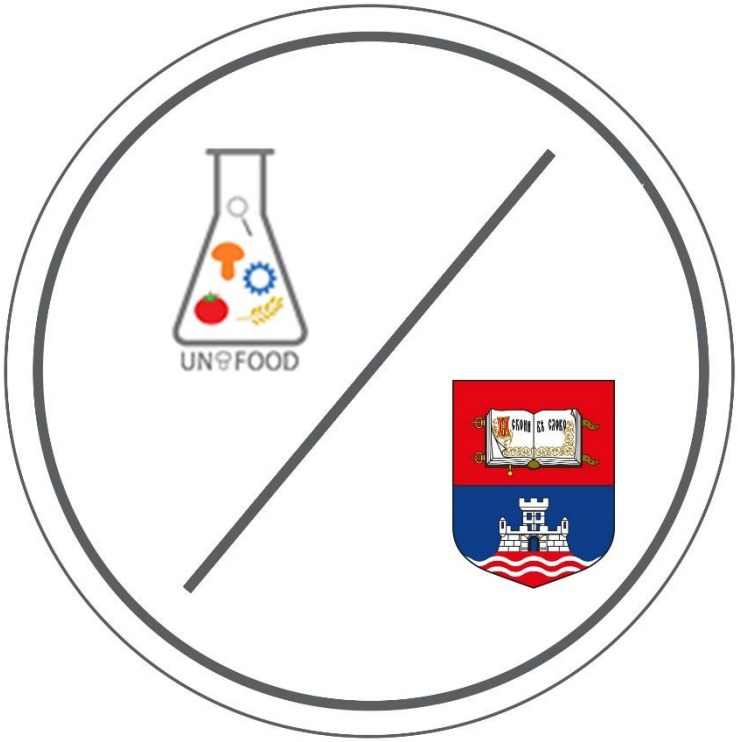
UNIFOOD CONFERENCE



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The word of welcome

Dear colleagues,

We would like to welcome you to the **2nd UNIFood International Conference –UNIFood2021.** We hope that this gathering will engage not only academics, but also the stakeholders from all the relevant industries and business sectors, serving as a meeting point and a platform for proliferation of new ideas and development of new partnerships.

The first UNIFood conference, organized as national, was established 2018. year as one of the events in honor of the **210th Anniversary** celebration of the **University of Belgrade** that ranked at Shanghai list on 35th place for the 2017 year in subject *Food Science and Technology*. The University of Belgrade has been recognized as a leading international scientific institution by LERU when it was selected to be a member of CE7, an informal network of seven Central and Eastern European universities collaborating with LERU on key research and education challenges. Furthermore, University of Belgrade joined European University Alliance Circle U. Following the European Commission's launch of the European Universities initiative, a group of research-intensive universities has entered into a Memorandum of Understanding with the intention of establishing a new university alliance: Aarhus University, Humboldt University of Berlin, King's College London, UC Louvain, University of Belgrade, University of Oslo and Université de Paris.

We are pleased that you have decided to take part in this mutual conversation, where many will present their recent work, through poster sessions, oral communications or simply by asking questions. One of the goals of this Conference is cooperation between academia and food industry. Food scientists, technologists, researchers, nutritionists, engineers and entrepreneurs will exchange their knowledge about the latest advances in all aspects of food production, processing, sustainability, safety and security, nutrition and health, hi-tech equipment, ethics and knowledge transfer supporting environment. At this meeting, over 200 participants from 23 countries will take part.

Belgrade, one of the oldest city in the Europe, always young, at the confluence of the Sava and Danube rivers, will be your host. At the confluence of new ideas and experiences we again wish you a warm welcome.

Sincerely,

Prof. Dr Mirjana Pešić

President of the Scientific Committee of UNIFood2021

Prof. Dr Ivanka Popović

Rector of the University of Belgrade

UNIFood2021 Conference

24th-25th September 2021 University of Belgrade

2nd International UNIfood Conference

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SEARCHING FOR BIOACTIVE COMPOUNDS AGAINST SARS-COV-2 FROM TRADITIONAL CHINESE MEDICINES

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Traditional Chinese medicine is a treasure in Chinese culture, which has been applied for thousands of years and accumulated numerous knowledge about the usages of herbs to improve human health. Since the breakout of coronavirus disease 2019 (COVID-19), traditional Chinese medicines play an important role in the fight against the infectious disease in China. Starting from traditional Chinese medicines with antivirus effects will greatly increase the possibility to find the lead compounds against COVID-19.

Based on the in-house multi-technology platform, a series of natural compounds with inhibitory activity against 3CL protease (3CLpro) were disclosed from antivirus medicinal herbs and formulas. For example, shuanghuanglian preparation, a Chinese traditional patent medicine (also called proprietary Chinese medicine) used for the treatment of acute respiratory tract infections since 1973, is a classical purified herbal preparation extracted from three Chinese herbal medicines, namely, *Lonicera japonica* Thunb., *Scutellaria baicalensis* Georgi, and *Forsythia suspense* (Thunb.) Vahl. A total of 27 compounds were quickly identified and separated from the three herbs, and assayed their inhibitory activities against 3CLpro. The results showed that nine compounds were identified as lower-micromolar-range inhibitors of 3CLpro. Among these, the binding of baicalin as well as baicalein with 3CLpro was comprehensively characterized, and a crystal structure of SARSCoV-2 3CLpro in complex with baicalein, the first noncovalent, nonpeptidomimetic small-molecule inhibitor, was also determined. The study provides a good example for exploring the in vitro potency of Chinese traditional patent medicines and identifying bioactive ingredients toward a specific target, and adds scientific evidence to support the clinical potential of Shuanghuanglian preparation, as well as two natural products for COVID-19 treatment.[1] We also discovered myricetin, a flavonoid found in many food sources, as a non-peptidomimetic and covalent inhibitor of the SARS-CoV-2 3CLpro. Crystal structures of the protease bound with myricetin and its derivatives unexpectedly revealed that the pyrogallol group worked as an electrophile to covalently modify the catalytic cysteine. The results provide detailed mechanistic insights into the covalent mode of action by pyrogallol-containing natural products and a template for design of nonpeptidomimetic covalent inhibitors against 3CLpro.[2] In addition, we tried an efficient discovery of potential inhibitors for SARS-CoV-2 3CL protease directly from the herbal extracts using a native MS-based affinity-selection method. It is a meaningful attempt to search for bioactive molecules in a quick and purposeful way.

Keywords: Traditional Chinese medicine and ethnodrug; Anti-SARS-CoV-2; bioactive compounds
Acknowledgements: We are thankful for the financial support from the Science and Technology Commission of Shanghai Municipality (20430780300).

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GREEN FOOD PROCESSING. PRESERVATION, TRANSFORMATION & EXTRACTION. FROM CONCEPTS TO RESEARCH, EDUCATION AND ECONOMIC OPPORTUNITIES

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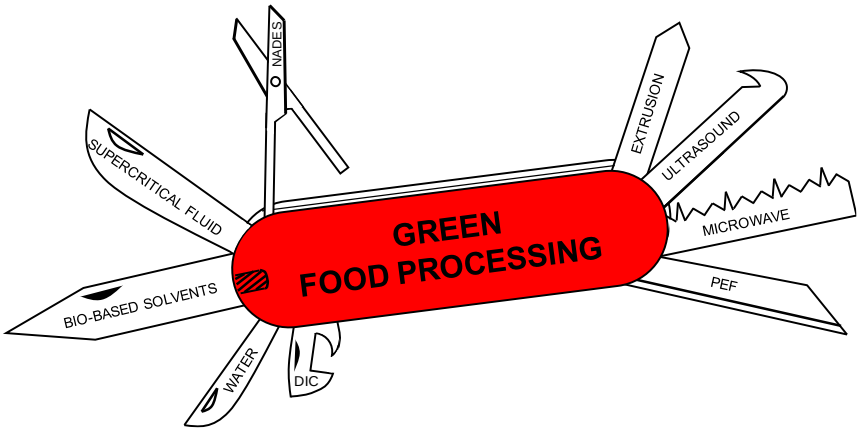
Food processing even preservation, transformation or extraction is a dynamically developing area in fundamental and applied research even in academia and industry, and takes an important place in manufacturing processes. Challenges and drivers launched by the environment protection, competitiveness of the globalized market, and more recently as requests by consumers and society, strongly require innovations that break away from the past rather than simple continuity. Green Food Processing could be a new concept to meet the challenges for the future of humanity on this strategic 21st century, to protect both the environment and consumers, and in the meantime, enhance competition of industries to be more ecologic, economic and innovative. This green approach should be the result of a whole chain of values in both senses of the term: economic and responsible, starting from the production and harvesting of food raw materials, processes of preservation, transformation, and extraction together with formulation and marketing. Green Food Processing could respond to these challenges of this 21st century for enhancing shelf life and nutritional quality of food products, to reduce energy and unit operations for processing, eliminating wastes and byproducts, reduction of water use in harvesting, washing and processing, use of naturally derived ingredients, the need of standardization, and more important eliminating hunger, food insecurity and malnutrition worldwide.



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PRODUCING PLANT ESSENTIAL OIL COMPOUNDS THROUGH INDUSTRIAL BIOTECHNOLOGY

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Plant essential oils play an important role in today's economy, for flavouring, fragrance, cosmetics and healthcare applications. Most of these oils can be extracted from ample waste materials such as citrus peels and pine trunks, or from dedicated plant cultivations, such as mint, thyme and patchouli. In some cases, the source of the oils is a more rare species, and may pose cultivation challenges. Examples of those include the Asian sandalwood tree (Santalum album) and the candeia tree (Eremanthus erythropappus), both of which represent trees which need more than 15 years before they can be harvested, and face extinction due to illegal cutting in the wild. In such cases, their main essential ingredients, which are often sesquiterpenes, can also be produced by microbial fermentation. Microbial production of sesquiterpenes is often mediated by terpene synthases derived from plants. These terpene synthases may behave with very different efficiencies when expressed in microbes, and for this reason it is important to be able to choose the most efficient terpene synthase for a specific product. In this work we aim to develop methods to identify the most efficient sesquiterpene synthases for specific products.

In a traditional approach, two very closely related genes were isolated from Cinnamomum camphora. One of these genes acts as a monoterpene synthase, when expressed in Nicotiana, while the other gene mediates production of santalenes, which represent important ingredients of sandalwood oil. A library of hybrids between the monoterpene- and sesquiterpene synthases leads to identification of enzymes with altered product specificity.

In a computational approach, the rapidly growing databases for plant genomes is interrogated for terpene synthases, and machine learning is applied to identify candidate synthases for a specific sesquiterpene, or a category of sesquiterpenes. This algorithm has been trained using a novel database of characterized sesquiterpene synthases (www.bioinformatics.nl/ sesquiterpene/synthasedb). Indeed, using the developed tool, a number of novel bisabolyl cation synthases from Citrus bergamia 'Femminello' were identified successfully from a cDNA sequence library.

Thus novel approaches have been developed for generating superior microbial production platforms, to replace vulnerable plant resources for the flavour and fragrance industry.

Keywords: Terpene, Biotechnology, synthase

Acknowledgements: This work is part of the research programme Novel Enzymes for Flavour and Fragrance with project number TTW 15043 which is financed by the Netherlands Organisation for Scientific Research (NWO).

ENCAPSULATION OF BIOACTIVES BY HIGH THROUGHPUT ELECTROSPRAYING ASSISTED BY PRESSURIZED GAS

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One of the most promising approaches to preserve bioactive compounds is their encapsulation within protective matrices. Recent developments in engineering and industrial investment have allowed our research group to develop an innovative encapsulation technique based on the combination of electrohydrodynamic technology and the pneumatic atomization process. This novel high-throughput technology, termed electrospraying assisted by pressurized gas (EAPG), is based on the atomization of the polymer solution by a pneumatic injector using compressed air that nebulizes within a high electric field. During this process, the solvent is evaporated at room temperature in an evaporating chamber and the encapsulated material is collected as a free-flowing powder. This technology is a versatile technique that presents multiple advantages compared to conventional encapsulation techniques. For instance, it is carried out at room temperature, which reduces the denaturation of bioactive compounds, produces particles with high encapsulation efficiency, results in reduced particle size with a narrow size distribution, does not require a subsequent step to separate the particles from the medium, and it is highly versatile in terms of the encapsulating materials and bioactive compounds that can be processed. In addition, by means of this technology, it is possible to achieve the production volumes required by commodity food applications.

The current presentation will introduce the technology and highlight some use cases for the stabilization, shelf-life extension, and controlled release of different bioactive compounds.

Keywords: EAPG encapsulation, omega-3, probiotics, polyphenols, functional foods, nutraceuticals.

Acknowledgements: This research was funded by H2020 EU projects CAPSULTEK (reference number 873827) and FODIAC (reference number 773872), and by the Spanish Ministry of Science and Universities (project RTI-2018-097249-B-C21).

UNRAVELLING THE MECHANISMS OF FOOD DIGESTION TO IMPROVE OUR KNOWLEDGE OF THE EFFECT OF FOOD ON HUMAN HEALTH

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Recent findings have demonstrated that the food matrix structure is one of the key drivers to control the fate of food in the digestive tract and, consequently, the kinetics of nutrient release.

As an example, using the pig as a model of human, we have clearly demonstrated that, at identical composition, differences in food macrostructure (milk vs acid and rennet gels) lead to differences in gastric emptying, protein hydrolysis in the gut and amino acid bioavailability. Compared to dairy gels, milk goes more rapidly through the stomach to reach the small intestine where protein will be quickly and extensively degraded. This generates a fast and intense peak of plasma amino acids.

Similarly, the food macrostructure also drives the kinetics of release of hydrophilic and lipophilic vitamins in the bloodstream.

When food composition and macrostructure are identical, microstructure can be a tool to modulate nutrient bioavailability. Three egg white gels were made at different ionic strengths and pH, generating gels with similar macrostructure but different microstructure (porosity, tortuosity). When given to pigs, these gels had different impact of the pH and the diffusion of acid secretion in the stomach, leading to differences in gastric proteolysis.

Controlling the time of residence of food in the stomach by playing on its structure should allow to design products with a fast release of nutrients particularly adapted for elderlies, athletes etc… whereas foods persisting in the stomach should induce satiety and be dedicated to overweight people. Ongoing experiments using biophysical methods will help us to understand the mechanisms of gel particle breakdown in the stomach in order to design foods of new generation perfectly adapted to the nutritional needs of specific populations

Keywords: digestion, food structure, nutrient, bioavailability

RECOVERY AND CHARACTERIZATION OF FUNCTIONAL INGREDIENTS AND BIOACTIVE COMPOUNDS FROM *Theobroma cocoa* BEAN SHELLS: AN OVERVIEW AMONG CHEMISTRY, TECHNOLOGY AND NUTRITION

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Cocoa and chocolate (*Theobroma cacao* L.) have been found to be rich plant-derived sources of antioxidant polyphenols (particularly flavonoids) with different beneficial properties. As well known, these favorable physiological effects include antioxidant activity, vasodilation and blood pressure reduction, inhibition of platelet activity, and decreased inflammation. Cocoa consumption is so correlated with reduced health risks of cardiovascular diseases, hypertension, atherosclerosis, and cancer; all the health-promoting effects of cocoa are mediated by phytochemicals.

Cocoa bean processing, particularly roasting process, leads to the production of wastes (cocoa bean shells, CBSs, often defined also as "husks") more properly currently considered as "by-products", rich in fibres and other bioactive polyphenols. According to the principles of green chemistry and circular economy, the recovery of bioactive compounds from by-products and wastes is a useful approach to formulate new "functional ingredients", a priority in modern food technology.

This lecture will explore all the findings related to the identification, extraction and characterization of bioactive compounds from CBSs, highlighting the technical approaches functional to their isolation, extraction and formulation.

An overview about the main outcomes obtained by our research group (and about the technical processing used) will focus on carbohydrates and polyphenols recovered from this interesting biomass.

METABOLOMIC-BASED APPROACH ON WINE AUTHENTICATION: A CASE STUDY ON VARIETAL DISCRIMINATION

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Wine metabolomics constitutes a powerful discipline towards wine authenticity assessment through the simultaneous exploration of multiple classes of compounds in the wine matrix. Over the last decades, wines from autochthonous Greek grape varieties have become increasingly popular among wine connoisseurs, attracting great interest for their authentication and chemical characterization. In this work a case study is presented, where 46 red wine samples from Agiorgitiko and Xinomavro grape varieties were collected from wineries in two important winemaking regions of Greece during two consecutive vintages and analyzed using ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QToF-MS). A targeted metabolomics methodology was developed, including the determination and quantification of 28 phenolic compounds from different classes (hydroxycinnamic acids, hydroxybenzoic acids, stilbenes and flavonoids). Moreover, 86 compounds were detected and tentatively identified via a robust suspect screening workflow using an in-house database of 420 wine related compounds. Supervised chemometric techniques were employed to build an accurate and robust model to discriminate between two varieties.

