth inhibition in Hepa 1-6 cell line after 48 h curcumin exposure was confirmed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Trypan Blue (TB) methods with significant effects in concentrations ≥2.5 mol/L and ≥1 mol/L, respectively. In relation to curcumin-treated cells, the cell culture treated with FCA showed a higher viability and proliferation rate. The inhibitory effects of FCA were observed at concentrations ≥35 mol/L determined with MTT and ≥75 mol/L with TB assay. Cytofluorimetric analysis confirmed notably elevated apoptotic cell fraction (late apoptotic/dead cells – 73 %; early apoptotic cells – 19 %) after treatment with 100 mol/L curcumin vs. control/untreated cells, while FCA did not affect cell viability (level of apoptotic and necrotic cell death was low; early and late apoptosis – 14 %). These findings suggest that curcumin, but not FCA, alters cellular progression and may be effective in suppressing liver malignant progression.

KEY WORDS: apoptosis; bioorganometallic derivatives; cell viability; curcuminoids; nutrition

The importance of quality control of microbiological media in the microbiological control of food

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Laboratory diagnostics is important in quality control and food safety because it provides objective information about food quality and its impact on health. Based on the obtained results, it is possible to influence the promotion and popularization of a healthy diet. Proper preparation of microbiological media is an important step in conducting microbiological testing on food. They are prepared according to the manufacturer's instructions, taking into account the principles of good laboratory practice. The aim of this study was to verify the method by measuring the chemical characteristics (pH values) of prepared selective media [xylose lysine deoxycholate (XLD) agar] in the laboratory, which are used for the isolation of *Salmonella* spp. and *Shigella* spp. requirements of standards HRN EN ISO 11133:2014 and HRN EN 12322:2008. The pH values were determined using a moderate pH meter, 913 pH Meter. The choice of verification parameters depends on the type and purpose of a particular method. In this method, the repeatability and intermediate precision were used. Repeatability is the determination of the same series by the same analyst and the pH value was measured in parallel three times on the same day over a period of 20 working days. Intermediate precision is the determination of the same microbiological medium, but different series, by two analysts. The pH value was measured in parallel twice on the same day in a period of two months. The results of measuring the pH value of XLD agar show reliability in operation and indicate high accuracy of the obtained results. Relative standard deviation (RSD, %) in the amount of 0.371 for repeatability and RSD 0.368 for intermediate precision were calculated. According to the obtained results, the method fulfills the purpose of application and is suitable for procedures of preparing microbiological media.

KEY WORDS: food safety; intermediate precision; pH value; quality control; repeatability verification

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The opinion on health benefits of fermented foods: a survey among Slovenians

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Fermented foods are defined as foods or beverages produced through controlled microbial growth, and the conversion of food components through enzymatic action. In recent years, fermented foods have undergone a surge in popularity, mainly due to their proposed health benefits. Fermented foods are part of the Food Pyramid recommended by the Slovenian Institute of Public Health. We investigated the opinion of Slovenians about the health benefits of fermented foods. Subjects were 336 healthcare professionals were questioned (average age 29.1 years). As many as 84 % were female and 16 % male. We conducted an online survey (30 questions) using the 1KA application and prepared the analysis of the results in percentage shares of total samples. Three quarters of the respondents had already heard of fermented foods. More than 80 % also knew the correct definition of fermented foods. Most of the respondents recognised yeasts and lactic acid bacteria as the most common microorganisms that are involved in the fermentation process. The respondents agreed that they would consume more fermented had they more knowledge. Almost 80 % of the respondents agreed that fermented foods have a positive health effect on the digestive system, modulation of the intestinal microbiota, enhancement of the immune system, reduction of diarrhoea, lowering cholesterol, and others. Several studies have shown that consumption of fermented foods has a good effect on health and provides support to the intestinal microbiota, so their intake is undoubtedly important. Our results show that health promotion of fermented foods is important for public health.

KEY WORDS: beneficial microbes; fermentation; healthcare professionals; intestinal microbiota; public health

Fruit-drug interactions: which fruit should be avoided for patients taking certain therapies?

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Fruit is known to be universally promoted as a healthy source of many essential nutrients, fibres, vitamins, and minerals. It has been well-established that some phytochemicals found in fruit can be very beneficial for human health, thus many physicians encourage their patients to eat higher amounts of fruit. However, healthcare workers as well as patients should be mindful that some phytochemicals found in fruit can influence the pharmacological activity of certain drugs. Many studies have already shown that the intake of grapefruit can affect the bioavailability of more than 60 medications, including some antiarrhythmics, antibiotics, and antihistamines. However, there are many other types of fruits and fruit juices that can change drug bioavailability. Grapes and Sevilla oranges have similar metabolic effects to the grapefruit, resulting in interactions with numerous drugs such as vinblastine, fexofenadine, glibenclemida, atenolol, ciprofloxacine, ciclosporine, celiprolol, levofloxacin, and pravastatin. Tangerine is known to interfere with nifedipine and digoxin, while apples can modify the effects of fexofenadine. Studies have also shown that mangos should not be taken together with midazolam, diclofenac, chlorzoxazone or verapamil. The majority of case reports and reviews about warfarin and fruit interactions concluded that cranberry juice can cause international normalized ratio (INR) instability. Although there is a number of published studies suggesting that consumption of certain fruit and fruit juices could lead to interactions with certain drugs, scientific evidence remains scarce. Nevertheless, both prescribers as well as patients on long-term medication should be aware of the possible risks and be advised to moderate their intake of certain fruits.

KEY WORDS: drug bioavailability; fruit diet; fruit juice; health; long-term medication

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The representativeness and shelf-life of the newly-designed artificial reference materials for rancid and winey/vinegary defects in virgin olive oils

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The Panel Test method for the organoleptic assessment of virgin olive oils classifies oils into quality categories on the basis of the intensity of predominant defect and fruity attribute. The setting up of artificial and reproducible reference materials (RMs) is one of the objectives of the OLEUM project in order to increase the proficiency of the sensory panels involved in the Panel Test. In this context, two new RMs, for the aroma of winey-vinegary and rancid defects, have been formulated by adding specific volatiles responsible for negative sensory attributes to the refined olive oil. In this study, newly-designed artificial RMs were assessed by six panels to evaluate their i) representativeness, ii) panel detection thresholds, and iii) shelf-life after 3 and 6 months of storage. Both of the tested RMs were considered representative of the related defects. The panels' detection threshold for the winey-vinegary and rancid defect corresponded to a perceived intensity between 0.3 and 1.0, and 0.3 and 1.1, respectively. Considering the shelf life, there were no significant differences in the evaluation of winey-vinegary RM during the storage, although, in the case of rancid RM, there was a clear intensity increase with time. The utilization of artificial RMs could improve the method effectiveness by offering advantages such as reproducibility and unlimited availability.

KEY WORDS: aroma; OLEUM project; organoleptic assessment; panel test; quality categories

Acknowledgement: This work was developed in the context of the project OLEUM "Advanced solutions for assuring authenticity and quality of olive oil at global scale" funded by the European Commission within the Horizon 2020 Programme (2014-2020, GA no. 635690).

Eight dietary recommendations for the elderly

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Healthy ageing is associated with physiological, cognitive, social, and lifestyle changes that influence dietary intakes and nutritional status. The diet of the elderly should be harmonized with the general recommendations of a healthy diet. The poster proposes eight dietary recommendations for the elderly. Elderly people should eat a variety of healthy and fresh foods and drink plenty of fluids every day so they can preserve their vitality and health.

KEY WORDS: lifestyle; nutritional status; vitality

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Soil sampling within the project "Food Safety and Quality Center"

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As part of the scientific research project "Food Safety and Quality Center", sampling of food, water, and soil samples in the Republic of Croatia is planned for the needs of making an isotope map and information database of Croatian indigenous and organic food products. The paper presents the comprehensiveness of soil sampling. Soil sampling was conducted in four selected regions: eastern Croatia, central Croatia, northern littoral, and southern littoral. Each geographical area has a specific and unique soil composition and a unique ratio of oxygen and hydrogen isotopes as elements that form water, as well as carbon as a representative of the organic component. Soil sampling was carried out during 2019 and 2020 by an accredited method according to the standard HRN ISO 18400-102: 2017. At each location, one representative composite soil sample up to a depth of 30-50 cm was sampled, consisting of five subsamples. Approximately one kilogram of soil was taken with a polished probe made of stainless steel. The coordinates of the sampling locations were determined by GPS. A total of 120 soil samples were sampled. Immediately after sampling, the dry matter, electrical conductivity, and pH value were determined in the samples and the rest of the sample was stored for other analyses such as heavy metal determination, pesticide determination, and isotope analysis. From the obtained pH values, it was determined that the soils in the north and south littoral were mostly alkaline, in eastern Croatia slightly acidic to neutral, while in central Croatia they ranged from acidic to alkaline.

KEY WORDS: composite soil sample; dry matter; electrical conductivity; isotope map; pH value

Acknowledgement: The activity was carried out within the "Food Safety and Quality Center" (KK.01.1.1.02.0004) project funded by the European Regional Development Fund.

The importance of monitoring polyphenols in olive oil

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The Mediterranean diet is often associated with longevity and a low incidence of chronic and degenerative diseases. Epidemiological studies have shown a lower incidence of certain diseases in the Mediterranean area compared to other continental regions. Until recently, the therapeutic effects of olive oil were attributed exclusively to the high presence of monounsaturated fatty acids, mostly oleic, which makes up 70-80 % of the fatty acids in the oil. Since some other vegetable oils rich in monounsaturated fatty acids have not proven effects similar to olive oil, attention has been focused on less represented ingredients, such as polyphenols. Oleuropein is one of the most studied polyphenolic compounds from olive oil due to its strong antioxidant action and the role of the main antioxidant in defending the plant from attack by various pests. The aim of this study was to determine the amounts of oleuropein in olive oils from the Adriatic area, using high performance liquid chromatography (HPLC). A total of 41 samples were analysed for oleuropein content (harvest 2019). Oleuroperine values ranged from 1-970 mg/kg. Due to its wide range of biological activities, including anticancer properties, oleuropein has attracted the attention of the scientific community and EFSA, which included olive oil in Regulation 432/2012 with the explanation "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress". This statement can be used for olive oil containing at least 5 mg of hydroxytyrosol and its derivatives in 20 g of olive oil, which is the maximum daily intake. The obtained results indicate the importance of monitoring oleuropein in olive oil and its health effects.

KEY WORDS: EFSA; health claim; mediterranean diet; oleuropein aglycone; olive

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Botanical origin and physico-chemical properties of winter savory (Satureja montana L.) unifloral honey

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Due to its specific Mediterranean and sub-Mediterranean climate, Dalmatia has long been recognised for nectariferous flora, which enables the production of rare types of honey, such as winter savory (*Satureja montana* L.) unifloral honey. The aim of this study was to determine the botanical origin, selected physicochemical parameters, and sensory profile of winter savory honey. In total, 38 samples were analysed. Water content, electrical conductivity, and hydroxymethylfurfural (HMF) were determined in accordance with the harmonised methods of the International Honey Commission. The qualitative melissopalynological analysis was performed according to the method by Von der Ohe (2004), while sensory analysis was performed by a panel of five honey assessors. Along with the pollen of *S. montana*, pollen of the Apiaceae family, *Rhamnus* spp., *Centaurea* spp., *Trifolium pratense*, *Allium* spp., Liliaceae, and *Lotus corniculatus* were found in the pollen spectra. The content of *S. montana* pollen ranged from 22 to 80 % with a mean value of 51 %. The average value of the water content in the analysed samples was 17.2 %, electrical conductivity 0.34 mS/cm, and HMF content 6.8 mg/kg. The results of sensory analysis revealed a unique organoleptic profile of the analysed honey samples. The colour varied from light to dark amber. The odour intensity was medium to strong, reminiscent of dry herbs and moist soil. The taste was persistent, medium sweet with low acidity and weak to moderate bitterness. Quality properties characterized by a defined physicochemical and organoleptic profile of winter savory honey imply its high commercial value as a rare honey type.

KEY WORDS: electrical conductivity; hydroxymethylfurfural; melissopalynological analysis; physicochemical parameters; sensory profile

Development of a DNA barcoding curated database for the identification of commercial clam species from Chile

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Including the scientific name of the species in the label of seafood products is important for preventing fraud, illegal fishing, and the presence of food safety hazards, such as the undeclared inclusion of allergens in the product. Mollusks are included in the allergen list of European legislation, but people can be allergic to only one species within a genus, which increases the need for correct species identification methods. DNA barcoding is considered a gold standard for performing species identification and testing seafood authenticity. Typically, the sequence of a standardized gene is used to interrogate a database of known sequences. This reference database must be curated to avoid errors due to inaccurate taxonomic identification, changes in taxonomy or insufficient quality of molecular data. In Chile, nine clam species *Ameghinomya antiqua*, *Protothaca thaca*, *Mulinia edulis*, *Eurhomalea rufa*, *E. lenticularis*, *E. exhalbida*, *Semele solida*, *Gari solida*, and *Tawera gayi* are in the list of commercial and scientific names from EU countries (https://ec.europa.eu/fisheries/cfp/market/consumer-information/names_en). However, there is no validated analytical method to identify these species when morphological traits are removed by food processing. We develop a taxonomically curated database sequencing the 18SrDNA, 16SrDNA, ITS-1, ITS-2, H3, and H4 barcode genes. Twenty individuals from each species were identified by an expert taxonomist, photographed and Sanger sequenced. This database is used to test canned clams manufactured in Chile.