Keywords: wine; metabolomics; HRMS; chemometrics; biomarkers

CAN DAIRY PEPTIDES PLAY A ROLE IN SATIETY?

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Dairy proteins are high quality proteins, containing all essential amino acids, are easily digestible and can release a plethora of bioactive peptides. Some of these peptides can modulate satiety signals in the gut. Gut satiety signaling plays an important role in appetite and food intake. This lecture summaries the *in vitro* evidence that dairy proteins, hydrolysates and peptides can increase secretion of various satiety signals from enteroendocrine cells. Whether this bioactivity can be lost, bolstered or protected during gut transit is also explored. Data from intervention trials in animal models and humans will also be reviewed to decipher if dairy proteins, hydrolysates or peptides should be used in foods to make one feel 'fuller for longer'.

OVERVIEW OF MARIE SKLODOWSKA-CURIE ACTIONS (MSCA) IN EU RESEARCH FRAMEWORK FOR 2021-2027 (HORIZON EUROPE)/ FOCUS ON INTERSECTORAL AND INTERNATIONAL MOBILITIES OF RESEARCHERS ("STAFF EXCHANGES" ACTION) WITH PROJECT EXAMPLES IN CHEMISTRY

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The Covid-19 crisis has highlighted once more the importance of the world' reliance on a highly skilled research-based human capital that is able to detect and tackle emerging challenges. In the frame of the new EU Research Framework Program (Horizon Europe, 2021-2027), the European Union (EU) will continue reinforcing its efforts to entice more young people to a research career and promote its attractiveness for top talents from around the world. To this end, Marie Skłodowska-Curie Actions (MSCA) are the main EU instrument financing excellent projects to support all stages of researchers' careers and encouraging their mobility. Within the period 2014-2020 (Horizon 2020 Program) the MSCA Actions supported around 65 000 researchers including 25000 PhD candidates.
During Horizon Europe, the following types of MSCA funding Actions will be managed by European Research Executive Agency of the European Commission

(i) MSCA **Doctoral Networks** supporting early stage researchers to participate in competitively selected joint doctoral programmes;
(ii) **MSCA Postdoctoral fellowships** supporting the mobility of experienced researchers in two year's postdoctoral projects;
(iii) **MSCA Staff Exchanges** promoting the intersectoral and international mobility of researchers, management and administrative staff at all career levels;
(iv) **MSCA COFUND** offering additional funding to regional, national and international PhD or postdoctoral projects
(v) **MSCA and Citizens** aiming to bring research closer to public at large through the European Researcher's Night event.

This presentation particularly focuses on Staff Exchanges that build partnerships between universities, research institutions and industrial partners both within and beyond Europe. This action promotes interdisciplinary collaboration through exchanging staff and sharing knowledge at all stages of innovation chain. Examples of successful MSCA projects in various fields of Chemistry and Applied sciences will be provided.

MUSHROOMS AS SOURCES OF THERAPEUTIC FOODS

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Edible mushrooms have been appreciated worldwide for their unique odour, taste and texture, as well as their beneficial nutritional and chemical properties. They have been used as food by Chilean tribes 13,000 years ago, whereas the most comprehensive data on their medicinal use are 3,000 years old and come from Traditional Chinese Medicine. Until recently, use of mushrooms for medicinal purposes has been marginalized in Western countries while only their organoleptic properties have been appreciated. Nowadays, the situation has changed significantly, as is indicated by extensive research studies in the field of medicinal mycology (over 50,000 scientific studies, 400 clinical trials and 15,000 patents in the last 30 years have been conducted), as well as increasing number of mushroom-based products on the market (nutraceuticals, functional food additives, supplements). Interest in these products has increased tremendously in the last few years, thus suggesting mushrooms have significant potential in pharmaceutical and food industry. The therapeutic potential of edible mushrooms may be attributed to their favourable nutritional value - high carbohydrate and protein content with low total fat share, as well as presence of numerous biologically active compounds: phenolics, terpenoids, steroids, vitamins, lectins, <fatty acids and many other. The abundance of these bioactives in edible mushrooms possibly allow their further use as prophylactic agents in diseases associated with poor nutrition, oxidative stress, infections, tumour cell development etc. With this in mind, all the acquired knowledge in mushroom science should be passed to the public with the aim to encourage use, cultivation and further studies of mushrooms, as well as development of proper mushroom-based medicinal products.

Keywords: medicinal mushrooms, edible mushrooms, nutritional value, biological activity

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CONTROLLED RELEASE SYSTEMS FOR FOOD APPLICATION

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Micro- and nano-particulate encapsulating systems have the potential to play a key role in the future of delivery of nutraceuticals which is an essential characteristic of functional foods. It is useful to control the rate and extent of their digestion in different regions of the gastrointestinal tract. The nature of a carrier material, as well as the nature of loaded active component are some of the key-factors determining delivery performance of these systems. In order to develop food additives with controlled release it is important to study the release characteristics of the encapsulated material from the particle matrix as a function of particle size, material properties and processing conditions. This lecture aims to give a short overview on the controlled released systems intended for delivery of active food compounds and its gastrointestinal fate which is currently one of the topics of nearly all journals on food science.

Keywords: controlled release, active compounds, functional food

FROM RAW STARCH DEGRADING α-AMYLASE TO TRANSGLYCOSYLASE BY SINGLE POINT MUTATION

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Understanding the structural peculiarities and properties of both, raw starch as a substrate and raw starch digesting amylases (RSDA) as an enzymes is needed for efficient application in food and other industries. Although knowledge of these structures and properties has increased significantly in recent years, the main requirement defining whether RSDA will be efficient in raw starch hydrolysis is still a riddle. We have recently identified the surface binding site (SBS) of a potent RSDA from *Bacillus paralicheniformis* ATCC 9945a (*Bli*Amy), by crystallographic study of its native form and in complexes with maltose, acarbose, maltohexaose and β-cyclodextrin. To understand role of this SBS, the two key residues identified, Phe257 and Tyr358, were mutated. Kinetic studies show that starch binding through the SBS is disrupted in the mutants and that both mutants contributed cumulatively to binding and degradation. Mutation of both sites resulted in at least 5.5-fold weaker binding and 5-fold lower efficacy with raw starch as substrate compared to the wild type *Bli*Amy suggesting that the ability of *Bli*Amy to hydrolyze raw starch with high efficiency is related to the level of its adsorption onto starch granule.

RSDA was further exploited for its robustness by changing its activity and converting this hydrolase into transglycosylase. The use of transglycosylases for synthetic purposes is limited since, unlike hydrolases, these enzymes are relatively rare in nature, and act on a limited substrate repertoire. To alter the activity of *Bli*Amy, His235 was replaced with Glu. The mode of action of the mutant enzyme was tested using substrates such as starch, amylopectin, maltooligosaccharides etc. Mutant exhibited transglycosylation activity, while wild type *Bli*Amy exhibited hydrolysis activity exclusively. Converting hyperthermostable *Bli*Amy into transglycosylase provides potent tool in the synthesis of starch derivatives. The production of starchy foods with slow digestion properties, and thus a low glycemic index, is therefore an important goal of the modern food industry.

Keywords: α-amylase, transglycosylase, raw starch, mutation

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INVESTIGATION OF ESSENTIAL OILS AND THEIR POTENTIAL USE IN THE FOOD INDUSTRY AND PHARMACY

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Essential oils are complex mixtures of volatile compounds produced by aromatic plants and characterized by distinctive, strong fragrance. Essential oils and their constituents are widely used in a number of food products and beverages, primarily as flavoring agents. Due to the antimicrobial and antioxidant properties of essential oils and their components, they may represent a promising natural alternative to chemical preservatives in the food industry. Similarly, the insecticidal/repellent potential of essential oils may be worth exploring in view of food storage. In addition to antimicrobial and antioxidant activity, essential oils demonstrate various pharmacological activities including anti-inflammatory, expectorant, spasmolytic, carminative, diuretic, rubefacient activity and are hence used in the treatment of respiratory, gastrointestinal, skin disorders as well as rheumatism, neuralgia and many other ailments. The investigation of essential oils is a wide field of research addressing different aspects. In light of these considerations, the essential oils of different plants, mainly from the flora of the Balkan Peninsula, were investigated in order to find new sources of raw material and constituents with potential use in the pharmacy and food industry. The study focused on the content, chemical characterization (e.g. *Valeriana* sp., *Anthemis* sp.) and evaluation of their antimicrobial (e.g. *Thymus* sp., *Achillea* sp., *Armoracia* sp.), spasmolytic (*Ferula* sp., *Cymbopogon* sp.), antiedematous and antihyperalgesic (*Matricaria* sp., *Laserpitium* sp.) properties, as well as their insecticidal properties (*Cymbopogon* sp.). Since many factors have an influence on the composition of essential oils, chemical analyses provide insight into the variability and possible existence of chemotypes, which is important from the aspect of defining raw material and their quality control. Although of natural origin and usually considered safe, some essential oils contain compounds that bear potential risks (e.g. essential oils of *Salvia officinalis*, *Artemisia absinthium*, *Ocimum basilicum*); therefore, the safety aspect of essential oils should not be neglected.

Keywords: essential oil, chemical composition, activity, pharmacy, food.

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MODERN PLANAR CHROMATOGRAPHY IN FOOD ANALYSIS

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The European Commission requires that fruit products distributed on the market are high quality and authentic. Lack of food control during processing and production can lead to low quality products. Therefore, it is highly demanding to establish a suitable method for quality control of food products based on chemical/biological profile as a verification of samples authenticity.

Due to simplicity, low costs, and opportunity for parallel analysis of several samples under same conditions, high performance thin-layer chromatography (HPTLC) has becoming more popular in food analysis. Furthermore, HPTLC combined with *in situ* enzymatic, biological and chemical assays provide fast screening of complex natural samples with regard to effects or bioactive components. After bioautography, bioactive bands could be identified using combination of HPTLC and structure elucidation techniques such as mass spectrometry (MS).

HPTLC chromatogram is reach source of data. Combination of high sophisticated multivariate techniques with planar chromatography allows extraction of full information from HPTLC chromatogram concerning to similarity/dissimilarity between samples, or identification of characteristics markers responsible for classification.

Combination of planar chromatography-bioautography-mass spectrometry and sophisticated multivariate analysis provides high-throughput quality assessment of food products regarding to potential health-promoting activities.

Keywords: Food adulteration, HPTLC fingerprint, green analytical method, multivariate analysis

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THE CHALLENGES IN DEVELOPMENT OF PRODUCTS WITH PROBIOTICS

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Lactic acid bacteria have been recognized as beneficial microbes for human health and wellbeing. However, poor survivability of probiotics, especially during storage and passage through the gastrointestinal system, limits their applications in food products. Hence, additional protection of probiotic cells is in many cases the necessity in order to maintain high cells number. The application of encapsulation techniques is a well-established approach for probiotic cells protection and their controlled delivery. Nevertheless, encapsulation procedures require optimization and test numerous carrier materials for establishing the best approach in regard to the properties of the final food product and preservation of cells during processing and storage.

In recent years, our research has been focused on the protection of probiotics using various encapsulation techniques such as freeze drying, spray drying, and cells entrapment into the gel matrix. Using these techniques and by combining carrier materials such as maltodextrin, inulin, alginate, and soy protein isolates it could be achieved up to 3.4 $x10^9$ CFU/g of encapsulate (in the dried form). The drying method and appropriate carrier material were found to be critical for cells viability. Spray drying inlet temperature should be up to 130°C, while maltodextrin showed more suitable properties as carrier material regarding cells protection and mechanical properties of encapsulates. Also, we are able to adjust encapsulates' size from several microns up to several millimeters, depending on final applications and targeted food product properties. Further, we optimized our research to produce encapsulates in sufficient quantities in order to test them in real food products. Although considered as the most suitable encapsulation procedure for preservation of probiotics high viability, our results indicate that after freeze drying the cell number was lower compared to spray drying, while the further processing of freeze dried encapsulates and incorporation into final products were more challenging.

Keywords: Probiotics, Encapsulation, Spray draying.

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TRADITIONAL DAIRY PRODUCTS AS A VALUABLE SOURCE OF LACTIC ACID BACTERIA WITH UNIQUE PROPERTIES

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Traditional dairy products, white brined cheese, fresh cheese, hard cheese, yogurt, produced in Serbia are made from raw milk, while kajmak is made from boiled milk. These products are made according to a traditional recipe without the use of commercial starter cultures. They represent a rich source of various microorganisms, including lactic acid bacteria (LAB). LAB are known to synthesize antimicrobial compounds, proteolytic enzymes, exopolysaccharides, aromatic compounds and have the ability to aggregate. Some of them may be probiotic strains which, among other things, may have an immunomodulatory effect. Natural LAB isolates, which originate from autochthonous dairy products, often have one or more unique properties that cannot be found in industrial strains, which is important from both a fundamental and an applicative point of view. Isolation, characterization, and identification of these strains, and then the methods of molecular genetics, enabled the discovery of new antimicrobial compounds as well as new mechanisms of their action. These results could be the basis for their potential application in the food, medical and pharmaceutical industries. Among these strains, there are candidates that could be used to formulate new starter cultures to obtain functional dairy products. Although LAB from dairy products has been studied for decades, the novelty is the application of new approaches in the quest for new strains with specific characteristics, such as *in silico* analysis followed by targeted isolation.

Keywords: natural bacterial isolates, antimicrobial compounds, health

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DETECTING THE ESSENTIALS OF FOOD – SUPER-HYPHENATIONS AND NANOGIT$^{+ACTIVE}$

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The beneficial effects of plant-rich diets are increasingly recognized in the treatment of civilization diseases due to the abundance and diversity of bioactive substances therein. However, the important active portion of natural food is presently not under control. Hence, a paradigm shift from quality control based on marker compounds to effect-profiles is postulated. A generic strategy was developed to evaluate the bioactivity profile of each food as complete as possible, and without overlooking, straightforwardly assign the most potent bioactive compounds [1, 2]. This biological-physico-chemical hyphenation is able to straightforwardly detect and control the essentials of food [3].

Sample components can not only be changed but also activated or inactivated via enzymatic or metabolic reactions. Identifying such activity conversions is the challenge. The newly developed all-in-one nanoGIT$^{+active}$ system substantially extends, and at the same time, miniaturizes the state-of-the-art technology [4]. It combines all relevant steps on the same adsorbent surface. On-surface metabolization, immediate separation, multi-imaging, and effect-directed detection is performed as all-in-one system. The conversions on surface were verified by comparison with state-of-the-art *in vitro* assays.

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Keywords: functional food, health food, effect, assay, bioactivity, on-surface metabolization, digestion

EVALUATION OF SUGAR AND POLYPHENOLIC PROFILES OF FRUITS OF TWO APPLE CULTIVARS GROWN IN AN INTEGRATED AND ORGANIC PRODUCTION SYSTEM IN A NORDIC CLIMATE.

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Fjord areas in Western Norway (60 º North) represents the most northerly tree fruit producing areas in the world. This area influenced by fjord and the Gulf Stream together with long daylight during summer days makes this location suitable for apple production. The aim of this study was to compare fruits, its size and chemical content of two main commercial apple cultivars ('Discovery' and 'Red Aroma') from both organic (OP) and integrated production (IP) grown in a high density planting system under the same agro-climatic conditions. Separations of sugars were performed using DIONEX ICS 3000 DP liquid chromatography system. Ultra high performance liquid chromatography (UHPLC) coupled with a diode array detector and connected to a triple-quadrupole mass spectrometer was used for determination and quantification of the polyphenols. OP fruits were smaller than IP for both cultivars. 'Red Aroma' fruits from IP were significantly larger than IP 'Discovery' (177 and 150 g, respectively). Over color of 'Discovery' fruits produced at an integrated way and 'Red Aroma' fruits produced at organic way were more intense. Among 17 sugars detected in the apple fruits, the most common were fructose, glucose, sucrose and galactose, which amounted around 43%, 33%, 11% and 4.5%, respectively. In total 13 other sugars (trehalose, arabinose, isomaltose, meslesitose, gentobiose, turanose, raffinose, ismaltotriase, maltose, panose, maltiose, ribose and zylose) and three sugar alcohols (sorbitol, galactitol and mannitol) were found too, but in low levels According to the levels of glucose, fructose and sucrose, the sweetness index was calculated and IP 'Red Aroma' and OP 'Discovery' fruits were much sweeter than its counterparts. From a total of 19 polyphenolic compounds detected, quercetin 3-*O*-galactoside and chlorogenic acid were the most abundant. Organically produced apple fruits stored higher level of the majority of detected polyphenolic compounds. The skin had higher contents of phenolic compounds than the mesocarp and the most abounded in the skin was quercetin 3-*O*-galactoside and phlorizin. No differences in the phenolic contents were found between cultivars or OP and IP grown. Total anthocyanin content was higher in organic apples (0.293 – 0.540 g/kg frozen weight) than in integrated (0.184 – 0.253 g/kg frozen weight).