KEY WORDS: metabarcoding; reference database; seafood authenticity; seafood products; species identification

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The perspective of 3D printing in the production of functional fruit juices

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The market of functional food products has experienced a growing trend as consumers demand more out of food products than just safety and high quality. Fruit juices are an especially interesting matrix for functional production as they can be conveniently enriched with various bioactive ingredients and probiotics. Widespread interest in additive technologies was also evident in the food industry, while it is ubiquitously labeled as 3D printing (3DP). 3DP allows the creation of unique food designs (as functional fruit juice) with respect to nutritive compositions, desired flavors and the products' shape. The major benefits of 3DP include product diversity, customization, and personalization. Currently, different types of 3DP technologies used by manufacturers in the food sector are extrusion-based printing, selective laser sintering, binder jetting and inkjet printing. Characteristics of the 3D printer, along with the properties of the food material, significantly influence the quality of the final 3D product. 3DP of juices, is the latest endeavor in the food industry since it is challenging to optimize technological operating conditions for liquid media. Hence, for fruit juices it is necessary to control water contents by the addition of natural thickening agents (e.g., starch). The next challenge for 3D-printed functional fruit juices is the retention of a microbiological safety during shelf-life that requires additional research. In conclusion, 3D printing of functional fruit juices will open new possibilities for consuming functional foods with the aim to personalize dieting and provide health benefits within a single serving.

KEY WORDS: additive technology; food design; functional food; quality; safety

Food and beverage digital marketing – a Croatian market research

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In order to establish the nature and the level of children's exposure to the marketing of food rich in fats, sugars, and salt in the Republic of Croatia, a research on marketing environment was carried out. The research comprised six TV channels, out of which two non-commercial and four commercial channels. While watching the contents broadcast by a given TV-channel, the total number of promotion messages targeted at children or advertising products that could be used by children, even though the promotion was not specifically targeted at them, was registered. The study was conducted from September to November 2018. During this time, commercials were watched three working days a week and during each weekend. On a working day, these commercials were watched from 6 am to 11 pm (17 hours of each channel's broadcasting). During weekends, watching time was prolonged until midnight (18 watching hours). Food products and beverages under monitoring were divided into categories. In the examined period, a total of 2193 advertisements was recorded. Commercial TV channels most frequently promoted confectionery (19.9 %), milk and dairy products (14.8 %), soft drinks (12.2 %), meat and meat products (9.0 %), savoury snacks (7.7 %), food supplements (7.4 %), and cakes and biscuits (5.4 %). On national television, the most advertised products were food supplements (39 %), water and mineral water (26.3 %), cakes and biscuits (10.0 %), meat products and hot drinks (10.0 %). The current situation regarding food advertisements for children in various media could be improved by following recommendations provided by the World Health Organization.

KEY WORDS: advertising; child nutrition; legislation; non-communicable diseases; obesity

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Pollen spectrum of *Hedera helix* (common ivy) honey

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Hedera helix (common ivy) honey is an autumnal honey rarely found in Croatia. Since monofloral honeys are considered as the highest quality product and are sold all over the world at higher prices than multifloral honeys, it is of great importance to ensure that it meets requirements for palinologycal, organoleptic, physical, and chemical properties. Common ivy is a perennial, evergreen climbing vine which can act as ground cover as well as a climber. It is also widely used as an ornamental green facade. Ivy produces nectar rich flowers from the end of summer until the end of autumn and this is the time when other food sources for honey bees are limited. Five samples were analysed and 300 pollen grains were identified and counted with an Olympus BX52 microscope at 400× magnification. According to Croatian legislation honey is considered unifloral if the pollen frequency of one plant is larger than 45 % with exceptions to this due to 'under' and 'over' representation. All five samples met the criteria, ranging from 46-82 % of Hedera pollen. The organoleptic analysis revealed fine chrystals in samples. Colour was light do dark brown and the scent herbal and of strong intensity.

KEY WORDS: colour; melissopalinology; monofloral honey; scent; organoleptic analysis

Research on the quality of olive oil from the territory of the Republic of Croatia

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Olive oil is a precious agricultural product rich in monounsaturated fatty acids, vitamin E, and polyphenols. The quality of olive oil is determined by the olive variety, climatic conditions during cultivation, but also the technological process of oil production. The aim of this study was to analyse the selected quality parameters of olive oil produced in 2019, sampled from the north and south Adriatic region of the Republic of Croatia, to determine if there are differences between sampling areas. Upon delivery all samples were stored in a dark place, away from sunlight or heat and analysed with accredited methods according to the requirements of ISO 17025, within 2 weeks. Determination of FFA (free fatty acids) and PN (peroxide number) was made by titrimetric method, and the K-number [K232, K279, and (ΔK)] was determined spectrophotometrically. A total of 77 olive oil samples were sampled and analysed, of which 38 were sampled in the north Adriatic and 39 in the south Adriatic area. A total of 84 % of samples were rated as extra virgin olive oil, 14 % of samples did not meet the requirements for extra virgin olive oil, of which 1 sample (1.3 %) as virgin olive oil and 11 samples (14.3 %) lampante oil. The median value is made for all parameters: FFA=0.23 g/100 g; PN=7 meqO₂/kg, K 232=2.1, K 279=0.17, while for all samples the ΔK was 0.01. From the north Adriatic region, 87 % of the samples were classified in the category of extra virgin olive oils, as were 80 % of the samples from the south Adriatic. From the obtained results, we can conclude that olive oil from the Croatian Adriatic area is mostly of exceptional quality, as well as that the oils sampled in the north Adriatic are on average of slightly better quality than those from the south Adriatic area.