Keywords*: Malus domestica Borkh.***,** *cultivars, fruit quality***,** *sugar profile***,** *phenolic profile*

FOOD BIOACTIVES FIGHTING RENAL CANCER PROGRESSION

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Many studies suggest a protective role of certain dietary patterns (e.g., consumption of cruciferous vegetables) against the risk of renal cancer. However, the current body of evidence is yet insufficient to establish a link between diet and the risk of renal cancer development and progression. Our work aims at contributing to fill this gap by dissecting the effects of two structurally distinct redox-active food components on kidney cell characteristics related to renal cancer progression. A special emphasis was given to cell motility due to its critical importance for the development of metastases. Thymoquinone is a monoterpene isolated from the oil of Nigella sativa seeds, which is widely used as a spice as well as in traditional medicine. We have shown that thymoquinone reduced the viability and promoted apoptosis of 786-O human renal cancer cells. At non-cytotoxic/genotoxic concentrations, thymoquinone significantly decreased the collective migration and the invasiveness potential of these cells. Erucin is an isothiocyanate that can be generated by *in vivo* reduction of sulforaphane or by enzymatic hydrolysis of glucoerucin. Contrarily to sulforaphane, limited studies have addressed the anticancer properties of erucin. Erucin induced a concentration-dependent decrease of cell viability, more pronounced in 786-O cancer cells than in the "normal-like" Vero-E6 cells. The exposure of cells to this bioactive led to an increase of the G2/M population. Collective cell migration, chemotaxis and chemoinvasion abilities, as well as cell adhesion, were impaired in erucin-treated cells. Additionally, erucin induced concentration-dependent changes on cell morphology and impaired tubulin polymerization. Overall, our results suggest that thymoquinone and erucin may have a beneficial impact in reducing renal cancer cells migration, contributing to explore the mechanisms of possible dietary approaches for chemoprevention.

Keywords: Renal cancer; thymoquinine; erucin; cell motility

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OLIVE LEAF EXTRACT IMPROVED RENAL FIBRONECTIN EXPRESSION RESULTING IN SLOWING DOWN THE PROGRESSION OF EXPERIMENTAL FOCAL SEGMENTAL GLOMERULOSCLEROSIS IN SPONTANEOUSLY HYPERTENSIVE RATS

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Introduction: Glomerulosclerosis and tubulointerstitial fibrosis develop as a result of the extracellular matrix accumulation in the glomeruli and tubulointerstitium, leading to the progression of focal segmental glomerulosclerosis (FSGS). Fibronectin is one of the major extracellular matrix proteins. Transforming growth factor-β (TGF-β) is a potent pro-fibrotic cytokine that induces fibronectin synthesis. Glomerulosclerosis, enhanced glomerular matrix accumulation and monocyte infiltration are associated with hyperlipidemia, especially with the rise of the low-density lipoprotein cholesterol (LDL), due to a stimulation of fibronectin production in the glomerular mesangium, contributing to the progression of chronic kidney disease. Previously, we showed that adriamycin (ADR) induced the enhancement of LDL in plasma in spontaneously hypertensive rats (SHR). Here, we aimed to investigate whether olive leaf extract (OLE, rich in phenolic compounds that possess powerful antioxidant properties) could ameliorate fibronectin expression and improve kidney structure in SHR with ADR-induced FSGS. **Experimental methods:** SHR were divided into three groups. Control rats received vehicle. FSGS and FSGS+OLE groups received ADR (2 mg/kg body weight, i.v.) twice in a 3-week-interval. After the second injection, FSGS+OLE received OLE (80 mg/kg/day) by gavage for six weeks. Proteinuria, fibronectin and TGF-β protein expressions (western blot) in the kidney homogenates were measured. **Results:** Proteinuria and fibronectin protein expression remarkably increased while TGF-β protein expression decreased in the kidney of SHR after ADR application. Chronic treatment with OLE reduced urine protein loss for 40%, fibronectin protein expression was significantly decreased to the level as in control, while TGF-β protein expression remained similar as in the model group. **Conclusion:** In our study, ADR-induced fibronectin overexpression has been completely abolished after OLE without changing the protein expression of TGF-β. We suggested that treatment with OLE reduced fibronectin expression independently of TGF-β.

Keywords: olive leaf extract, TGF-β, fibronectin, adriamycin nephropathy, hypertension

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SPASMOLYTIC AND ANTIRADICAL ACTIVITY OF *THYMI HERBA* HYDROSOL

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Hydrolates (syn. aromatic waters, hydrosols, floral or distillate waters) are the side products of essential oils' isolation by steam distillation. They have a long history of application, especially in Persian nutrition culture and folk medicine, as safe refreshing or medicinal beverages (depending on the plants used for their production). Nevertheless, scientists are only nowadays starting to study hydrolates, especially their antimicrobial capacity. Apart from antifungal and antibacterial activities, significant antiviral and antioxidant properties of Thymus vulgaris hydrolate were reported. This study was designed to estimate antiradical capacity and spasmolytic activity of T. vulgaris hydrolate.

Radical scavenging activity was determined using a stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) and different concentrations of hydrolate (10-60%). The spasmolytic effect was tested in in vitro conditions using a model of spontaneous isolated rat fundus contractions. Results of both tests were presented as the IC50 values, which indicates the sample concentration (in µg/ml) required to remove 50% of DPPH free radicals or to reduce spontaneous fundus contractions for up to 50%.

Demonstrated inhibition of the spontaneous fundus contractions (IC50 = 13.81 ± 2.45 µl/ml) and antiradical activity (63.87 ± 2.21 µl/ml) could contribute to the possible overall action of this hydrolate on the gastrointestinal system. Application of T. vulgaris hydrolate (0.01–100 µl/mL) produced a decrease in the tone of spontaneous fundus contractions in a dose-dependent manner. After washing the tissue preparation with fresh Tyrod's solution and following rest for the duration of 15 minutes spontaneous tissue contractility was not returned. Thus, it could be concluded that T. vulgaris hydrolate shows prolonged blockage of spontaneous fundus contractions, and the mechanism of such activity should be elucidated.

Although most hydrolates are acidic liquids with pH ranging from 4.5 to 5.5 with pleasant to unpleasant and from similar to dissimilar odour to the essential oil, T. vulgaris hydrolate has pH 6.16 and very characteristic fresh smell and hot taste. Alongside these characteristics, shown antiradical and spasmolytic activities make it a potential functional beverage.

Keywords: aqua aromatica, tissue bath, rat fundus, DPPH, pH.

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BIOACTIVITY PROFILING OF VARIETAL WINES AND COUPAGE: *IN VITRO* ANTI-INFLAMMATORY POTENTIAL

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There are many evidences that support health benefits of moderate wine consuption. From the biochemical point of view, there is certain doubt: which one is „healthier", coupage, obtained by mixing wines or varietal wine? Herein we present a part of results related to extensive investigation of biological activities of wines and coupage, showing some aspects of mechanisms underlying their anti-inflammatory activity: modulation of expression of enzymes involved in cyclooxygenase pathway of arachidonic acid metabolism, which are responsible for the production of prostaglandin E_2 (PGE_2) and thromboxane A_2 (TXA_2), important lipid mediators in normal physiology and inflammatory response. In an *in vitro* model of early stages of inflammation (LPS-stimulated human U937 monocytes), potential of *Merlot* and *Cabernet Sauvignon* wines, one commercial coupage and three coupage prepared by mixing base wines in different ratios (1:1 (*K1*), 3:1 (*K2*) and 1:3 (*K3*)), to modulate mRNA expression of phospholipase A_2 (PLA_2), cyclooxygenase-1/2 (COX-1/2), PGE_2 and TXA_2 synthases (mPGES-1/2, cPGES, TXAS) was monitored by RT-qPCR. Analysis of wine polyphenols was done by HPLC-UV/VIS techniqe.

The most abundant polyphenols were gallic acid (41.1–66.4 mg/L) and catechin (15.3–24.5 mg/L), while malvidin-3-*O*-glucoside (3.1–6.0 mg/L) was leading anthocyanin. Some differences regarding contents of vanillic, caffeic and syringic acids, in addition to rutin, quercetin and piceid were found. Resveratrol was not detected in commercial coupage, while in other samples it was quite low (0.2–0.3 mg/L).

Wines and coupage have ability to modulate mRNA expression in applied model-system: the most notable was suppression of COX-2 mRNA expression by *Merlot* and *K1* (3.7 and 2 folds, respectively). Principal component analysis revealed reasonable separation of samples, particularly K3, due to the weakest overall activity.

Obtained results implicate that one interesting strategy in creating new products can be used: finding right ratios of varietal wines to achieve enhanced biological activity of final coupage.

Keywords: Merlot, Cabernet Sauvignon, coupage, inflammation.

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GRAPEFRUIT JUICE EXERTS A COMPLEX NUTRIGENOMIC EFFECT THAT IS INVERSELY ASSOCIATED WITH AN INCREASE IN ARTERIAL STIFFNESS

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Grapefruit is regularly consumed worldwide and is a rich source of polyphenols, particularly flavanones. Dietary flavanones have been suggested to exert diverse health-promoting effects. We have previously reported that consumption of flavanone-rich grapefruit juice for 6 months in postmenopausal women reduced pulse wave velocity, a measure of arterial stiffness that is a risk factor for cardiovascular disease development. Although complex genomic modulations by polyphenols have been shown, the mechanisms underlying the cardioprotective effects of flavanones are generally unknown. Thus, this study aimed to identify molecular mechanisms of action of flavanones in humans by performing multi-omics analysis in peripheral blood mononuclear cells of volunteers consuming flavanone-rich grapefruit juice and flavanone-free control drink for 6 months. Microarrays were used to assess gene and miRNA expression profiles. Bioinformatic analyses were performed to identify differentially expressed genes, miRNAs, their functions, putative transcription factors, their interactions, and correlations with previously observed changes in pulse wave velocity. Grapefruit juice modulated the expression of 1401 genes and 6 miRNAs. Integrated analysis of differentially expressed genes and miRNA-target genes showed their involvement in the regulation of inflammation, immune response, cell motility, and metabolism. Bioinformatic analysis identified transcription factors (e.g., STAT3 or NfkB) potentially mediating the observed genomic response to flavanones, and *in-silico* docking showed a possible binding of flavanone metabolites to identified transcription factors and cell signaling proteins. Correlation analysis between changes in gene expression and pulse wave velocity revealed 34 significant correlations. Also, there was an inverse correlation between the global gene expression and gene expression profiles linked with arterial stiffness and hypertension. In conclusion, this study revealed molecular mechanisms underlying vasculo-protective effects of flavanones, including interactions with transcription factors and modulations in the expression of protein-coding genes and miRNAs that are negatively correlated with gene expression changes associated with cardiovascular risk factors.

Keywords: Flavanones, Genomics, Bioinformatics, Vascular function

APPLICATION OF YOGURT PRODUCED USING APPLE POMACE FLOUR GRANULATED WITH LACTIC ACID BACTERIA AS A FUNCTIONAL BEVERAGE AND A POTENTIAL ADJUNCTIVE TREATMENT FOR DEPRESSION AND ANXIETY

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Yogurt, a favored fermented dairy beverage among the wellness generation, is comprised of three main functional components: prebiotics, probiotics and biogenics. It has been characterized with immunomodulatory and brain modulatory effects. Functional features can be further improved by enrichment with fruit pomace. Following the idea of sustainability in food production, apple pomace flour (APF) premix for fabrication of a fresh yogurt fortified with natural dietary fiber and antioxidants has been designed. APF was obtained from apple varieties: Idared, Jonagold, Golden Delicious and Granny Smith by squeezing, dehydratation and grinding (>500 μm) and granulated with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* in 5 % w/w lactose solution by fluidized bed. Fluidized bed granulation enabled employment of APF as immobilization support for lactic acid bacteria (LAB). Particle size distribution was determined and improvement of flowability confirmed. Bacterial viability of stored APF (0-205 days) was estimated by the spread plate technique before and after *in vitro* digestion. The high viability of LAB was maintained upon immobilization and storage (>log 8) while digestion reduces viability. Granulated APF and neat LAB were employed to prepare yogurts. pH, syneresis and color of yogurt were analyzed. Less pronounced syneresis and lower initial pH were ascribed to APF yogurts (3% w/v) in comparison to those traditionally produced. Produced yogurt has been intended as functional beverage for modern consumers as well as potential adjunctive treatment for depression and anxiety. Due to the fact that humans and canines share most of the causative factors and the mental/physical epidemiology, APF yogurt was tested in order to be introduced in feeding plan of companion animals as well. Palatability assessment, trial with dogs (the two-bowl test) and trial with human panelist with depression and anxiety (yogurt taste test), was carried out. Acceptable palatability properties for humans and dogs were noted.

Keywords: dairy beverage, apple pomace flour, fluidized-bed, sustainability, depression

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HEPATOPROTECTIVE POTENTIAL OF SWEET BASIL (*OCIMUM BASILICUM* L.) EXTRACT IN ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN RATS

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The liver is the center of the metabolic transformation of drugs and other xenobiotics and detoxifying processes. The aqueous and ethanolic extract of *Ocimum basilicum* had a protective effect against liver damage. Observed effects were mediated by significant radical scavenging activity, therefore suppressing free radical-mediated lipid peroxidation leading to the accumulation of lipid-derived oxidation products that cause liver injury. The aim of this study was to evaluate the *in vivo* effects of basil extract pre-treatment on liver function in acetaminophen-induced liver injury.

Effects of basil extract on biochemical markers of liver injury were determined in an *in vivo* model of acetaminophen-induced liver injury in 24 Wistar rats. To estimate the extent of liver damage after acetaminophen administration, the following parameters were determined in serum: aspartate aminotransferase activity (AST), alanine aminotransferase activity (ALT), direct bilirubin level.

Administration of acetaminophen resulted in a statistically significant difference in ALT and AST levels in comparison to the control group ($p<0.01$), confirming the onset of liver injury. The ALT values in the group pre-treated with basil extract prior to acetaminophen administration were lower than those treated with saline and acetaminophen, but without statistical significance. On the other hand, levels of AST were statistically significantly lower in the basil pre-treated acetaminophen group in comparison to the group treated with saline and acetaminophen ($p <0.01$). Seven-day pre-treatment with aqueous basil extract prevented the increase in the direct bilirubin levels, implying preserved excretory liver function.

The present study demonstrated the significant hepatoprotective potential of aqueous basil extract in a model of acetaminophen induced liver injury. Hepatoprotective effects were apparent through the decreasing serum activity of liver transferase enzymes as well as preserved excretory liver function in animals pre-treated with basil extract.

Keywords: basil, acute liver injury, laboratory animals, acetaminophen.

A NUTRITIONAL COMPARISON OF DAIRY MILK AND PLANT BASED MILK ALTERNATIVES

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Milk and dairy products are present in the everyday diet of millions of people all over the world. While some consume it for its pleasant taste, others are well aware of the many health benefits that come up with daily milk consumption. Yet, during the last couple of years, milk consumption in Western countries has been slightly decreasing for different reasons. This trend is mostly caused by a vegan type of diet which has been very popular recently, allergens present in milk, and environmental problems related to the emission of greenhouse gasses. Knowing all this and having in mind milk is not available for most of the human population (especially in developing countries) it is easy to come up with a conclusion that it is necessary to find an adequate alternative for this drink. A potential solution is the usage of plant-based milk substitutes, which represent colloidal suspension (or emulsion) made of dissolved particles of plant material in water.

The aim of this work was to provide as much information about the nutrient composition of plant-based dairy alternatives and to summarize some of the most important properties of these products compared to milk. Although consumption of these dairy alternatives has many benefits for the human body, it is necessary to be careful if there is a tendency to completely replace milk with some of the products from this group because milk is rich in essential nutrients (primarily calcium and vitamin D) that are necessary for the proper growth and development of the organism. As it has long been known, the key to a healthy diet lies primarily in a well-balanced and varied selection of foods consumed daily, and the inclusion of milk and dairy alternatives on the regular menu greatly contributes to the ultimate and most important goal - preserving human health.

Keywords: Milk, Dairy alternatives, Milk analogue, Diet

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RED BLOOD CELL FATTY ACID PROFILE AND ADHERENCE TO THE MEDITERRANEAN-STYLE DIET AMONG TESTICULAR CANCER SURVIVORS

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Testicular cancer (TC) is the most common type of solid tumor among men aged 15–40 years. Due to chemotherapy-induced toxicities and potential hypogonadism, TC survivors may experience metabolic disturbances and long-term cardiovascular sequelae. Substantial scientific evidence confirms the health benefits of a Mediterranean diet (MedD) pertinent to a plethora of chronic diseases, including cardiovascular, neurodegenerative diseases and cancer. Red blood cell (RBC) fatty acid (FA) profile, comprehensive biomarker reflecting long-term dietary FA intake and tissue status, provides predictive data for cardiovascular risk assessment. This study recruited a sample of patients followed after curative treatment for TC at the Clinic of Urology, University Clinical Center of Serbia. Validated 55-point MedDietScore index, based on the intake of 11 food groups (wholegrain cereals, fruits, vegetables, legumes, meat and meat products, poultry, fish, olive oil, dairies, and alcohol) was used to evaluate the MedD model adherence. RBC FA composition was analyzed by gas chromatography. Based on self-reported dietary intake, participants (n=24, $\bar{x}$=38.54±8.18 years) reached low to moderate MedDietScore $\bar{x}$=27.70±5.11 (range 20-39). Correlation analysis revealed a strong positive relationship between the MedDietScore and RBC eicosapentaenoic acid (EPA, C20:5n-3) ($r=0.600$; $p<0.01$), docosahexaenoic acid (DHA, C22:6n-3) ($r=0.560$; $p<0.1$), Omega-3 Index, i.e. EPA+DHA ($r=0.662$; $p<0.001$) and total polyunsaturated FAs ($r=0.476$; $p<0.05$). Two-thirds of subjects displayed Omega-3 Index <4% suggesting high cardiovascular risk, while the rest were allocated in the intermediate hazard-category. MedDietScore was inversely associated with total saturated FAs ($r=-0.415$; $p<0.05$) and arachidonic acid (AA, C20:4n-6) to EPA ratio ($r=-0.537$; $p<0.01$). This is the first study to report on erythrocyte FA profile in men successfully treated for TC. Given the importance of promoting the cardiovascular health of TC survivors and the protective effect of MedD, targeted interventions addressing major modifiable risk factors, i.e., food consumption habits may exert a beneficial impact on achieving favourable cardiometabolic status in this vulnerable group.

Keywords: Testicular cancer, Fatty acid profile, Mediterranean diet, Omega-3 Index

SOURDOUGH FERMENTATION AS A TOOL TO ENHANCE THE FUNCTIONAL AND NUTRITIONAL FEATURES OF *TRITORDEUM* BREAD

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Traditional sourdough fermentation of *Tritordeum* (*Triticum durum* x *Hordeum chilense*) flour was characterized for its microbiological, biochemical, and nutritional properties. Viable plate counts of presumptive lactic acid bacteria, the ratio between lactic acid bacteria and yeasts, the rate of acidification, biochemical (pH, total titratable acidity, sugars and organic acids) features, the number of operational taxonomic units (OTUs), and diversity indices by 16S and 26S metagenome sequencing, altogether demonstrated the maturity of the sourdough during 10 days of propagation. The novel cereal *Tritordeum* flour was mainly contaminated by genera (*Acinetobacter, Pantoea, Pseudomonas, Comamonas, Enterobacter, Erwinia*, and *Sphingomonas*) belonging to the phylum Proteobacteria or Bacteroidetes (genus *Chryseobacterium*). Their relative abundances after 5 days of propagation were almost completely inhibited. Although members of the phylum Firmicutes were present at very low or intermediate relative abundances in the flour, they became dominant soon after 1 day of propagation. Lactic acid bacteria were almost exclusively representative of the Firmicutes by this time. *Weissella confusa* already dominant in *Tritordeum* flour and stably persisted, though it was later (from day 5 onwards) flanked by facultative heterofermentative LAB (e.g. *Lactobacillus plantarum*). Yeast diversity seemed to be consistent throughout the sourdough propagation with *Saccharomyces cerevisiae* as the dominant population. The *Tritordeum* bread prepared with sourdough had improved digestibility *in vitro* in comparison to baker's yeast bread as demonstrated by changes in gut microbiota by 16S metagenome sequencing and metabolomic profile by NMR analysis dominated by short chain fatty acids (SCFAs). Overall, this study revealed the potential of sourdough fermentation by the exploitation of a sustainable cereal, *Tritordeum.*

Keywords: Sourdough, Tritordeum, in vitro digestibility, SCFA

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ANALYSIS OF OPIATES IN HORSE FEED BY GAS CROMATOGRAPHY-MASS SPECTROMETRY

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Opiates such as morphine and codeine are substances often misused to improve the performance of racing horses during competitions and are therefore on the International Federation for Equestrian Sports' list of banned substances. However, a positive antidoping test may be due to the consumption of feed (mainly alfalfa or oats) contaminated by poppy seeds containing the alkaloids morphine and codeine. In order to determine whether a positive antidoping test is the result of an intentional abuse of opiates or ingestion of feed contaminated by poppy seeds, we optimised conditions for the extraction of morphine and codeine from dehydrated alfalfa and developed and validated a gas chromatographic-mass spectrometric method for the simultaneous determination of both analytes. The most efficient extraction of morphine and codeine from dehydrated alfalfa was achieved using a citrate buffer pH4 followed by solid phase extraction. The method showed good linearity (R^2>0.9980) in the tested concentration range (50-1600 ng/g), as well as good precision (RSD<4 %), accuracy (95.5-97.7 %), and sensitivity (limit of detection=22 and 25 ng/g for morphine and codeine, respectively). The proposed method was used for analysing a sample of dehydrated alfalfa that contained 1510 ng/g morphine and 327 ng/g codeine. The literature data indicates that consumption of 10 g of poppy seeds containing 732 μg of morphine gives a detectable concentration of morphine in the serum and urine of horses up to 4 hours after consumption of poppy seeds. Since the 1 g of alfalfa we analysed contained 1.51 μg of morphine, for the amount of 732 μg of morphine, a horse needs to consume only 485 g of alfalfa for the positive results of serum and urine for morphine in an antidoping test. The use of the proposed analytical method will enable the exclusion of horse feed as a cause of positive antidoping tests.

Keywords: morphine, codeine, alfalfa, gas chromatography-mass spectrometry, antidoping test

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IDENTIFICATION OF PUTATIVE ALLERGENS IN NOVEL FOODS VIA LC-ESI-MS/MS ANALYSIS AND MATLAB-BASED *"ALLERT"* WORKFLOW

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Due to its increasing incidence, food allergy is now recognized as an important public health problem. While allergenic proteins responsible for side reactions of the most common allergenic foods are well known and recognized, their identification on uncommon foods is greatly demanded. An emerging and interesting alternative for putative allergens identification is represented by *in-silico* studies to evaluate amino acid sequences similarities among proteins recognised as allergens. Some databases are already available, such as AllergenOnline (http://www.allergenonline.org) which allows the recognition of putative allergenic epitopes through an overlapping between the amino acid sequences of the protein under examination and the allergenic protein sequences present in the database, depending on established parameters. However, the pipeline is time-consuming and cannot be automatized for high-throughput analysis.

A Matlab-based workflow for Allergens idenTification (AllerT) was developed to predict the putative allergenicity of novel foods by exploiting the sequence similarity with a data set of known allergenic proteins obtained combining several available allergen databases. Data collected on protein digests after reversed-phase liquid chromatography (RPLC) coupled to electrospray ionization (ESI) and high resolution tandem mass spectrometry (MS/MS) analysis were processed with a dedicated workflow; the obtained protein identification codes were used to divide food proteins into three groups: (I) annotated known allergens, (II) proteins with identity >70% with listed allergens, and (III) proteins with identity >50%, i.e. threshold value of cross-reactivity. AllerT can perform *in-silico* digestions of putative discovered allergenic proteins, identifying similar or identical peptides overlapping known allergenic proteins. Herein, the application of a developed AllerT workflow to some common and novel foods is reported.

Keywords: Allergens, LC-MS, Proteomics, Matlab, superfoods

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DEVELOPMENT AND VALIDATION OF PESTICIDES RESIDUES DETERMINATION METHOD IN FRUITS AND VEGETABLES THROUGH LIQUID AND GAS TANDEM MASS SPECTROMETRY (LC-MS/MS AND GC-MS/MS) EMPLOYING MODIFIED QuEChERS METHODS AND A CENTRIFUGAL VACUUM CONCENTRATOR

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Plant protection product or pesticide is any substance or mixture of substances with widespread use, worldwide, with the main purpose of controlling organisms, unwanted plants and animals that cause damage to plant organisms. Accumulation of pesticide residues in food makes a necessity their determination in order to prevent the negative effects they may have on human health and environment. The purpose of this work is the development of fit for purpose methods for determination of pesticide residues in fruits and vegetables, validated according to SANTE/12682/2019 guidance. Five different categories of fruits and vegetables were selected, namely four from different high-water content commodity groups (apple, onion, lettuce, tomato) and one from the high acidity and high-water content group (orange). The methods developed are based on the QuEChERS method, slightly modified mainly in the cleanup step for each commodity. Appropriate d-SPE reagents were selected for each commodity and a Multi-Tube Vortexer was used for better and more repeatable agitation and separation of the sample phases. The methods developed are common up to the cleaning step. One part of acetonitrile extract was evaporated with a centrifugal vacuum concentrator, reconstituted with acetone/hexane mixture, followed by GC-MS/MS quantitative and qualitative analysis. The second acetonitrile extract was just acidified and injected in LC-MS/MS. Validation results satisfied the requirements (linearity, specificity, repeatability, reproducibility, sensitivity, recovery, retention time, uncertainty) set by SANTE/12682/2019 for more than 130 pesticide substances for each commodity. Limit of Quantification was set at 0.010 mg/kg for each analyte. Successful participation in proficiency testing at different commodities, as external quality assessment (requirement in standard ISO/IEC 17025), proved that the methods developed were fit for purpose.

Keywords: QuEChERS, tandem mass spectrometry, SANTE/12682/2019, centrifugal vacuum concentrator

FOOD VULNERABILITY TO FRAUDS: ASSESSMENT OF DIFFRENT FOOD INDUSTRIES

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Food fraud is common in the food industry. All industries are susceptible to food fraud, but there are certain industries where the potential for fraud is greater than others. The aim of this scientific paper is to determine where the highest probability of food fraud occurs, using a methodology for assessing the vulnerability of a given industry.

The method used to assess the vulnerability of food industries to frauds is an adapted SSAFE **(Safe Supply of Affordable Food for Everyone Everywhere)** tool for assessing vulnerability to fraud.

The assessment was made on the following industries:

A) Chocolates and Waffles
B) Milk and dairy products
C) Meat and meat products
D) Fruits and Vegetables

Results were obtained after the conducted risk assessment for vulnerability by industries, the risks and the level of risk by industry were determined. The obtained results are compared and it is determined that the highest level of risk or in other words the most vulnerable industry for fraud is the chocolate industry, in second place is the milk and dairy industry and at the same level are meat and meat products and fruits and vegetables.

The vulnerability to fraud in a given industry is variable and depends on many external factors such as the social, political and financial situation of the markets where the products are sold or the markets from which the raw materials are sourced. Increasing the number of markets and suppliers increases the risk of fraud. Globalization significantly increases the possibility of fraud.

Keywords: Fraud, Risk, Assessment, Vulnerability, Methodology

APPLICATION OF ROSEMARY AND EUCALYPTUS ESSENTIAL OILS AND THEIR MAIN COMPONENT FOR PRESERVATION OF APPLE AND PEAR FRUITS

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Nowadays, increase fruit losses are being reported due to the development of fungal postharvest diseases. In an attempt to reduce the use of synthetic fungicides, a turn towards natural products such as essential oils (EOs) and natural compounds has been made. The objective of this study was to investigate the effects of eucalyptus (Euc), rosemary (Ros) EO, their mixture (50:50 v/v) and their common main component (i.e. eucalyptol) on the quality parameters, fruit response and inhibition of blue rot (*Penicillium expansum*) in apple and pear fruits during their shelf life. The results of the present study revealed that fungal colony growth decreased *in vitro* with exposure at eucalyptus EO (Euc-300 μL/L), rosemary EO (Ros-300 μL/L) and their mixture (Euc+Ros 100 and 300 μL/L). The exposure at Ros-100 μL/L stimulated spore production, whilst Euc+Ros (100 and 300 μL/L) and eucalyptol (100 and 300 μL/L) decreased spore germination. Moreover, the *in vivo* applied treatments resulted in decreased lesion growth of *P. expansum* in apple and pear fruits. Respiration rate increased with the application of Euc+Ros at 300 μL/L and eucalyptus EO (Euc-100 μL/L and Euc-300 μL/L) for both assessed fruits. On the other hand, no significant differences were reported on apples and pears total soluble solids and acidity values. The application of Euc+Ros-300 μL/L in apples increased hydrogen peroxide (H_2O_2) levels, whilst Euc-100 and Euc-300 μL/L increased lipid peroxidation levels. Regarding pear fruits, exposure to Euc-100 μL/L and Ros-100 μL/L resulted in increased H_2O_2 whereas, Euc-100 μL/L, Ros- (100 and 300 μL/L) and eucalyptol (100 and 300 μL/L) also increased lipid peroxidation. The findings of this study indicate that the investigated natural products can be explored for the preservation of fresh apples and pears, as alternative natural fungicides.

Keywords: Apples; Pears; Essential oils; Fruit quality; Penicillium expansum

SODIUM CHLORIDE AND ORGANIC ACIDS ELEVATE *BACILLUS CEREUS* RESPIRATORY ACTIVITY IN CULTURE MEDIA

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Bacillus cereus is the leading etiological agent of toxin-induced foodborne diseases. Next to the pathogenic properties of infective strains, the environment plays a significant role in the efficiency of toxin production. Available nutrients, sodium chloride (NaCl), pH and additives can affect the total pathogenic potential of *B. cereus*. Acids and salts induce a secondary-oxidative stress response that leads to the formation of reactive oxygen species (ROS) in bacteria, which might be followed by an elevated respiratory activity. To evaluate perturbation of *B. cereus* respiratory activity in the presence of NaCl, acetic, citric, malic and tartaric acid in real-time, we developed a novel assay using a Seahorse XF24 Analyzer (Agilent, USA). Prior to analysis, minimum inhibitory and bactericidal concentrations were estimated for each compound. After basal respiration measurements, compounds were sequentially added, and oxygen consumption rate (OCR) was assessed in PPS and TSB media. Results showed that basal respiration rates for *B. cereus* strains variate from 448-512 pmol/min. Treatment with 10% NaCl resulted in a marked recession of OCR in PPS to 300 pmol/min in 5 min. However, NaCl accelerated respiration up to 850 pmol/min in TSB, indicating that the present nutrients influence the bacterial metabolic activity and inhibit bactericidal effects. The minimal inhibitory concentration of organic acids caused the gradual recession of OCR at pH 5.30 in PPS after exposure to acetic, malic and tartaric acid. In opposite, citric acid dynamically elevated respiratory activity up to ≈1600 pmol/min in 180 min. Our data indicate that NaCl and organic acids induce different metabolic changes and perturbation of cellular respiration, which consequently may affect the efficacy of preservatives against *B. cereus* in different food environments.

Keywords: Bacillus cereus, Seahorse XF24 analyzer, sodium-chloride, organic acids

SANICULA EUROPEA PHENOLIC ACIDS AND COUMARINS CONTENT AND ANTIOXIDANT PROPEERTIES

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Sanicula europea L., wood sanicle (Apiaceae), is a perennial herbaceous plant with a significant role in folk medicine in European countries like France, Austria, and Switzerland. Its leaves and roots are used for the preparation of the teas for internal and external use. Prepared with water or wine, infusions are used for the healing of gastrointestinal and respiratory tract or kidney problems. Since sanicle possesses healing effects, this study was focused on the determination of phenolic acids and coumarins content and antioxidant properties of aqueous, 70% ethanolic, methanolic, and ethyl acetatic extracts obtained by ultrasonic extraction from leaves and stems of the plant in the fruiting phase. The phenolic acids quantity in extracts (C=1 mg/mL) was determined spectrophotometrically using Arnow's reagent. The highest content was measured in ethanolic (227.9 mg equivalents of caffeic acid (CAE)/g of dry extract (DE)), and methanolic sample (212.5 mg CAE/g DE). The total coumarin content was expressed as milligrams of coumarin equivalents (CE) per gram of DE. The highest coumarins concentrations were found in the ethanolic and methanolic samples (271.9 and 211.8 mg CE/g DE, respectively). Spectrophotometric β-carotene-linoleic acid and FRAP assays were applied for the determination of antioxidant activity and standards BHT and BHA were tested for comparison. In the β-carotene-linoleic acid test, the results were presented as IC_{50} values. The best activity had methanolic (0.70 mg/mL) and ethanolic extracts (0.85 mg/mL), still, lower than BHT (0.017 mg/mL) and BHA (0.019 mg/mL). Similar results were obtained in FRAP assay, but methanolic (174.41 μmol Fe^{2+}/g DE) and ethanolic extracts (200.63 μmol Fe^{2+}/g DE) were stronger than BHT and BHA (154.66 and 160.71 μmol Fe^{2+}/g DE). Obtained results suggest that the future emphasis for potential consummation should be on ethanolic extract and wine infusion rather than on aqueous extract and tea.