KEY WORDS: free fatty acids; K-number; lampante oil; peroxide number; quality parameters

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Risks assessment and opportunities of laboratory activities

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The Andrija Štampar Teaching Institute of Public Health has introduced an "Integrated Management System" which includes the standards ISO 9001, ISO 14001, ISO 15189, ISO 45001, ISO/IEC 17025, and ISO/IEC 17065 and is therefore in accordance with these standards' prescribed risk and opportunity management. In the Institute, the FMEA analysis (Failure Mode and Effect Analysis) is used as a tool for risk assessment for the purpose of risk assessment and opportunity. The purpose of this paper is to present risk management and opportunity in accredited laboratories of the Department of Environmental Protection and Health Ecology, in accordance with the requirements of ISO/IEC 17025:2017 and ISO 9001:2015 in order to continuously improve the quality management system. The laboratories of the Department identified the risks of their activities and processes, and analysed them. The risk identification process identifies risks that may affect the overall activity of the laboratory. After identifying the risks, a risk analysis was performed, which consisted of determining the consequences and their probability. Risk assessment involves comparing the assessed risk levels with the defined risk criteria, in order to determine the significance of the level and the type of risk. The result of the FMEA analysis procedure is a risk level assessment (RPR) for each potential hazard. FMEA analysis, beside recognizing, analysing and evaluating risks, also recognizes opportunities to improve laboratory activities.

KEY WORDS: accredited laboratories; FMEA (Failure Mode and Effect Analysis); quality management system; risk level; standards

Content and variability of minerals in dry-cured ham

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The manufacturing of dry-cured ham is mostly based on the addition of salt (NaCl) and the dehydration process. These procedures lead to changes of mineral content over time with the largest focus on sodium and health impact. For the purpose of this study, 20 dry-cured hams were manufactured and analysed for the content of sodium, potassium, phosphorus, magnesium, calcium, zinc, iron, copper, and manganese by flame atomic absorption spectroscopy. As expected, it was found that sodium content was the highest with an average value of 2350.54 mg/100 g. Thus, it was the limiting mineral for higher consumption of dry-cured ham especially for people experiencing issues with high blood pressure, because a consumption of 100 g exceeds the entire recommended daily intake. Besides sodium, a significant amount of potassium (441.81 mg/100 g) and phosphorus (242.44 mg/100 g) was noticed. Calcium was the mineral with the lowest variability (CV=8.31 %), followed by magnesium (CV=9.39 %) and phosphorus (CV=9.76 %), while the most variable minerals were iron (CV=54.75 %) and copper (CV=52.38 %). Respecting the higher mineral bioavailability than from plant foods and average mineral content, it can be concluded that 100 g of dry-cured ham is the source of a significant amount of phosphorus [34.63 % of the Recommended Dietary Allowance (RDA)], zinc (22.50 % of the RDA) and potassium (22.09 % of the RDA). On the other hand, dry-cured ham is a very poor source of manganese (1.25 % of the RDA) and calcium (1.92 % of the RDA).

KEY WORDS: flame atomic absorption spectroscopy; food composition; nutritive recommendation; Recommended Dietary Allowance; sodium

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Inactivation kinetics of cytochrome P450 3A4 by acacetin

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Flavonoids are secondary plant metabolites found in fruits and vegetables. Cytochrome P450 enzymes are the most significant metabolic enzymes of xenobiotics, including food constituents, toxic substances, and medications. Some flavonoids can interact with other drugs by inhibiting cytochrome P450 enzymes. The objective of this study was to determine the inhibition kinetics of cytochrome P450 3A4 by acacetin. Acacetin is one of the major polyphenols present in honey, which is believed to be associated with the prevention of heart disease. Enzyme activity was determined using testosterone and nifedipine as marker substrates, and generation of respective metabolites 6β -hydroxytestosterone and nifedipine-oxide was monitored by high performance liquid chromatography coupled with a diode array detector. In the testosterone assay, the values of inactivation kinetic parameters were as follows: IC_{50} value of $10.9\pm0.3~\mu$ mol/L, inhibition constant $6\pm3~\mu$ mol/L, inhibition rate constant 0.036 ± 0.006 per min. Similar results were obtained in the nifedipine assay. Further *in vitro* studies are needed to determine the reactive intermediate responsible for inactivation of cytochrome P450 3A4 enzyme, as well as *in vivo* studies to determine the possible clinical significance of this inhibition.

KEY WORDS: flavonoids; honey; HPLC-DAD; inhibition; polyphenol

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Prevalence of *Escherichia coli* cefotaxime-resistance strains isolated from poultry faeces

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The aim of this study was to investigate the prevalence of cefotaxime-resistance in *Escherichia coli* strains isolated from poultry faeces in the primary production phase in the Zenica-Doboj Canton, Bosnia and Herzegovina. Between September and October 2019, 108 faecal samples (cloacal swabs) were obtained from 25 different poultry farms of Zenica-Doboj Canton. The swabs were subcultured on MacConkey agar supplemented with cefotaxime (2 g/L). Antibiotic susceptibility was determined using the disk-diffusion method. Phenotypic detection of extended spectrum beta-lactamases (ESBL) and AmpC production was performed using double disk synergy and the phenyl-boronic acid test, respectively. Among 108 swabs, 75 (69.4 %) were positive for *E. coli*, of which 27 (36.0 %) were cefotaxime-resistant. All cefotaxime-resistant *E. coli* isolates were positive in the phenotypic test for ESBLs and five (out of 27; 18.5 %) were positive for AmpC beta-lactamase production. More than 70 % of cefotaxime-resistant isolates were resistant to cephalosporins 1st, 2nd, 3rd, and 4th generation, and more than 30 % of isolates were resistant to fluroquinolones. Low prevalence of resistance was observed for amynogligosides and sulphamethoxazole-trimethoprim. There were no isolates resistant to imipenem, meropenem, and colistin. Twelve (44.4 %) of the cefotaxime-resistant isolates were multi-resistant (more than three clases of antibiotics). It is very important to develop national surveillance system of resistance in order to prevent any possibility of emergency situations and the spread of multiple resistant strains among animals, as well as between animals and people.