Keywords: Wood sanicle, Phenolic acids, Coumarins, β-Carotene, FRAP

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LIPIDOME CHARACTERIZATION OF ALGA WAKAME (*UNDARIA PINNATIFIDA*) BY LIQUID CHROMATOGRAPHY COUPLED TO ELECTROSPRAY IONIZATION AND TANDEM MASS SPECTROMETRY

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Undaria pinnatifida is a pluricellular life-form belonging to the group of Algae, composed of more than 30,000 aquatic, oxygen-evolving and photosynthetic autotroph organisms also identified by its Japanese name Wakame [1]. Wakame is nowadays consumed around the world, including Western countries, as a nutraceutical food or supplement. With regard to lipid fraction, several studies on brown algae have been focused on minor arsenosugar phospholipids [2] while glycolipids (GL) and phospholipids (PL) are important in red algae [3] but they have been only partially investigated in brown algae [4]. This study is focused on the characterization of these compounds by liquid chromatography (LC), either hydrophilic interaction LC (HILIC) or reversed-phase LC (RPLC), coupled to electrospray ionization (ESI) and mass spectrometry (MS), operated both in high and in low resolution mode. Through the acquisition of single (MS) and tandem (MS/MS) mass spectra more than 200 PL and GL of *U. pinnatifida* extracts prepared following the Bligh Dyer protocol [5] were recognized in terms of lipid class, fatty acids composition (length and number of unsaturations) and regiochemistry, namely 16 sulfoquinovosyl diacylglycerols (SQDG), 6 sulfoquinovosyl monoacylglycerols (SQMG), 12 diglycosyl diacylglycerols (DGDG), 5 diglycosyl monoacylglycerols (DGMG), 29 phosphatidylglycerols (PG), 8 lysophosphatidylglycerols (LPG), 19 phosphatidylinositols (PI), 14 phosphatidic acids (PA), 19 phosphatidylethanolamines (PE), 8 lysophosphatidylethanolamines (LPE), 38 phosphatidylcholines (PC) and 27 lysophosphatidylcholines (LPC). The palmitic acid was the most abundant saturated acyl chain, whereas the monounsaturated oleic and the polyunsaturated C18:2 and C20:4 chains were the prevailing ones. Odd-numbered acyl chains, i.e., C15:0, C17:0, C19:0, and C19:1, were also recognized. The developed analytical approach might open the way to extend lipidome investigation also for other edible marine algae, thus emphasizing their potential role as source of bioactive lipids.

Keywords: Wakame, ESI-LC-MS/MS, lipidome, novel foods

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DETERMINATION OF ANTIOXIDANT POTENTIAL OF DEHYDRATED SOUP ENRICHED WITH LYOPHILIZED MUSHROOM EXTRACTS

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The aim of this study was to determine the antioxidant potential of dehydrated soup enriched with different types of lyophilized mushroom extracts. Three mushroom species were collected from the Republic of North Macedonia: *Suillus granulatus*, *Coriolus versicolor* and *Fuscoporia torulosa*. After their determination and drying, aqueous and ethanolic extracts (50% EtOH) were prepared. Moreover, both extracts were lyophilized showing high antioxidant potential, determined through the ability to capture free DPPH radicals (73.38–81.60%) and the conjugated diene method (69.25–77.06%). Both types of lyophilized extracts were added in industrially produced dehydrated vegetable soup in order to improve its quality properties, as well as the biological activity. Thus, ten samples of dehydrated soups were produced. Higher values for all antioxidant tests were obtained in all tested soup samples enriched with lyophilized mushroom extracts, compared to the control sample. According to the DPPH radical test (54.14–61.41%) and the ability to chelate iron ions (36.68–43.99%), higher values were obtained in soups enriched with lyophilized aqueous extracts, while the method of conjugated dienes showed higher values in soups enriched with lyophilized ethanolic extracts (47.27–60.60%). From the aspect of product traceability during storage, it can be noticed that in all antioxidant tests the values were constant until the 30^{th} day after production, and then they decreased minimally until the 45^{th} day after production. Namely, on the 45^{th} day after production, the largest decrease in antioxidant activity of soups enriched with lyophilized aqueous extracts was observed with the conjugated diene method (43.17–54.79%). The antioxidant activity of all tested samples on 0^{th}, 15^{th} and 30^{th} day after production differed statistically significant ($p<0.05$) compared to 45^{th}, 60^{th} and 90^{th} day after production. Therefore, it can be concluded that the obtained product showed certain antioxidant potential which is significantly higher compared to the control sample.

Keywords: mushroom, extract, soup, antioxidant potential.

EFFECT OF *ARBUTUS UNEDO* FRUIT SYRUP SUPPLEMENTATION ON YOGURT QUALITY AND ANTIOXIDANT PROPERTIES

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Arbutus unedo is wide spread in the Mediterranean basin; it is endowed with antiseptic and diuretic properties and laxative effects. This study is a contribution to the assessment of antioxidant and antibacterial activities of *A. unedo* fruits and its valorization as syrup which was incorporated in a bilayer yogurt.

Chemical characterization of *A. unedo* fruits and syrup was conducted on the total polyphenols by Folin Ciocalteu method, total flavonoids using $AlCl_3$ method, total tannins via HCl/ butan 1-ol assay and total anthocyannins using differential pH method. The antioxidant activity of the 80%ethanol extracts was evaluated using three methods namely two radical scavenging assays against 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) and ferric reducing antioxidant power (FRAP) method. The antibacterial activity was tested against seven pathogenic bacteria using agar well diffusion method in which inhibition diameters were measured.

A bilayer yogurt was prepared by addition of 10 and 20% of *A. unedo* syrup, while physicochemical, rheological and microbiological analysis were carried out during storage in addition to the antioxidant activity assessment.

The total polyphenols contents were 33.84±0.60 and 25.05±0.07 mg GAE/gof extract for fruits and syrup, respectively. The total tannins and anthocyannins were the most affected after syrup preparation with a loss percentages of 91 and 75 % respectively.Both *A. unedo* fruits and syrup showed a high antioxidant potential, they had also an important antibacterial activity against tested bacteria, the inhibition diameter reached 40.33±0.17mm against *Aoromonas aerugenosa* for the fruits ethanolic extract at 100 mg/mL. The prepared yogurts showed stability for the physicochemical and microbiological properties during storage. The rheological analysis revealed that addition of *A. unedo* syrup increases the yogurt consistency. The evaluation of the antioxidant activity showed that fermentation improved radical scavenging ability of the added *A. unedo* syrup at the beginning. This activity has been decreased during storage. This study showed that *A. unedo* fruits present a source of bioactive molecules which keep their biological activities under heat treatment and lactic fermentation.

Keywords: Arbutus unedo, syrup, bioactive compounds, functional yogurt.

PRODUCTION OF β-GLUCAN ENRICHED STRAWBERRY SNACKS BY VACUUM IMPREGNATION TECHNOLOGY: PROCESS OPTIMIZATION AND QUALITY ASSESSMENT

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Increased consumer interest in healthy snacks, especially during the COVID-19 pandemic, has accompanied by the development and production of functional foods. Nowadays, researches have increased interest in development and production of functional snacks that contain β-glucan, which exhibit properties like a prebiotics effects and immune support. Clinical studies have shown that daily consumption of 250-500 mg β-glucan has positive impacts on the immune system. Functional fruit-based products can be produced by vacuum impregnation (VI) technology while preserving quality. This technology rapidly introduces solutions of bioactive compounds into porous structures of fruit tissues under the controlled process conditions. VI, an osmotic treatment, can obtain minimally processed fruits, intermediate moisture fruits, and functional foods and could improve different food preservation processes. In this study, fresh strawberries were enriched with yeast β-glucan by VI technology for the first time to enhance the immune system and produce functional and healthy strawberry snacks. Thus, fresh strawberries were impregnated with apple juice concentrate contained β-glucan by VI treatment. VI process parameters were optimized by Response Surface Methodology (RSM), and the optimum point was determined as 215 mbar of vacuum level, 40 °C of process temperature, and 10 min of vacuum time. After the production was conducted at the optimum point, β-glucan enriched strawberries were dried in a vacuum oven until the water activity <0.65. Also, Osmotic Dehydration (OD)-applied strawberries were produced for comparison. Quality analyses were performed on total soluble solids, pH, moisture, water activity, acidity, total phenolic compounds, antioxidant activity, color, texture, microbiological, and sensory analysis. As a result of this study, it can be concluded that the functional whole fruit snack obtained by VI technology is a promising concept and could be the basis for the future developments of functional food products.

Keywords: β-glucan, Vacuum impregnation, strawberry snacks, functional snacks, immune support

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ADVANCES IN FOOD TECHNOLOGY FOR SHELF LIFE PROLONGATION

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The adoption of non-conventional technologies for food preservation is gaining great attention among researchers. In particular, ionizing radiation (Gamma ray; Electrobeam and X-ray) is a technology based on non-thermal mechanism of action with considerable potential for food decontamination. X-rays are generated by a machine sourced-ionizing radiation and no radioactive substances are involved in the system. X-rays do not produce radioactive waste and are able to pass through thick materials. Ionizing radiations are able to inactivate not only various microorganisms but also pathogenic bacteria, parasites and viruses. The inactivation occurs as a result of the direct or indirect effect of the radiation energy by damaging most often the genetic material, thus preventing multiplication and most cellular functions. More than 60 countries have approved the use of irradiation. All these features make this technology suitable to treat food already packaged, avoiding re-contamination. The present study focused the attention on X-rays to treat artisanal and industrial ricotta cheese. Packaged samples were subjected to X-ray treatment at 0.5, 2 and 3 kGy. The effectiveness of this treatment to prolong cheese shelf life was evaluated in terms of microbiological and sensory quality. Results highlight that artisanal ricotta irradiated at the two highest intensities (2 and 3 kGy) remained acceptable for more than 20 days, whereas the untreated samples became unacceptable after only 3 days of storage. The shelf life of the product irradiated at 0.5 kGy was limited to 14 days, due to the appearance of sensory defects. The industrial product irradiated at all X-rays intensities recorded a significant shelf life prolongation up to 84 days compared with the control, which was rejected after 40 days due to sensory defects. Therefore, the results show that X-ray treatment can significantly prolong the shelf life of ricotta cheese, boosting the marketability of this fresh dairy product far from the local production sites.

Keywords: non-thermal technologies, mild technique, cheese preservation, ricotta shelf life

WHEAT GERM AS A SOURCE OF PROTEIN IN FORTIFIED PASTA

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Protein is one of the basic nutrients necessary for the proper functioning of the human organism. When introducing new sources of protein to the human diet, one should base it on well-known and widely accepted products. An ideal carrier of new protein sources is pasta, which is a universal product, easy to prepare and whose consumption is constantly increasing. The aim of this study was to improve the protein content and amino acid composition of pasta by adding wheat germ or wheat germ protein concentrate. Wheat germ is a by-product of wheat processing. However, it is a good source of protein and essential amino acids, including lysine, which is the limiting amino acid in pasta. The research model included analysis of the chemical composition of raw materials and pasta samples (raw and cooked), pasta texture and cooking quality parameters, and evaluation by a consumer panel. Study showed that the addition of wheat germ (5/10/15/20/25%) and germ protein concentrate (4/8/12%) significantly increased the protein content of the products. The addition of 25% wheat germ and 8% of wheat germ protein concentrate made it possible to obtain a high-protein product according to the EU definition. The lysine content of the sample with the addition of 25% wheat germ increased almost 2.5 times, while with the addition of 12% protein concentrate by more than threefold. The addition of wheat germ also increased the fat and dietary fibre content of the samples, while the addition of protein concentrate resulted in a decrease in the content of these macronutrients. Pasta samples with both germ components had good cooking quality characteristics and high consumer acceptance, but pasta with protein concentrate performed better than pasta with wheat germ.

Keywords: pasta, wheat germ, protein concentrate, aminoacids.

APPLE POMACE AS SOURCE OF BIOACTIVE COMPOUNDS FOR MEAT ENRICHMENT

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Apple pomace, the press cake resulting from apple juice production, contains plenty of compounds with nutritional and health properties, among which dietary fibre, minerals and phenolic compounds. The present research aimed at investigating the composition of this by-product, with particular interest for the phenolic fraction, and developing functional products based on beef meat added with dried apple pomace. Ultrasound-assisted extraction was used to isolate the phenolic compounds, then the extract fractionation was performed and the free and bound phenols, together with the hydrolysed fractions in acid and alkaline conditions, were investigated. Ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometer detector and ultraviolet spectrophotometric detector was used to obtain the qualitative and quantitative profile of phenols. Phloridzin and different glycosylated forms of quercetin were the most represented among the phenolic compounds. The antioxidant properties of apple pomace extract were measured by in vitro tests, able to evaluate both the antiradical and the reducing capacity. The second part of the study was relative to the addition of dried apple pomace to minced beef meat, with two addition levels, 4 and 8%. Then apple pomace added burgers were prepared together with non-added burgers as control. The prepared burgers were evaluated for quality attributes including physical characteristics and shelf-life test. Moreover, the samples were cooked in a domestic microwave and then subjected to sensory evaluation. The sensory properties and overall acceptability were higher for burgers added with 4% apple pomace, but satisfactory results have been obtained also for 8% added burgers. The results of this investigation are promising as the addition of vegetable by-products, rich in fibre and phenolic compounds, improves the nutritional and health properties of the meat. In fact, low-fat, fibre rich and antioxidant/antimicrobial rich products can be prepared, avoiding or reducing the addition of additives as nitrites and nitrates.

Keywords: vegetable by-products, antioxidants, high-performance liquid chromatography, enriched meat, sensory properties

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EFFECTS OF ELECTRICAL TREATMENTS ON MILK PROTEINS AND FAT GLOBULES

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Milk is regarded as a wholesome food due to its valuable contents of protein, fat, lactose, minerals, and vitamins. Fresh milk needs to be sterilized for safety and shelf-life. Milk with a longer shelf-life is required to have access to markets of distant countries. Food consumers are seeking safe, healthy, and minimally processed food with a "fresh-like" taste. Such a market demand initiated intense competition in the food industry to develop alternative processing technologies that can ensure safety while keeping the "fresh-like" characteristics of the foods. In this regard, researchers have explored the applicability of emerging food processing technologies.

One of these novel electrical emerging technologies is Ohmic heating. It is based on the principle of heating by passing an alternating current through the product between two electrodes. The electrical energy conducted through the food is converted into thermal energy owing to the electrical resistance of food leading to volumetric and instantaneous heating. Researches have shown that electricity poses both thermal, which is ohmic heating, and non-thermal effects on the microorganisms. Another electrical heating process regarded as a moderate electric field (MEF) has been used as a novel non-thermal treatment. These novel technologies for milk processing may provide the milk industry with reliable, efficient and clean processing systems for drinking milk manufacture.

Conventional treatments applied to milk can disrupt the native structure of the milk fat globule and lead to adsorption of casein micelles and whey proteins onto the milk fat globule membrane surface. The changes that occurred through heating, such as denaturation of proteins, the interaction of denatured whey proteins with casein micelles, and casein micelle dissociation, can affect the functionality of milk. In this review, the effects of ohmic heating and MEF systems on milk proteins and fat globules, either under experimental examination or already commercially available, were summarized.

Keywords: ohmic, mild electric field, milk.

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EFFECT OF SURFACTANTS ON THE RELEASE KINETICS OF CATECHINS ENCAPSULATED IN WATER-IN-OIL-IN-WATER (W1/O/W2) DOUBLE EMULSIONS

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Catechins, a class of polyphenolic compounds, have recently attracted great attention due to their beneficial effects on human health. However, their use in modern therapy is limited because of their low bioavailability and limited stability at physiological pH, with rapid degradation under alkaline, oxygen and light conditions.

This study was designed to evaluate the effect of catechins encapsulation in a double emulsion water-in-oil-in water on the stability and the release kinetics during storage. Thus, various formulations using the ultrasonic process and different concentrations of surfactants namely chitosane and sodium caseinate were developed.

The obtained results showed that the used surfactant agent and the storage temperature significantly influenced the release rate of catechins from double emulsions ($p<0.05$). For all double emulsion formulations, the increase in temperature led to higher leakage percentage of catechins during storage. At 5°C, only 6.80% of catechins leaked after 60 days of storage, which is about a half of the amount leaked at 25°C (13.08%). Thus, to limit leakage, it is advantageous to store the double emulsions at refrigerated temperature.

Likewise, the employed surfactant agent influenced the stability of the double emulsions. Indeed, it was observed that the release of catechins is minimal for the casein and chitosan-based formulations and maximal in the absence of a chitosan. The determination of kinetic parameters of catechins double emulsion showed that the energy of activation (E_a) increased when chitosane was employed as a surfactant agent.

The use of the double emulsion technique was efficient for the encapsulation of catechins. Although, the stability depends on the nature of the surfactant agent. Double emulsion containing chitosan and sodium caseinate was the most effective.

Keywords: Catechins, double emulsion, surfactant, stability, release kinetics.

Acknowledgements: The authors express their gratitude for the support of the Department of Physics at the Faculty of Sciences of Tunis for the double emulsion preparation.