KEY WORDS: AmpC beta-lactamases; disk-diffusion method; ESBLs; MDR; national surveillance system

Detection of Salmonella spp. as a colistin resistance strain isolated from fresh minced meat and poultry faeces

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Colistin is clearly associated with a selection of resistant strains in livestock which can spread from animals to humans by direct contact, or indirectly, through the food chain. Thirty-two *Salmonella* spp. strains were isolated. Food samples and poultry faeces in primary production phase were analysed according to the Rulebook in accordance with International Standards (EN ISO 6579-1:2018) and the Manual for Reporting 2018 – data on antimicrobial resistance from Decision 2013/651/EU. The isolates were identified by colony morphology, standard biochemical, and serological tests. Antimicrobial sensitivity testing was carried out using disk-diffusion and minimal inhibitory concentration (MIC). Among 32 *Salmonella* spp. strains, seven were isolated from food samples – fresh minced meats from the markets, and 25 from the poultry faeces. Using agar dilution method test, seven (21.9 %) *Salmonella enteritidis* strains were resistant to colistin, two (out of 7; 28.6 %) from fresh minced meats and five (out of 7; 71.4 %) from poultry feecs. The MICs values for seven colistinresistant isolates were in the range of 4 to 8 μ g/mL. Six isolates (out of seven) were resistant to cefazolin (85.0 %) and one isolate was resistant to ciprofloxacine and nalidixic acid with MIC values of 0.25 and \geq 128 μ g/mL. There is a need for careful monitoring of colistin usage in animal husbandry, as well as to seek for alternative, effective, less expensive antibiotics, competitive with colistin in the treatment of animal gastrointestinal infections. The detection of *mcr* genes should be introduced as well.

KEY WORDS: agar dilution method; disk-diffusion method; food; mcr-like genes; minimal inhibitory concentration

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Determination of cannabinoid content in industrial hemp

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Hemp (*Cannabis sativa* L.) is one of the oldest plants to be widely used in different industries. It contains cannabinoids some of which are psychoactive like tetrahydrocannabinol (THC). In the Republic of Croatia, it is allowed to breed varieties of industrial hemp in which the THC content does not exceed 0.2 %. This paper presents the results of determining the THC content in different varieties of industrial hemp during 2020, in order to determine health safety. In addition to THC, the following cannabinoids were analysed in the samples: cannabidiol (CBD), cannabinol (CBN), and cannabigerol (CBG). From the homogenized sample the cannabinoids were extracted with absolute ethanol. They were analysed by gas chromatography with a flame ionization detector (GC-FID) and quantified by the method of internal standard (tribenzylamine, TBA) using a calibration curve. The method was developed in the laboratory, validated, and accredited. Relative standard deviation (RSD) for repeatability of sample injection is from 0.30 to 0.62 %, while repeatability of sample preparation is ranges from 0.88 to 2.17 %. In our laboratory during 2020, 103 samples of hemps were analysed, of which 10 samples showed a mass fraction of THC higher than 0.2 %. Samples with a content of THC higher than 0.2 % were analysed by confirmatory technique, liquid chromatography coupled to mass spectrometry (LC-MS/MS). This technique confirmed that of all the samples positive using GC-FID; only one sample had a content of THC higher than 0.2 %.

KEY WORDS: GC-FID; LC-MS/MS; mass fraction; THC; tribenzylamine

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Monitoring of dithiocarbamate pesticides in fruits on the Croatian market by a validated GC-MS method

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A healthy and balanced diet typically requires plenty of fruit in order to supply the human organism with vitamins, minerals, and bioactive compounds such as phytochemicals. Fruits are usually consumed fresh. Recently, freshly squeezed juices or technologically produced juices have been gaining more popularity. Dithiocarbamates (DTCs) were commercially used as fungicides during World War II, but they can also be used as pesticides. They are characterised by high chemical and biological activity, low production costs, low/moderate toxicity to humans, animals and crops, and they act against a broad spectrum of microorganisms. DTCs can be classified into three groups: dimethyldithiocarbamates (DMDC: ziram, thiram, and ferbam), ethylenebisdithiocarbamates (EBDC: maneb, zineb, and mancozeb) and propylenebisdithiocarbamates (PBDC: propineb). For DTC market monitoring, 104 samples of fruits (strawberries, apples, blueberries, pears, plums, peaches, nectarines, grapefruits, oranges, kiwis, and bananas) were sampled and analysed by gas chromatograpy-mass spectrometry (GC-MS). DTCs are not stable and cannot be extracted or analysed directly. Therefore, the method for the determination of intentionally produced carbon disulphide (CS2) in laboratory by control reaction in chemical bottle was developed and validated. Results obtained by this method satisfy the given criteria of acceptability. Obtained results showed that 21 samples had DTCs above limit of quantitation (LOQ), but none was above the maximum residue limits (MRLs) established by EU regulation (2017/171).

KEY WORDS: dimethyldithiocarbamate; ethylenebisdithiocarbamate; maximum residue limit; propylenebisdithiocarbamate; public health

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Residual pesticide active substances in fruit juice: Occurrence and risk estimates for the Serbian population

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Occurrence of pesticide residues in food and the consequent health risks attract the attention of both public health professionals and the general public. The current study comprised a total of 139 samples of fruit juice available on the Serbian market. Out of 69 pesticide active substances analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS) with electrospray ionisation, 19 were detected, most frequently carbendazim, acetamiprid, and pyrimethanil. Almost 60 % of the 71.2 % of the positive samples contained multiple residues, up to 6 in an individual sample. Residues of unapproved pesticides were detected in 2.9 % of the samples, whereas the proportion of samples not in compliance with the allowed maximum levels was 2.2 %. Juice with 50 % fruit content showed a more favorable profile of pesticide residues than 100 % juice, as well as apple juice in comparison with other fruit. The deterministic method applied to carry out a risk assessment provided adequate reassurance of safety. The probability of exceeding the toxicological reference value was insignificant for any of the pesticide active substances assessed in any of the consumer groups considered, with the exception of adolescents acutely exposed to carbofuran. Chronic cumulative exposure to triazole pesticides showed very low risk, regardless of the applied approach – Hazard Index or Relative Potency Factors used to scale the exposure relative to the cyproconazole as an index compound for hepatotoxic effects. Estimate of the lifetime cancer risk attributable to the presence of carcinogenic pesticides in fruit juice was sufficiently protective, corresponding to a total of 0.0112 additional cancer cases annually in the Serbian population.

KEY WORDS: cancer risk; LC-MS/MS; pesticide residues; public health; risk assessment

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Occurrence of pesticide residues in mushrooms in Serbia, 2015-2020

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Pesticides are a common part of conventional food production, although they carry acute and chronic health risks. Dietary intake is the main route of pesticides exposure for the general population. Mushrooms have a place in healthy diets as they are low in fat and calories, modest in fiber, and rich in several minerals and vitamins. The aim was to determine the frequency of pesticide residues in mushrooms from the Serbian market and the distribution of non-compliance samples. Analysis of data of pesticide presence and compliance with defined maximum residue levels (MRL) from the results of testing mushroom samples [by gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) techniques] obtained from the Annual National Pesticide Residue Monitoring Programme (2015 to 2019). In the observed period, the total number of analysed mushroom samples was 40. Thirteen different pesticide residues were detected in 25 (62 %) samples, 20 (50 %) samples had multiple residues (2-5), and 4 (10 %) samples were non-compliant. The most commonly detected pesticides were carbendazim (16 samples), prochloraz (12), metrafenone (9), chlothianidin (6), and only chlothianidin among them (in 2 samples) was above the MRL (found in the range 0.006-0.130 mg/kg) as well as tetramethrin (found in 1 sample – 0.033 mg/kg) and alethrin (found in 1 sample – 0.078 mg/kg). There is a need for the continuous collection of data on the presence of pesticide residues in food through regular monitoring programmes as an important prerequisite for comprehensive risk assessment of the population long-term exposure to multiple pesticide residues.