EVALUATION OF TECHNO-FUNCTIONAL AND PHYSICOCHEMICAL PROPERTIES OF HOT AIR-DRIED JACKFRUIT (*Artocarpus heterophyllus* Lam) POWDER

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Jackfruit (*Artocarpus heterophyllus* Lam) is an evergreen tree native to tropical Asia with many food and non-food uses. As fresh ripened fruits are highly perishable, developing products in the form of fruit powders would be a good approach to retain stability over a long storage time and reduce wastage. Jack fruit powder was produced from a firm-flesh variety available in Sri Lanka by subjecting the flesh to hot air drying at three temperatures (70^0C, 80^0C and 85^0C) and techno-functional and physicochemical properties (moisture, pH, particle size distribution, color, flowability, rehydration properties-solubility, wettability, sinkability and dispersibility) of the powder were evaluated. Retention of natural color and typical aroma were observed in powder obtained at 80^0C. Powder obtained at 70^0C showed a clumping behavior and an unpleasant aroma while powder obtained at 85^0C gave off a cooked aroma.All jack fruit powder samples had desirable moisture contents (M_d<5%). The highest acidity (pH 4.75±0.015) was observed in the powder obtained from flesh dried at 70^0C. Powder from sample dried at 85^0C showed good flowability properties according to the Carr index (12.50) and Hausner ratio (1.14). The highest median particle size (D50) was observed in powder obtained from sample dried at 80^0C (949.9 nm) and the lowest value was recorded in the powder obtained at 85 ^{0}C (871.2 nm). In the powder from the sample dried at 80^0C, sinkability was 7.45±0.08s and the solubility was highest (70.06%±0.19). The powder obtained at 85^0C recorded the highest wettability (1.48s±0.04) and dispersibility values (21.78s±0.24). According to the chroma analysis, lightness was highest (81.56±0.50) in the powder obtained at 80^0C and redness was highest (5.85±0.27) in powder obtained at 85^0C. Jack fruit powder has a very hygroscopic nature and the adsorption isotherm corresponds to the Type IV isotherm shape revealing a swellable hydrophilic character. It can be concluded that hot air drying is a suitable method for preserving ripen jack fruit and according to color, aroma and solubility properties, the effective temperature for drying is 80^0C.

Keywords: jack fruit, hot air drying, particle size, powder, rehydration properties

EFFECTS OF A BACTERIAL CONSORTIUM ON DROUGHT STRESS RESPONSE OF DIFFERENT TOMATO CULTIVARS

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Twelve tomato cultivars (Roma VF, Red Cherry, Pantano, Costoluto Fiorentino, Cuor di Bue, Rio Grande, Principe Borghese, Redpear Sel. Franchi, S. Marzano, Beefsteak, Dattero Ibrido F1 and Marglobe) treated with a bacterial consortium containing 5 strains isolated from the *Ramonda* sp. rizosphere and selected based on their PGP characteristics (Rs1 – *Brevibacterium frigotolerans*, Rn2 - *Micrococcus luteus*, P3 – *Pseudomonas vranovensis*, P4 – *Pseudomonas putida* and Rn5 – *Bacillus licheniformis*) were screened for enhanced drought stress tolerance. Seeds of each cultivar were incubated in a mixture of five overnight cultures (treated) or a pure growth medium (control) and sown into pots. All plants were grown under well-watered conditions for three weeks. Half the plants were subsequently exposed to drought by completely withholding watering for the next two weeks. In order to investigate the response of the cultivars to water stress, leaf relative water content (RWC), lipid peroxidation, and proline content were estimated. Cultivar Principe Borghese treated with the consortium showed the greatest improvement in RWC (29.6%) as well as the greatest decrease in proline content (12.3x) under drought stress conditions compared to the control group. Higher RWC and lower proline content in treated drought-stressed plants was also observed in cultivars Red Cherry, Beefsteak, Redpear Sel. Franchi and Dattero Ibrido F1 (12.16%, 6.52%, 5.62%, 3% and 1.95x, 1.52x, 1.37x, 1.34x, respectively). Interestingly, no changes in lipid peroxidation intensity were observed in treated plants. These preliminary findings suggest that the consortium alleviates drought stress in some tomato cultivars through mechanisms that do not involve an increase in proline biosynthesis or oxidative stress attenuation. Moreover, the ability of the consortium to induce drought stress tolerance is shown to be cultivar-specific, with the most prominent effect on cultivar Principe Borghese. The results presented here indicate an important role plant genotype appears to have in establishing productive interactions with beneficial bacteria.

Keywords: Tomato cultivars, Bacterial consortium, Drought, Drought stress tolerance.

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DNA PROTECTIVE POTENTIAL OF FORSKOLIN ON ETHYL METHANESULFONATE-INDUCED GENOTOXITY

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Forskolin, a diterpene with multiple physiological effects, found in the root of the plant *Coleus forskohlii* (Willd.) Briq., is used in traditional medicine in the treatment of various diseases. Also, it has been proposed as a natural weight loss supplement. Considering the widespread usage for therapeutic purposes and as a weight loss agent, a sex-linked recessive lethal (SLRL) assay was performed in germ cells of *Drosophila melanogaster* to assess genotoxic and possible DNA protective effects of forskolin on ethyl methanesulfonate (EMS)-induced DNA damage. The genotoxic effect of forskolin was tested at a concentration of 100 ppm for exposure periods of 24 h. To assess the DNA protective effect, *D. melanogaster* males were treated with EMS (0.75 ppm), 24 h prior to forskolin (100 ppm). Treated *D. melanogaster* males showed significant reductions in the frequency of sex-linked recessive lethal mutations in all three broods in comparison with the negative and positive controls. The genotoxic evaluation of forskolin by *in vivo* SLRL assay indicated that this compound did not induce any genotoxic effect and also exhibited a DNA protective potential against EMS.

Keywords: Forskolin, SLRL test, in vivo, genotoxicity, antigenotoxicity

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CYTOTOXIC ACTIVITY OF WATER EXTRACTS OF TWO FUNGAL SPECIES FROM NATURE AND SUBMERGED CULTIVATIONS

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The therapeutic activities of medicinal mushrooms have been documented almost 7.000 years ago through traditional medicine practice in China. Fungal fruiting bodies were mainly used in preparation of soups, stew and tea, as hot water extract. Nowadays, numerous of studies are focused on research of medical activities of water fungal extracts – particularly anticancer activity. This study aimed to investigate cytotoxic potential of water extracts of two indigenous edible species *Coprinus comatus* and *Coprinellus truncorum*, towards human hepatocellular (HepG2) and rat pancreatic (Rin-5F) carcinoma cell lines. Cytotoxic activity of both species was estimated for fungal fruiting bodies (FB) from nature and submerged cultivated mycelia (M) and extracellular medium - filtrate (F). Cytotoxicity analysis was determined using a colorimetric MTT assay while fungal extracts were tested in concentration range of 33.33-900 µg/mL for 24 h and 72 h. The cell viability was expressed in percentages (%) after comparison with control (untreated cells), which was 100% viable. The determined effect of the extracts on the cell viability were time and dose dependent. The strongest effect on the decrease viability of tested cell lines was expressed by M extract of both species. After 72 h of treatment, at 900 µg/mL M extracts of *C. comatus* (*C. truncorum*) reduced cell viability to 24% (13%), for HepG2 cells and to 17% (22%) for Rin-5F cells, respectively. FB and F extracts of both species did not show substantial effect on reducing cell viability (63% and 98% respectively). The results indicated that both analysed M water extracts stood out as cytotoxic agents against HepG2 and Rin-5F cancer cell lines. Therefore, submerged cultivation of mycelia represents a sustainable way for producing valuable fungal biomass with bioactive components with antiproliferative activity.

Keywords: Coprinus comatus, Coprinellus truncorum, submerged cultivation, cytotoxic, MTT assay

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DIETARY INTAKE OF VITAMIN B12

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Vitamin B12, also known as cobalamin, is an important water-soluble vitamin. It plays an essential role in the production of red blood cells and DNA, as well as the proper functioning of nervous system.Vitamin B12 is naturally found in animal foods, including meats, fish, poultry, eggs and dairy. However, it can also be found in products fortified with B12, such as some varieties of bread and plant-based milk.

Unfortunately, B12 deficiency is common. You're at risk of deficiency if don't get enough from diet or aren't able to absorb enough from the food eat.Vitamin B12 deficiency is associated with a number of neurological, haematologican and gastrointestinal diseases. Recent research has also studied its relationship with colorectal cancer (CRC).

The aim of this study was to analyse the dietary intake of vitamin B12. 100 respondents participated in this research, of which 78 were female and 22 male. Respondents ranged in age from 16 to 31, with 92% of respondents being within 20-27 years.

Respondents were asked to fill semi-quantitative food frequency questionnaire, consisting of foods considered to be the best dietary sources of vitamin B12, i.e. meat, fish, milk and products, and eggs.

Median intake of vitamin B12 is the result is expressed in μg/day. Respondents got most of their intake of B12 from meat. Gender significantly affected vitamin B12 intake in both regions. Young people (16-31 years) from the Banja Luka region who participated in this survey in a large percentage (83%) consume enough vitamin B12 from food.

Better dietary habits of respondents were positively correlated with B12 intake**.**

Keywords: vitamin B12, students, food sources

UNDERSTANDING THE MOTIVES FOR TRADITIONAL FOOD CHOICE IN SERBIA

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Traditional foods are an expression of lifestyle, culture. and history. Traditional foods, consumed by people over a long period of time, play an important role in establishing local identity, culture, and custom, and they transfer cultural heritage from generation to generation. It is hypothesized that the relationship between external influences and actual food choice behavior is mediated by food-related attitudes and beliefs. The objective of this research is to better understand consumers' attitudes, expectations, and behavior toward traditional food in Serbia. The research also presents a picture of the profile of Serbian traditional food consumers in terms of their socio-demographics, attitudes, lifestyle orientations, and behavioral characteristics. The method used for the data collection was a face-to-face interview using a structured questionnaire. In total, 100 respondents (students of the second year of bachelor studies at the Faculty of Agriculture, University of Belgrade) participated in the survey. Surveyed consumers expressed a positive attitude toward traditional food, pointing out that traditional food associates them with home and family (32%) and that the main reason for consuming traditional food is its ordinary use in a diet at their homes (59%). For the majority of respondents, the source of nutritional information about traditional foods is the Internet (43%) and the vast majority of respondents believe that fraud is possible on the market in terms of the authenticity of traditional foods (85%). The price of the food is not the most important for selecting a food (64%) and the vast majority of respondents are willing to pay more for good quality food (83%). Generally, young people are very satisfied to consume traditional foods and consider that they connect them to the history of a particular locality (89%).

Keywords: Serbian traditional food, survey questionnaire

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DROSOPHILA MELANOGASTER AS A MODEL SYSTEM IN STUDYING NUTRITION – THE KNOWLEDGE OBTAINED IN WORK WITH FLY STRAINS MAINTAINED FOR MORE THAN TWO DECADES ON DIFFERENT DIETS

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Fruit fly *Drosophila melanogaster* has been representing one of the most suitable model systems for studying underlying mechanisms in various biological research for more than a hundred years. Findings in the field of genetics and genomics certainly paved the way for this model system to be used in the study of the mechanisms underlying complex human diseases, such as Alzheimer's, Parkinson's and others. It can be also used in studying the effects of nutrition and/or malnutrition, and to some extent, be extrapolated to humans. In the year 2000, we collected *D. melanogaster* flies from natural population and established and maintained five "nutritional" strains. Flies were reared on standard cornmeal laboratory food and four fruit/vegetable diets. All substrates were analysed for protein/carbohydrate ratios, and their antioxidant properties were established. During the years of running *in vivo* studies, we have shown that diet can affect individuals on many levels. As the most pertinent to the fitness, we observed the impact of diet on body morphology (the size, shape and symmetry of certain body traits), the chemistry of odors essential for social and sexual recognition, behavior, and life history traits. We also considered food choice in those flies in order to find out how they maintained nutritional homeostasis. In addition, we experimented with standard *Drosophila* diet by adding chemicals and some bioactive plant components in growing substrates (such as the black chokeberry fruit extract), widely used as a supplementary source in human diet and medicine. Here we will present a short overview of the most important results of our research, dealing with the fruit fly nutrition. Having in mind the key similarity in some metabolic pathways in *Drosophila* and mammals, our studies could be applicable for understanding the possible effects of various diets, drug-treatments and their potential benefits for humans.

Keywords: Drosophila, nutrition, human health benefits

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TRADITIONAL USE OF PLANTS IN THE HUMAN DIET IN TIMOK REGION

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The Valley of Timok River is located in the Eastern parts of Serbia with a special geographical location and rich biodiversity. There are parts of rural areas with a high percentage of unpolluted nature with a huge potential for the production of healthy food. The investigation was conducted on the territory of 10 Timok villages (located in the valley of the Timok River, Crni Timok and Beli Timok). The survey has included 94 respondents, 33 men and 61 women, which reported 1549 uses of plants. A semi-structured, anonymous ethnobotanical interview has been used for the data collection. The respondents indicated that they use 108 plants species in total, 52 species in nutrition. From 1549 use-reports of the Timok local population 435 use-reports shown that plants are used as fresh, cooked, as juice, jam, marmalade or compote. The most commonly used are the next species: *Armoracia rusticana* Gaertn.,C.A.Mey.&Scherb. (33 use-reports), *Sambucus nigra* L. (27 use-reports), *Fragaria* x *ananassa* Dush.F. (24 use-reports), *Urtica dioica* L. and *Vitis vinifera* L. (22 use-reports). A fresh root of *A. rusticana* is used for treatement of migraine, sinusitis, productive cough, abdominal pains, improvement of appetite, chills, fever and improve immunity. The local population use juice from the flower of *S. nigra* in the case of bronchitis and productive cough. The respondents use the fruit of strawberry in the diet in form of juice, jam, marmalade for improving immunity and cardiac insufficiency. Aerial parts or the leaves *U. dioica* are used fresh or cooked in the diet for immunity improvement, cardiac insufficiency and anaemia. Fruit or fresh juice *V. vinifera* are used in the treatment of abdominal pains, constipation, as well as the improving immunity. This ethnopharmacological survey gives us the possibility to save the knowledge about the use of plants in the Timok region, and increase the awareness of people about the health aspect of these plants.

Keywords: Timok region, Traditional use, Plants, Food

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THE SUPEROXIDE DISMUTASE AND CATALASE - LIKE ACTIVITIES OF ROSMARINIC ACID IN L-NAME INDUCED HYPERTENSIVE NEPHROPATHY

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Spontaneously hypertensive rats (SHR) chronically treated with an inhibitor of NO synthase, NG-nitro-L-arginine methyl ester (L-NAME), develops chronic kidney disease characterized by massive albuminuria, proteinuria, arteriolar fibrinoid necrosis and glomerular sclerosis. Previously we showed that rosmarinic acid (RA) supplementation could improve albuminuria in the L-NAME/SHR model of hypertensive nephropathy, independently of NO bioavailability. Because albuminuria is associated with increased in oxidative stress, we evaluate whether chronic consumption of RA could affect kidney oxidative stress in L-NAME/SHRs by altering the antioxidant enzyme expression and activity. Male SHRs, aged 6 months and weighing about 300 g, were divided into four groups. The first group, that served as control (SHRC), received tap water. The second group, SHR+RA, was treated with rosmarinic acid (RA, 15mg/kg/day, gavage, 4 weeks). The third group, L-NAME/SHR received L-NAME (10 mg/kg/day) in drinking water during the next 4 weeks. The fourth group, L-NAME/SHR+RA, received rosmarinic acid (RA, 15mg/kg/day) by gavage in the following 4 weeks, at the same time with L-NAME (10 mg/kg/day) dissolved in drinking water. Urine albumin, kidney superoxide anion (O_2^-), TBARS, superoxide dismutase (SOD) and catalase (CAT) activity were measured spectrophotometrically, and kidney SOD and CAT expressions were detected by western blot. Correlations between obtained parameters were also examined. The expression and activity of both enzymes, SOD and CAT, were significantly reduced in L-NAME/SHR, while O_2^- and TBARS were significantly elevated compared to SHRC. RA significantly elevates SOD and CAT activity without changing their expression and reduces O_2^- and TBARS in L-NAME/SHR+RA to control levels. TBARS exhibits a significant positive correlation with O_2^- and albuminuria, while negatively correlates with SOD and CAT activities. Our results indicate a close link between oxidative stress and renal dysfunction in hypertensive nephropathy and that the antioxidant efficacy of RA stems from its SOD and CAT mimetic activities.