KEY WORDS: food; health; maximum residue level; monitoring program; risk

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Metabarcoding for detection of potential toxin-producing algae and pathogenic bacteria from mariculture environment

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The Adriatic coastal area, where most of Croatia's aquaculture activities are carried out, is often under various anthropogenic influences. Throughout 2018, in Mali Ston Bay, where bivalve and cage fish farms are located, seasonal sampling of seawater and sediment was performed within the scope of selected commercial cage European sea bass farm. The aim of this study was to detect potential pathogenic bacteria and harmful bloom producing algae (HABs), by using high-throughput metabarcoding methods. Physicochemical parameters were measured in situ, while concentrations of chlorophyll a, total nitrogen, and phosphorus were measured in the laboratory. Total DNA was extracted from the samples and 16S rRNA and 18S rRNA high-throughput sequencing was performed. Bioinformatics and statistical analysis were performed in QIIME2 and RStudio. The bacterial community in seawater had a higher relative abundance of Gammaproteobacteria, especially in the summer (Vibrio 41.58 %; Acinetobacter 37.80 %). Additionally, the sediment in the fish cage vicinity was enriched with phylum Firmicutes (Clostridia). Potential fish pathogens of the genera Tenacibaculum and Photobacterium species were below 1 % (rare taxa). Known antagonistic bacteria such as Bacillus and Roseobacter were also detected. The seawater harboured a significant percentage of Dinoflagellata (40.99-67.14 %), and sediment Bacillariophyta (32.24-62.25 %). Potential HABs of the genera Alexandrium, Gonyaulax, Gymnodinium, and Heterocapsa were detected in seawater and sediment. The genera Amphidinium and Nitzschia were predominantly found in the sediment, while the genera Takayama, Chrysochromulina, and Pseudonitzschia in seawater. The detection of these taxa coupled with monitoring of environmental parameters could serve as an important biomarker for aquaculture management and an early warning of health status, protecting both farmed animals and human consumers.

KEY WORDS: 16S rRNA; 18S rRNA; aquaculture; European seabass; sediment

Presence of sulfite-reducing Clostridia in bee pollen

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The aim of this paper was to emphasize the importance of microbiological testing in food most often used raw. Microorganisms in the bee pollen, that most commonly contaminate dried food, mainly belong to sporogenic species of bacteria that can pose a danger to human health and these are most often bacteria of the genus *Clostridium*. Along with *Clostridium botulinum*, one of the most common isolates of this genus in samples of bee products, and especially bee pollen, is *Clostridium perfringens*. Bee pollen has a complex chemical composition and contains numerous vitamins, minerals, proteins, and amino acids. Collected fresh bee pollen contains a large proportion of moisture, as much as 35-45 % and it needs to be dried and frozen. As bee pollen is used dissolved in water, tea or other beverages for several hours, favourable conditions are created for the growth and multiplication of bacteria. Bee pollen samples were tested according to the standard HRN ISO 15113:2003 – Horizontal method for determining the number of sulfite-reducing bacteria under anaerobic conditions. The paper presents the test results of *Clostridium perfringens* presence in bee pollen samples in relation to water activity. This study shows that bee pollen samples which are not sufficiently dried, frozen or adequately stored have a greater predisposition for *Clostridium perfringens* growth and propagation.

KEY WORDS: bee product; Clostridium botulinum; Clostridium perfringens; propagation; water activity

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Presence and antimicrobial resistance of *Staphylococcus* aureus in milk from vending machines

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Consumption of milk from vending machines is popular in Croatia, but it is necessary to evaluate the potential risks to consumers in terms of microbiological safety. The aim of this study was to examine the presence and counts of *Staphylococcus aureus* in raw milk from vending machines (n=319) and to test the susceptibility of isolates to antimicrobials over a one-year period. The number of *S. aureus* was determined according to HRN EN ISO 6888 and the assessment of resistance to kanamycin, erythromycin, nitrofurantoin, ampicillin, levofloxacin, teicoplanin, ciprofloxacin, penicillin, cefotaxime, tetracycline, trimethoprim/sulfamethoxidolol, linezolid, chloramphenicol, and ceftazidim by disk diffusion method. The average number of *S. aureus* by months of sampling ranged from 2.04 to 3.09 log CFU/mL. A total of 88 samples contained these bacteria (27.58 %). Sensitivity to antimicrobials was tested in 60 isolates and the percentage of resistant isolates varied from 33.3 to 100 % depending on the sampling month. A total of 41 isolates were resistant to at least one antimicrobial agent (68.33 %). The majority of isolates were resistant to penicillin, ampicillin, and ciprofloxacin, followed by linezolid, ceftazidime, kanamycin, and levofloxacin. Based on our results, there is a strong need to implement appropriate control measures in order to reduce microbiological risk and within connected antimicrobial resistance in the milk distribution chain through vending machines.

KEY WORDS: disk diffusion method; isolates; microbiological safety; milk distribution chain; raw milk

Reducing the risk of *Campylobacter jejuni* transmission through modulation of bacterial adhesion using *Lactobacillus* spp.

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Campylobacter spp. are a major cause of bacterial food-borne diarrhoeal disease acquired through eating undercooked poultry. Bacteria readily colonize a wide variety of livestock, which may serve as a reservoir of infection for humans. Due to the substantial burden of campylobacteriosis, it is important to reduce the prevalence of Campylobacter spp. at farm level where good hygiene and biosecurity practices are not effective enough. The consequent reduced transmission from animal carcasses to humans will lower the risk to consumers. This needs to be achieved without the use of antibiotics and for that reason alternative strategies for the reduction of colonization of Campylobacter spp. are needed. The addition of probiotic bacteria might promote competitive adhesion to epithelial cells, thus reducing Campylobacter jejuni colonisation. Effect of Lactobacillus spp. (PCS20, PCS22, PCS25, LGG, PCK9) on C. jejuni adhesion, invasion, and translocation in chicken small-intestine cell lines (B1OXI) was investigated. One-way ANOVA followed by Dunnett's multiple comparison tests was performed to compare the C. jejuni counts between the control and tested probiotics in the anti-adhesion assays. Overall, Lactobacillus spp. significantly impaired C. jejuni adhesion in a three-dimensional B1OXI cell model. Also, C. jejuni did not translocate across B1OXI cells when co-incubated with probiotics. Among the selected probiotics, Lactobacillus rhamnosus LGG was the strain that reduced adhesion efficacy of C. jejuni most significantly. Our results showed that using Lactobacillus spp. might be an effective strategy to target Campylobacter adhesion, prevent Campylobacter colonisation, reduce transmission, and minimize the risk of bacterial spread to humans.

KEY WORDS: Campylobacter spp,; chicken B1OXI cells; food-borne diarrhoeal disease; Lactobacillus rhamnosus LGG; probiotics

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In silico prediction of ADMET properties of opium poppy alkaloids found in food

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Individuals subjected to drug testing should avoid food containing poppy seeds, which are commonly used in cakes and pastries because it might be prepared with alkaloid-contaminated poppy seeds and could cause a positive opiate drug test. It is of forensic importance to assess whether and to what extent the intake of poppy seed-containing products is associated with the excretion of measurable amounts of these alkaloids and their metabolites. The study aimed to evaluate the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of opiate alkaloids and their safety profile using the ADMET Predictor™ to elucidate their impact on the environment and health. The obtained results revealed that the main biotransformation pathways, catalyzed by cytochrome P450 (CYP) enzymes, for phenanthrene alkaloids (morphine, codeine, thebaine, and oripavine) are O- and N-demethylation, whereas O-demethylation and further oxidation of formed catechols to the corresponding toxic o-quinones are characteristic for biotransformation pathways of papaverine and noscapine. The highest scores of ADMET_Risk were predicted for papaverine and no risk for noscapine. Likewise, the highest scores of Absn_Risk (due to physicochemical and biological properties) and CYP-Risk were also predicted for papaverine, whereas rat acute toxicity and hepatotoxicity were predicted for papaverine and oripavine. The qualitative estimation of reproductive/developmental toxicity (Repro_Tox) was predicted for papaverine and noscapine, the chromosomal aberration for morphine and oripavine, whereas androgen and estrogen receptor toxicity were predicted for all phenanthrene alkaloids. All of the investigated alkaloids are nonbiodegradable molecules with the highest ecotoxic potential of oripavine to *Daphnia magna*, codeine to *Tetrahymena pyriformis*, and papaverine to the fathead minnow (*Pimephales promelas*).

KEY WORDS: bioconcentration; biotransformation; ecotoxicity prediction test tools; safety; toxicity

Determination of metals in honey

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Honey is a natural supersaturated sugar solution composed of a complex mixture of carbohydrates. The composition and properties of honey depend on the plants that bees visit and the climatic characteristics of the area from which the honey originates. Honey contains important micro and macro elements, but may also contain potential contaminants. The concentrations of individual metals in different types of honey depend on the presence of elements in the flowers, which is associated with the botanical and geographical origin. Samples of honey from different locations were collected for analysis. In this research, honey samples were analysed for the presence of certain metals. Samples were destroyed by a microwave oven using nitric acid and hydrogen peroxide. The concentrations of elements were determined by inductively coupled plasma mass spectrometry (ICP-MS). The obtained results showed that the concentrations of Cr, Cu, Ni, Cd, and Pb in the analysed samples were less than 1 mg/kg. Concentrations of the other elements were found in the following ranges (mg/kg): Na 64.5-273.5; Mg 7.0-71.9; Al 34.1-73.8; K 265.0-1780.0; Ca 40.3-177.0; Mn 0.163-1.47; Fe 0.71-3.45; Zn 0.454-1.49; Rb 0.372-2.56; Sr 0.086-0.519. Comparing the obtained results on the presence of metals in honey at different locations, it is noticeable that there are differences between the measured concentrations of individual metals, but also between the samples from different locations. From the aforementioned it can be concluded that honey can serve as an indicator of environmental pollution and its geographical origin can be determined by the content of metals.

KEY WORDS: contaminants; elements; geographical origin; ICP-MS; microwave oven

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Method optimization for fatty food sample preparation for GC-ECD analysis of organochlorine pesticides

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Fatty foods are a complex matrix and it is very challenging to find an appropriate method of sample preparation that can obtain extracts with a minimum proportion of interference. Gel permeation chromatography (GPC) has proven to be the most effective method for purifying fatty food samples in the past. More recently, as an alternative to the GPC method, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method has gained popularity. At the Andrija Štampar Teaching Institute of Public Health, the method for fatty food samples preparation for analysis of 29 organochlorine pesticides by gas chromatography with an electron capture detector (GC-ECD) was optimised. The method is based on the extraction of samples with acetonitrile and purification through two dispersive solid phase extraction (dSPE) columns [Enhanced Matrix Removal_Lipid (EMR_Lipid) and EMR Polish] that removes fat and water from the samples. To increase the extraction efficiency of the pesticide, acetonitrile: ethyl acetate=80:20 was used instead of pure acetonitrile. The method was validated where the matrix influence, sample preparation repeatability, influence of evaporation extract to dryness, limits of quantification, and recovery were examined. Acceptance criteria for validation experiments were taken from document SANTE/12682/2019. By validating the method, satisfactory results were achieved for linearity (k >0.98), repeatability of sample preparation (RSD <20 %), limit of quantification (0.005 mg/kg) and recovery (40-140 %). Given the results obtained and a satisfying proficiency testing result (EUPT AO15; z score <1±21 for rapeseed oil), the method is suitable for routine analysis of fatty food samples with a fat content >30%.