Keywords: hypertensive nephropathy, rosmarinic acid, superoxide dismutase, catalase, oxidative stress

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BILE ACIDS – A PHARMACO-NUTRACEUTICAL APPROACH

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Due to amphipathic properties, bile acids (BAs), a major constituent of the bile, have several physiological functions including solubilization and absorption of dietary lipids and lipophilic xenobiotics, solubilization of cholesterol in bile, stimulation of bile flow and biliary phospholipid secretion and cholesterol excretion. BAs also have antibacterial properties, significantly influencing the composition of intestinal bacterial flora and maintaining the sterility of the biliary tree. The aim of this study was to summarize the present knowledge in the innovative field of BAs pharmacology, to reveal novel mechanisms of their action, focusing on clinically-relevant aspects. A detailed and comprehensive search of PubMed and Scopus databases was carried out for original and review articles. Initial mainly mechanistic function of BAs has been expanded to diverse and versatile regulatory functions involving cell homeostasis, hepatic and extra-hepatic metabolic processes, regulation of cell proliferation and death, and carcinogenesis. BAs are now recognized as signaling molecules capable to activate specific nuclear and membrane receptors, multiple cellular kinase signaling pathways. BAs can directly and through epigenetic mechanisms regulate the expression of genes involved in integrative metabolism and homeostasis. Disturbances in BA homeostasis contribute to the development of intestinal dysbiosis, dyslipidemia, obesity, inflammatory diseases, common metabolic diseases such as diabetes, non-alcoholic fatty liver disease, liver cirrhosis, hepatobiliary and intestinal disorders, carcinogenesis, even the disorders of the central nervous system. The field of research of natural and semisynthetic derivatives of BAs and BA-receptors ligands has been extensively expanding and several BA-based therapeutics have been approved for the treatment of liver and intestinal diseases. These biomolecules may also influence drug bioavailability and metabolism, by interacting with nuclear receptor-transcriptional networks, the expression of membrane transport proteins and drug-metabolizing enzymes. Bile acids can be utilized in the formulation of conventional dosage forms, but also of novel micellar, vesicular and polymer-based therapeutic systems.

Keywords: metabolic syndrome, nuclear receptor, microflora, inflammation, carcinogenesis

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DIETARY SUPPLEMENTATION WITH BILBERRY EXTRACT MODULATES THE EXPRESSION OF GENES IN MICE HIPPOCAMPUS

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Dietary anthocyanins, phytochemicals abundantly found in berries and berry-derived products, have been linked with lower cognitive decline, improved cognitive performance and protective effects against neurological diseases. Still, the molecular mechanisms underlying these benefits are not fully established. The aim of this study was to evaluate the effects of anthocyanin-rich bilberry extract on gene expression in the hippocampus to contribute to unveiling the mechanisms of reported neuroprotective action of anthocyanin-rich foods. Male ApoE-/- mice were fed a control diet with or without 0.02% anthocyanin-rich extract for 12 weeks. At the end of the experimental period, hippocampi were collected to examine the global gene expression using microarrays. Bioinformatics analyses were conducted to explore the functions of modulated genes and identify potential mediators of the nutrigenomic effect. We observed that anthocyanin-rich bilberry extract differentially modulated the expression of around 1700 genes in the hippocampus. Bioinformatics analyses revealed that these genes are involved in the regulation of neurogenesis, synaptic function, inflammation, cell adhesion, and metabolism, but also Alzheimer's and Parkinson's disease pathology and cognitive dysfunction. Several miRNAs, including mir-124-3p, mir-340-5p, mir-15a-5p, mir-9-5p, mir-329-3p and mir-181a-5p, were identified as potential mediators of the genomic effect to anthocyanin-rich extract supplementation. Bioinformatics analyses also revealed putative transcription factors such as RELA, TRP53, SP1, JUN, and *in-silico* analyses suggested that anthocyanins could bind to them and potentially affect their regulation of gene expression. Taken together, this study showed a multi-target mode of action of anthocyanins in the hippocampus and provided novel findings of the potential molecular mechanisms of neuroprotective effects associated with anthocyanin-rich food sources. Further mechanistic studies are, however, needed to validate these mechanisms *in vivo*.

Keywords: Bilberry, Anthocyanins, Gene expression, Bioinformatics

CAFFEIC ACID STIMULATES EXTRAVILLOUS TROPHOBLAST CELL MIGRATION

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In the everyday diet of pregnant women, plant products with high concentrations of polyphenol compounds are relatively common. Caffeic acid is a natural polyphenolic compound deriving from various fruits, spices and beverages present in nutrition, with proven antioxidative, anti-inflammatory and anti-cancer activities. In the western world, daily intake of caffeic acid goes as high as 260 mg, 90% of which can be resorbed in the digestive system. Having that in mind, it is of great importance to explore the effects of caffeic acid on placental function. Extravillous trophoblast cells are specific placental cells that invade into the uterine stroma and spiral arteries and directly contact the mother's tissue. This study was undertaken to explore the effects of caffeic acid on trophoblast cell migration and the levels of matrix metalloproteinases (MMP)-2 and -9, essential for the trophoblast invasion process. HTR-8/SVneo extravillous trophoblast cell line was treated with caffeic acid at 10 µM and 100 µM. Cell viability was assessed by MTT assay. The effect of caffeic acid on HTR-8/SVneo cell migration was determined using a „wound healing" scratch assay. The levels of MMP-2 and MMP-9 were assessed by SDS-PAGE gelatine zymography. The results of the study show that caffeic acid stimulated HTR-8/SVneo cell migration to 120% of control ($p<0.001$) and 113% of control ($p<0.05$) for 10 µM and 100 µM of caffeic acid, respectively. Densitometric analysis of obtained zymograms showed that following treatment with caffeic acid MMP-9 levels increased to 144% of control ($p<0.01$) for 10 µM and to 148% of control ($p<0.01$) for 100 µM of caffeic acid applied. MMP-2 wasn't significantly changed by treatment and neither was cell viability. The results point to potential beneficial effects of food and/or supplements rich in caffeic acid consumption during early pregnancy.

Keywords: Polyphenols, Placenta, Caffeic acid, MMP, Trophoblast

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ANTITUMOR ACTIVITY OF LAMIACEAE PLANTS FREQUENTLY USED IN SERBIAN FOLK MEDICINE AND CUISINE

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Recently, cancer research has focused on searching for new and more effective antitumor agents of natural origin that can activate multiple defence mechanisms and selectively damage transformed cells. The goal of this research was to assess different antitumor mechanisms of ethanolic extracts of 18 Lamiaceae species traditionally used in Serbian folk medicine and cuisine, as well as their genotoxic potential towards HCT-116 (colorectal cancer) cells. The viability of treated HCT-116 cells was assessed by MTT assay; the production of reactive oxygen species (ROS) by treated HCT-116 cells was determined using NBT assay, while their production of nitric oxide (NO) was evaluated using Griess assay. The genotoxic activity of the extracts on HCT-116 cells was tested in Comet assay, using etoposide as a positive control. The results indicated that lavender, basil, and rosemary inhibited the proliferation of these cells, significantly lowering their viability. Moreover, lavender and thyme extracts displayed a significant increase in ROS production, whereas ground-ivy, hyssop, lemon balm, peppermint, basil, rosemary, sage, and winter savory have significantly lowered their production. The results of the Griess assay suggested that lavender, motherwort, peppermint, basil, rosemary, sage, winter savory, ironwort, and thyme have significantly increased the production of NO. Furthermore, Comet assay results pointed out that motherwort, peppermint, basil, oregano, marjoram, winter savory, ironwort, wild thyme, thyme, and mountain germander possess genotoxic potential towards HCT-116 cells, while only basil had genotoxic activity statistically similar to etoposide. The obtained results are in accordance with our previous findings, which indicated that these extracts have antigenotoxic and genoprotective activities towards normal cells. Finally, it can be concluded that these traditionally valued plants might act as potent antitumor agents by modulating the proliferation and production of ROS and NO by cancer cells, as well as by expressing significant genotoxic properties towards cancer cells.

Keywords: Lamiaceae, ethanolic extracts, antitumor activity, immunomodulation

Acknowledgements: This work was supported by the Ministry of Education and Science of the Republic of Serbia (Contract number: 451-03-68/2020-14/200178).

ETHNOBOTANICAL STUDY ON TRADITIONAL USE OF MEDICINAL PLANTS IN NIŠAVA DISTRICT, SOUTH-EAST SERBIA

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Ethnobotanical studies are the first step in collecting data on the traditional use of medicinal plants, which allows further steps in developing of new drugs or functional food. Since medieval times, medicinal plants have been used for the treatment of numerous health problems in Serbia. Our study was conducted in the area of Nišava districts which is located in the South-East part of the Republic of Serbia. It is covering an area of 7.292 square kilometers being the second district in the Republic of Serbia by surface. Also, the region is the second district in the Republic of Serbia according to the number of inhabitants. Considering diversity of landscapes and natural resources, poorly developed infrastructure in rural areas and depopulation of rural regions, we found Nišava district interesting for our study. Semi-structured ethnobotanical interviews were conducted during 2018. A total of 168 informants were interviewed, 94 female and 74 males. All informants were of Serbian nationality (100%). The informants were between 16 and 83 years old (7 < 20 years; 53 from 21-40 years; 70 from 41-60 years; 36 from 61-80 years and 2 > 80 years). The informants provided data for 172 medicinal plants belonging to 69 families. *Lamiaceae*, *Asteraceae* and *Rosaceae* were the predominantly used families in the Nišava district. The highest use value was recorded for the following species: *Mentha piperita* (116), *Matricaria chamomilla* (111) and *Hypericum perforatum* (102). The most frequently reported medicinal uses were noticed for gastrointestinal ailments, respiratory problems and skin diseases. The application was primarily oral, followed by topical applications. Aerial parts were the most exploited, but the use of all other plant parts was recorded. According to our results, traditional medicine in the Nišava district is mainly applied in primary health care for healing minor illnesses or as an addition as food.

Keywords: Ethnopharmacology, Traditional use, Nišava district, Medicinal plants

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-9/2021-14/200003).

ETHNOBOTANICAL STUDY ON TRADITIONAL USE OF MEDICINAL PLANTS IN URBAN AREAS OF OSIJEK BARANJA DISTRICT (NORTH-EASTERN CROATIA)

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The ethnobotanical survey presents the first step in acquiring data on the traditional use of medicinal plants and is a preliminary screening tactic of great importance in the identification of new bioactive compounds. This study aimed to collect and document data on the traditional use of medicinal plants in Osijek Baranja district, located in North-Eastern Croatia. Ethnobotanical study was carried out in June 2019, using semi-structured interviews. A total of 200 informants were interviewed (133 females and 67 males) and the age of informants was between 16 and 83. Quantitative ethnobotany factors were calculated, allowing us to discuss the results. The informants reported data on 73 medicinal plants belonging to 32 families. The most locally used families were *Lamiaceae, Asteraceae and Malvaceae*. *Matricaria chamomilla, Tilia cordata* and *Urtica dioica* were found to be the species with the highest number of use reports. Several ways for preparation and administration of cited species were reported. Most of the plant species (65.28%) were consumed internally, 20.83% were used both internally and externally, while 13.89% were used only externally. Among plant parts, flowers (43.94%) and leaves (39.06%) were dominantly used, while the most frequent type of preparation form was infusion. The species with most diverse uses were *Urtica dioica, Matricaria chamomilla* and *Taraxacum officinale*. A high number of informants (96%) confirmed that they collect plants by themselves and only 25 informants (12.5%) use literature related to medicinal plants. According to our results, traditional medicine in urban areas of Osijek Baranja district is mainly applied in primary health care for healing minor illnesses or as food consumption.

Keywords: Ethnopharmacology, Traditional use, Osijek, Medicinal plants, North-Eastern Croatia

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-9/2021-14/200003).

ANTIOXIDATIVE AND IMMUNOMODULATING POTENTIAL OF THE MUSHROOM *Phellinus linteus*

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Phellinus linteus is a popular medicinal mushroom that is widely used in Asian countries. A number of studies have confirmed that *P. linteus* possesses exceptional biological potential useful for pharmacological applications, including anticancer and anti-inflammatory activities, as well as antidiabetic, hepatoprotective, and neuroprotective effects. The objective of the present study was to evaluate antioxidant and immunomodulatory activities of hot water polysaccharide extract obtained from the medicinal mushroom *Phellinus linteus* (Berk. et Curt.) Teng. FT-IR was used to study the polysaccharide profile of the extract. Its antioxidant potential was measured by the conjugated diene method in the linoleic acid model system. Immunomodulation was tested *in vitro* by measuring the synthesis of interferon-gamma (IFN-γ) in healthy human peripheral blood mononuclear cells (PBMCs) using enzyme linked immunosorbent assay (ELISA). The FT-IR spectrum of *P. linteus* hot water extracted polysaccharides showed a typical carbohydrate pattern. A small amount of proteins was also observed with characteristic absorptions at 1635, 1540 and 1412 cm^{-1}. Measurements of antioxidant properties in linoleic acid model system revealed relatively high antioxidant activity with EC_{50} value of 7.11 mg/mL. After 48 h of *P. linteus* polysaccharide extract incubation, the IFN-γ titer displayed immunosuppressive effect, 32.6 pg/mL. The IFN-γ titer for the suspension of PBMCs in PBS, which was used as a positive control, was found to be 135.2 pg/mL. Differences in IFN- γ contents in *P. linteus* extract vs. model control were strongly significant ($p < 0.05$). The results of this study suggest that the polysaccharide extract of *P. linteus* acts as a natural antioxidant and possesses immunomodulatory properties. Therefore, it can be a suitable raw material for the development of antioxidant food additives. In addition, due to the possible immunosuppressive effect *P. linteus* polysaccharide extract is particularly interesting and could find application in suppression of autoimmune diseases such as rheumatoid arthritis.

Keywords: Phellinus linteus, Medicinal mushroom, Antioxidant, Immunomodulation

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WALNUTS CONSUMPTION MODULATES ENDOGENOUS METABOLIC CONVERSION TOWARDS LONG-CHAIN FATTY ACIDS AND AFFECTS INDIVIDUAL FATTY ACID CONTENT IN PLASMA AND LIVER OF FRUCTOSE-FED RATS

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Disturbed plasma and tissue fatty acid profiles have been linked with metabolic syndrome, a cluster of metabolic abnormalities associated with increased cardiovascular disease risk. Walnuts are rich in dietary fats, and growing evidence suggests various cardiometabolic benefits of their consumption. However, no previous study investigated the metabolic breakdown of fats contained in walnuts following their consumption. Therefore, this study aimed to evaluate the impact of 6-week walnut consumption on plasma and liver fatty acid metabolic conversion toward longer-chain products and individual fatty acid composition in rats with a cluster of metabolic disturbances. Three-week-old male Wistar rats were fed for 9 weeks a standard diet with or without 10% fructose in drinking water. Afterward, a diet of half of the animals of each group was supplemented with walnuts (2.4 g/day) for additional 6 weeks. Total lipids were extracted from plasma and liver and fatty acids determined by gas chromatography. Our results revealed that walnut consumption decreased arachidonic/linoleic and palmitoleic/palmitic acid ratios in plasma and liver total lipids. It also significantly affected the docosahexaenoic/alpha-linolenic acid ratio, with up to a 4-fold decrease in the animals at metabolic risk. We also observed that walnuts consumption induced changes in profiles of individual fatty acids. It increased linoleic and eicosapentaenoic acid levels and decreased palmitoleic acid levels in rat plasma, while increasing liver linoleic and docosahexaenoic acid levels. Independently of the fructose-induced metabolic risk, walnuts induced up to a 3-fold increase in alpha-linolenic acid and decreased arachidonic acid in both tissues. They also reduced palmitic, oleic, and adrenic acid levels and increased docosapentaenoic acid content. Taken together, these results suggest the beneficial effects of walnuts on fatty acid profiles in rats and highlight the promising potential of walnuts in the prevention and treatment of the metabolic syndrome.

Keywords: Walnuts, Fatty acid profiles, Plasma, Liver, Metabolic syndrome

COMPARISON OF PHENOLICS CONTENT AND *IN VITRO* BIOLOGICAL ACTIVITIES OF PARSLEY AND BEAR'S GARLIC LEAVES EXTRACTS

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Epidemiological evidence shows that the population of Mediterranean countries, where the daily consumption of significant quantities of culinary herbs and spices is common, has lower incidences of chronic diseases and higher life expectancy than those adapted to the western lifestyle. Parsley (*Petroselinum crispum* (Mill.) Nym) and bear's garlic (*Allium ursinum* L.) have been widely used in diet as a source of micronutrients and to enhance the taste of foods. In addition to the well-known diuretic effect of parsley and hypolipidemic effect of bear's garlic, ascribed to flavonoids and sulfur compounds (methiin and alliin), respectively, they are also used in traditional medicine as an antioxidant, antimicrobial and antidiabetic agents. In the present study, dry hydroethanolic extracts of *P. crispum* (PC) and *A. ursinum* (AU) leaves were compared in terms of total phenolics content, antioxidant and enzyme inhibition activity. The total phenolics content (TPC) was measured by the Folin–Ciocalteu method, while the antioxidant capacity was determined by the radical scavenging (ABTS) and reducing power (FRAP) assays. Spectrophotometrically *in vitro* assays were performed to evaluate α-amylase and α-glucosidase inhibition activity. Quantitative analysis revealed that PC had higher TPC than AU (28.51 ± 1.10 vs. 19.85 ± 0.66 mg GAE/g of extract), which was in accordance with exhibited better antioxidant activity of PC compared to AU (132.21 ± 3.28 vs. 75.40 ± 4.84 µmol TE/mg of extract for ABTS; 2.63 ± 0.18 vs. 1.95 ± 0.08 µmol TE/mg of extract for FRAP). The extracts showed concentration-dependent inhibition of α-amylase, with IC_{50} values of 19.83 ± 1.41 mg/ml for PC and 10.07 ± 0.12 mg/ml for AU, while they didn't show valuable α-glucosidase inhibition activity. These findings support the traditional use of parsley and bear's garlic as beneficial antioxidants and mild hypoglycemics, and as spices, they could be considered integral for the implementation of diabetes diet therapy.