KEY WORDS: analytical validation; dispersive solid phase extraction; enhanced matrix removal; QuEChERS; SANTE

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Irrigation water quality in urban gardens of Zagreb

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Urban gardens are a form of urban, modern agriculture integrated into cities offering users the possibility of food production. The quality of water used for irrigation has implications for agricultural productivity and human health. The aim of this study was to determine the irrigation water quality used in Zagreb urban gardens. The main physicochemical [pH, electrical conductivity (ECw), Cl⁻, NO₃⁻, NO₂⁻, P, HCO₃⁻, NH₄⁺, Na⁺, K⁺, Ca²⁺, Mg²⁺] and microbiological parameters (total coliforms, *Escherichia coli*, intestinal enterococci, colony count at 36 and 22 °C, *Pseudomonas aeruginosa* and *Clostridium perfringens*) were determined in groundwater used for irrigation. Water was sampled and analysed during 2016-2020 from 12 locations according to an accredited and standardised methodology. The physicochemical and microbiological parameters were interpreted according to FAO quality standard and DIN 19650 classification. The results indicated that the most analysed chemical parameters were under no degree of restriction on use. Only the ECw value in 50 % of water samples fell under a slight to moderate degree of restriction on use. For microbiological analysis, the samples were classified into four classes, according to the number of present *E. coli* and enterococci. As many as 85 % of the samples were in the first class, which is equivalent to drinking water, 11 % samples in the second, and 2 % in the third and fourth class. The continuous monitoring of water quality indicators for irrigation is important to ensure the adequate quality of water applied to agricultural lands in urban areas. Monitoring would prevent the occurrence and harmful effects of potential inorganic and organic pollutants.

KEY WORDS: human health; microbiological parameters; monitoring program; physicochemical parameters; urban agriculture

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Food allergy awareness among people at social risk

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Through a unique system of social assistance, the City of Zagreb institution "Dobri dom" offers food for its citizens of a wide socio-demographic spectrum who are potentially immunocompromised persons due to their weaker material status. The purpose of this paper was to show the awareness of users about the presence of allergens in food, with the aim of improving the quality of service and health protection of users. The paper presents the results of surveys on the presence of food allergies in users obtained by personal surveys on a sample of 120 respondents at four locations of public dining rooms, one of which included the homeless. The questionnaire contained questions about the personal predisposition to allergies of users and their family members and listed the most significant food allergens. Users were asked if they knew where they could request information about food allergens before taking a meal and if they ever did so. The results of the surveys showed a very low level of information of both users about food allergens and about the data that are available at any time at the request of users, and relate to allergens in ready meals. The institution has ISO 9001 and ISO 14001 certificates, and in accordance with the legal obligation, a list of allergens has been made for all meals prepared for users. From the results, it can be concluded that there is a need to educate users about food-derived allergens through cooperation with public health institutions.

KEY WORDS: education; food allergens; health protection; legal obligation; service quality

Pollen and food: the Pollen Forecast app

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Pollen allergies are a significant public health problem with an impact on health life quality, social interactions, work performance, and school attendance. Cross-reactivity of pollen and food of plant origin can cause food allergies and symptoms can last from a few minutes to half an hour. Severe forms occur very rarely. The oral allergic syndrome is an allergic reaction, most often with symptoms of irritation at the level of the oral cavity and perioral area, but the occurrence of symptoms is also possible at the level of the digestive, skin, and respiratory system. It occurs after the consumption of fresh fruits and vegetables in people with allergies to plant pollen. People who are sensitized to a certain type of allergenic pollen can react to another type of pollen, without having been in contact with it before. With the aim of informing and educating citizens, the PLIVAzdravlje portal, in cooperation with the Andrija Štampar Teaching Institute of Public Health and county institutes developed the mobile application Pollen Forecast. The application contains daily information on the concentration of pollen of certain allergenic plants in the air of Croatian cities where measurements are performed, allergenic plants, and the degree of allergenic potential, as well as information on cross-reactions of pollen and food of plant origin. This way, allergic people can adjust their diet and daily activities and actively influence their health. The application is available free of charge to all iOS and Android device users.

KEY WORDS: aeroallergens; Croatia; mobile application; pollen allergy syndrome

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Migrations of substances from synthetic and biodegradable coatings in disposable cups and their safety assessment as food contact materials (FCMs)

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Paper and cardboard are materials often used for packaging food products. Due to the cellulose in them, they easily absorb water, which is why they are coated with hydrophobic materials such as polyethylene (PE), polyvinyl alcohol or fluorocarbons in order to enable resistance to moisture. "Disposable paper cups" are the most common representatives of this type of packaging. Since it seems that they are made only of paper, consumers are confused that they cannot be recycled. This is because of the synthetic coating that is applied to the inside of the cup, so such products end up mainly in landfills and become a major environmental problem. Their permanent ban is being considered, and a solution may well be in the use of renewable and biodegradable biopolymers. Whether synthetic or biodegradable coatings, they must comply with the legal framework [Regulation (EC) No 1935/2004] in terms of health safety and quality that all materials or articles that come into direct contact wth food must provide before being placed on the market by its maximum inertness in order to exclude the transfer of the substance to the food in quantities which may endanger human health or cause unacceptable changes in the composition of the food. For this purpose, we conducted research on 10 types of disposable cups from different manufacturers to determine possible irregularities in their quality and health safety. The presence of formaldehyde above the specific migration limit (SML) and changes in sensory properties in terms of color penetration from the outer surface were determined.

KEY WORDS: formaldehyde; health safety; maximum inertness; paper packaging; synthetic coatings

Changes in the antioxidant status of tobacco plants upon exposure to silver nanoparticles and silver nitrate

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Silver nanoparticles (AgNPs) have a wide application in consumer products due to their antimicrobial properties. However, their increasing release into water or soil represents a potential environmental hazard. In this work, *in vitro*-grown tobacco (*Nicotiana tabacum*) plants were exposed to AgNPs stabilized with cetyltrimethylammonium bromide (CTAB) or polyvinylpyrrolidone (PVP) coating, and to ionic silver (AgNO₃) of the same concentrations (25, 50, and 100 µmol/L). The aim of the study was to investigate the generation of reactive oxygen species (ROS) and changes in activities of antioxidant enzymes with regard to different treatments, thereby revealing if plants were exposed to oxidative stress. Silver uptake and activities of antioxidant enzymes catalase, ascorbate, and pyrogallol peroxidase and superoxide dismutase were determined in leaves and roots extracts and compared to non-exposed, control plants. The obtained results showed that uptake of silver in roots after either AgNPs or AgNO₃ treatment was significantly higher than in leaves, while there were no significant differences in silver uptake after AgNP or AgNO treatments of the same concentrations. An increase in ROS level was observed only in roots after AgNP treatment. Significantly higher enzyme activities were observed in most treatments compared to control, although the increase was most prominent after exposure to AgNP-CTAB. Concentrations of silver as well as the form in which silver is applied seemed to be correlated with changes in the activities of antioxidant enzymes. The obtained results suggest that oxidative stress is associated with AgNP toxicity in plants and cannot be ascribed merely to the dissolution of silver ions.

KEY WORDS: nanosilver; oxidative stress; phytotoxicity; reactive oxygen species; silver uptake

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