Keywords: Petroselinum crispum, Allium ursinum, phenolics, antioxidant, α-amylase

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LOW DIETARY POLYPHENOLS INTAKE IN PATIENTS WITH CHRONIC KIDNEY DISEASE-IS SUPPLEMENTATION NECESSARY?

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Polyphenols are important constituents of the human diet, which are involved in antioxidant and other biological activities. Dietary intake of fruits and vegetables representing polyphenol rich-foods are restricted in patients with chronic kidney disease (CKD) to reduce the risk of hyperkalemia. Nevertheless, intervention studies with polyphenol supplements in hemodialysis patients support their use to counteract high levels of oxidative stress. The objective of this study was to estimate the dietary intake of total polyphenols in patients with CKD. This study enrolled 73 randomly selected subjects, 37 pre-dialysis (PD) and 36 hemodialysis patients (HD) from the Clinic of Nephrology, Military Medical Academy, Belgrade, Serbia. Dietary intake assessment was based on repeated 24-hour dietary recalls. Food consumption was recorded for the dialysis day, the day after dialysis, and one weekend day. Dietary questionnaires were processed using Diet Assess & Plan, an advanced nutritional software tool. Consumption data were converted to nutrient intake estimates according to the Serbian Food Composition Database. The estimated mean dietary intake of polyphenols was 789 ± 418 mg/day in CKD patients. No differences were observed regarding the total polyphenol intake by gender or between PD and HD groups. Dietary intake of less than 500 mg/day polyphenols was found in 22%, between 500 and 1000 mg/day in 53%, and more than 1000 mg/day in 25% of studied CKD patients. Estimated mean dietary intakes of prominent polyphenol-rich food groups, i.e., vegetables, fruits and non-milk beverages were 254 ± 174 g, 145 ± 115 g, and 266 ± 191 ml, respectively. In conclusion, the findings of this study revealed low dietary intake of polyphenols among both patients undergoing hemodialysis and those with 3-5 stage CKD. Higher dietary consumption of certain fruits and vegetables abundant in polyphenols and low in potassium may be a valuable strategy in disease-progression management, especially in pre-dialyses patients.

Keywords: polyphenols, chronic kidney disease, hemodialysis, fruits, vegetables.

ASSOCIATION BETWEEN THE TEMPORAL EATING PATTERNS AND DIETARY QUALITY IN A SERBIAN POPULATION SAMPLE

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Meal timing, regularity, and frequency of eating occasions may have a significant impact on health due to the complex interplay between dietary intake, circadian rhythms, metabolic and physiological factors. This study aimed to evaluate whether meal timing is related to diet quality in a Serbian nationally representative sample as determined by the latest census and three stratification layers (age, gender, and region of residence). Dietary intake assessment was based on two interviewer-administered non-consecutive 24-h recalls conducted with an interval of at least one week. Questionnaires were processed with Diet Assess & Plan, advanced nutritional software, and nutrient intake calculations were performed using the Serbian Food Composition Database. The dietary quality score (DQS) was computed for each participant and it integrated one point for meeting each of the EU Science Hub recommendations for 5 items: fruits and vegetables, fiber, saturated fat, sugar, and sodium. The dietary intakes of children aged 10–17 (n=74) and adults aged 18-74 (n=260) were similar and high in total fat intake, with an average of 40% of energy derived from fat. Mean fruit and vegetable intakes (463±288 g/day) exceeded the threshold of EU recommendation. Only 12 adults and one child had a maximal DQS of 5, and 78% of adult subjects had scores of 3 or lower. The most worrisome aspects of the Serbian diet were high intakes of saturated fat, sugar and sodium. Lunch was the meal with the highest mean content of energy, followed by breakfast and dinner. A later meal pattern with the largest meal consumed after 20:00, representing a shift from the traditional Serbian eating pattern, was more prevalent in younger individuals, men, and working adults. The findings of this study provide novel insight into temporal eating patterns among individuals residing in Serbia. Further research is warranted to explore the associations of these eating behaviors with health outcomes.

Keywords: Meal timing, Diet quality, Diet Quality Score, Temporal eating pattern

THE EFFECTS OF INFANT FORMULA COMPARED TO BREASTFEEDING ON THE WEIGHT GAIN OF INFANTS, IN SHKODËR, ALBANIA.

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Infant formula means a breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding. Infant formula is a product based on milk of cows or other animals or a mixture thereof and/or other ingredients that have been proven suitable for infant feeding. Infant formula prepared ready for consumption in accordance with instructions of the manufacturer shall contain per 100 ml not less than 60 kcal (250 kJ) and not more than 70 kcal (295 kJ) of energy.

An observation study was conducted through a prepared questionnaire and included 500 mothers of different ages, in Shkodër, Albania. A study was based on infant formula compared to breastfeeding for babies aged 0-12 months. The aim of this study is to monitor the effects of different regimens on the body weight gain of infants.

During the statistical processing of the data collected, It is observed that female children predominate with 51.75% compared to male children with 48.25%. In the comparison on the frequency of feeding infants, It was observed that in breastfeeding, mothers feed their infants 8-12 times a day while mothers with formula feed the infants mostly 2-4 times a day. Breastfed babies have gained about 4-6 kg after the first 6 months of life compared with formula infants have gained about 6-8 kg because the formula is riched in nutrients where in some cases cause weight gain higher than needed. Most of the interviewees answered that powdered milk is the type of formula their babies have preferred 92.11% and 30.77% of them have preffered cow's milk based formulas.

If mothers are not able to breastfeed their babies, they should choose the right formula according to the standards.

Keywords: Formula, Breastfeed, Infant, Mother.

IMPACT OF TYPE-2 DIABETES MELLITUS ON ALTERNATIONS OF ATP-SENSITIVE POTASSIUM CHANNELS IN INTERNAL MAMMARY ARTERY

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The prevalence of type-2 diabetes mellitus (T2DM) is closely related to the occurrence of obesity. At the same time, there are more people with T2DM who required bypass surgery and the status of their blood vessels used as bypass grafts are crucial for successful surgery. The knowledge that T2DM can impact those blood vessels and caused decreased potential of vasodilatation leading us to the adequate treatment of those grafts and patients. Pinacidil is a well-known vasodilator which mechanism of action includes interaction with smooth muscle ATP-sensitive potassium (K_{ATP}) channels. In case when the endothelium is damaged, which has been observed in diabetes, K channels are the most important mechanism of relaxation of blood vessels. Previously, we have shown that pinacidil relaxed human internal mammary artery (HIMA) obtained from patients with T2DM, but K_{ATP} channels were not involved in this process. Thus, the aim of our study was to detect differences in the expression of K_{ATP} channel subunits in HIMA obtained from patients with/without T2DM. The expression of K_{ATP} subunits (Kir6.1, Kir6.2 and SUR2B) was detected by western blot and immunohistochemistry using segments of HIMA obtained from patients who were undergoing coronary bypass surgery. All three types of K_{ATP} subunits are expressed on HIMA from patients with/without T2DM. While there are no differences in the expression of the SUR2B subunit, the expression of the Kir6.1 and Kir6.2 subunits are lower in HIMA obtained from patients with T2DM ($P < 0.05$). K_{ATP} channels are expressed in the vascular smooth muscle of HIMA, but they are not involved in the dilatation of HIMA induced by pinacidil in T2DM patients. It seems that pinacidil has an additional mechanism(s) of action. Also, this could implicate that the presence of diabetes decreasing level of the expression of Kir6.x subunits.

Keywords: type-2 diabetes mellitus, bypass grafts, human internal mammary artery, potassium channels

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DETERMINATION OF CYTOTOXICITY EFFECT OF MISTLETOE EXTRACTS OBTAINED BY SUBCRITICAL WATER

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European mistletoe (*Viscum album* L.) is a semi-parasitic evergreen plant that can be found on host trees during all year periods. Its extracts have been used in traditional medicine for decades in curing or managing a wide range of diseases (cancer, diabetes mellitus, chronic cramps, stroke, stomach problems, heart palpitations, to lower blood pressure, difficulties in breathing, and hot flushing in menopause). Furthermore, aqueous extracts from mistletoe are widely used in complementary cancer treatment as immunomodulating agents. The specific components (i.e., the cytotoxic mistletoe lectins) present in mistletoe extracts lead to destruction of the tumours and metastases. In order to fully exploit the biopotential of the plant, a properly choose extraction technique is of utmost importance. Recently, more attention has been paid to green solvents and modern extraction techniques (such as extraction with water in a subcritical state) that can offer high bioactivity plant extracts while preserving the environment and human health. The aim of this study was to evaluate the cytotoxic effects of subcritical water extract of mistletoe leaves. Extraction procedure was performed in a batch reactor at elevated temperature of 120°C and pressure of 40 bar. Cytotoxicity of the obtained extract was determined on 2 different strains of cancer cells. The obtained results indicate that subcritical water extract has a high cytotoxic effect, which can be linked with high polyphenolic content. According to the obtained results, subcritical water extracts of mistletoe possess a high biopotential to be used in the pharmaceutical and food industries.

Keywords: Mistletoe, Subcritical water extraction, Cytotoxicity, Bioactive compounds

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FOOD INTERACTIONS WITH LEVOTHYROXINE

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Levothyroxine, a synthetic thyroid hormone, was the most prescribed drug in the USA in the past three years. Levothyroxine is applied as substitution therapy in hypothyroidism (both primary and central) and also recommended after a thyroidectomy (without hypothyroidism) for the prevention of recurrences, for the inhibition of nodular goitre growth, after an operation of differentiated thyroid carcinoma, as well as after the neck radiation (in cancer prophylaxis). The main aim of this study was to determine the possible interactions of different food with levothyroxine by searching the Pubmed database.

The absorption of levothyroxine may be decreased by foods such as soy, walnuts, dietary fibres, cottonseed meal, calcium carbonate and iron supplements. Grapefruit juice delayed the absorption of levothyroxine, but did not affect its overall availability, according to a randomized cross-over study. Soy milk reduced the thyroxine plasma levels in two women with congenital hypothyroidism on levothyroxine substitution therapy. Both dietary fibres and calcium carbonate by nonspecific adsorption decreased the levothyroxine bioavailability. Concomitant ingestion of levothyroxine and iron supplements has resulted in the need to increase the levothyroxine dosage in pregnant women. The administration of levothyroxine swallowed with coffee/espresso lowered the peak values of thyroxine in the blood plasma, suggesting that the coffee interference with levothyroxine absorption.

Optimal levothyroxine absorption occurs when the stomach is empty; hence patients should take levothyroxine on an empty stomach 60 minutes before any food intake. The simplest regimen is for the patients to take the levothyroxine daily dosage immediately on waking in the morning with water and an hour before any food intake.

Keywords:levothyroxine, interactions, soy, grapefruit, coffee

ANALYSIS OF THE ATTITUDES AND KNOWLEDGE REGARDING FOOD ALLERGY

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Despite concerning statistics, there is substantial scientific evidence suggesting knowledge deficit and lack of preparedness with reference to appropriate ways of addressing food allergic reactions. Responsibility for the prevention and management of food allergies is posed not only to healthcare professionals but also to patients themselves and their family members, as common first responders. The aim of this study was to explore the knowledge and attitudes regarding food allergies in a sample of the Serbian general population. In this cross-sectional study, conducted in May 2021, data was collected via an online self-reported questionnaire, from 184 people (65.2% female and 34.8% male), different ages (mean age 28.2±10.1), and of various educational backgrounds. Among participants only 32 (17.4%) have reported food allergy, and, for the majority of these subjects (66.0%), the condition was not confirmed by the clinician. Foods that were mostly reported as the cause of allergic reactions were nuts (36.4%), soybeans, and peanuts (15.2% each), followed by dairy products and strawberries (12.1% each). More than a third of surveyed people (37.1%) expressed concern that the restaurants are especially risky for allergen ingestion due to the limited opportunity to exert dietary control. Furthermore, 82.1% of participants agreed that consumers should carefully read food labels in order to prevent exposure to potentially harmful items. Only half of the respondents (56.5%) identified proper procedures in treating common symptoms of allergic reactions, including rashes and anaphylaxis, while 64.1% knew that the food to which a person is allergic, must be strictly avoided. The findings of this study imply that food allergy knowledge level and certain attitudinal determinants among the general population require improvement. Given the growing prevalence of food allergies, tailored educational programs might be beneficial for the familiarization with the condition, early recognition of symptoms, and promotion of appropriate reactions. Further research is warranted to identify subpopulations with particularly poor knowledge or misbeliefs, which should be targeted for interventions.

Keywords: Food allergy, Attitudes, Knowledge, Education

BIOAVAILABILITY OF BIOACTIVE FOOD INGREDIENTS—BIBLIOMETRIC INSIGHTS ON CURRENT RESEARCH FOCUSED ON THE ROLE AND APPLICATION OF BILE ACIDS

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Bile acids participate in the complex mechanism of solubilization and absorption of lipid molecules in the intestines. Although they are approved for supplementation in several diseases and often investigated as drug carriers, their use as additives to improve the bioavailability of compounds in food is not common. This bibliometric study aimed to examine the publication records of research on improved bioavailability of food compounds, and to search for the applications of the bile acids and their derivates in the field of food science technology. A literature search was done in August 2021 using the Web of Science (WOS) online database (Clarivate Analytics, Philadelphia, PA, USA), choosing the following set of keywords: Topic = (food OR nutrient* OR dietary OR nutraceutic*) AND (bioavailability OR bioaccessibility OR liberation OR absorption). Only research articles assigned to the WOS category Food Science Technology were taken into consideration. A total of 10,098 publications were obtained, published in the period from 1994 to 2021. An increasing trend in the annual number of publications was observed, which reached its maximum in 2020 with 1171 publications. Most of publications came from China and the USA, followed by Spain, India and Brazil. The publications were supported by various funding agencies such as the National Natural Foundation of China (8%), European Commission (4%) and National Council for Scientific and Technological Development, Brazil (3%). Further limitation of the WOS search by Title= *cholic OR bile acid, gave 19 results, ranging from 2004 to 2021, with most publications (4) published in 2020. Seven publications examined the role of fiber-enriched foods in bile acid binding, while others evaluated the effects of food ingredients on bile acid transporters, synthesis, gene expression, or effects on intestinal microbiota. Only one study suggested improvement of polyphenols bioavailability due to bile acids binding. This study showed that the research on food compound bioavailability is current, while the application of the bile acid micellar systems as food additives is not widely recognized and gives space for development by implementing the knowledge gained in field of drug delivery.

Keywords: bile acids; bioavailability; foodcompounds; additives; bibliometric analysis

TREATMENT OF PSORIASIS: BIOLOGIC VERSUS SYSTEMIC DRUGS

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Psoriasis is a chronic skin disease that affects between 0.91 and 8.5 % of people across the world. Of these, approximately 10% suffer from different severe forms. Therapeutic drugs in treating moderate to severe psoriasis have been limited. Three of the so-called "non-biological systemic therapies" were the most commonly prescribed systemic drugs worldwide: *Methotrexate* (methotrexate), *Neotigason* (acitretin), and *Sandimmun Neoral* (cyclosporine). Clinical trials for systemic medicines have shown high rates of severe infections in psoriasis. Still, these have not been confirmed in all trials because of the lack of statistical power to demonstrate differences from placebo. Biological drugs which have been authorized for use in severe and moderate forms of psoriasis are: *Stelara* (ustekinumab), *Humira* (adalimumab), and *Cosentyx* (secukinumab). A recent systematic review stated that there was still insufficient evidence about the risk of severe infections from biological drugs in psoriasis patients in long-term and daily use and that further observational studies were needed. It is confirmed that a large number of people in Serbia have severe psoriasis. Due to the minimal number of patients in Serbia who use biological therapy, a detailed case study was performed with 70 patients with moderate to severe psoriasis. The background and roots of the case study in medicine and clinical practice are covered. Given the complexity of the factors involved in non-biological systemic therapies, changing health paradigms, patient/practitioner interactions, multiple treatment modalities, and multiple symptom profiles, the case study approach offers systematical and essential information for investigating and generating findings in this area. Reliability and validity of data collection, data reduction, and interpretation were achieved. The most frequent types of psoriasis between patients were listed, including the benefits and side effects from systemic and biological drugs. 100% of patients answer that biological treatment ultimately helped in treating even severe forms of psoriasis.

Keywords: Psoriasis, Ustekinumab, Adalimumab, Sekukinumab.

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INTERPLAY BETWEEN THE GUT MICROBIOTA AND ESSENTIAL MINERALS

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Intestinal microbiota performs many functions that support human health. Minerals have a role to play in the formation of a gut microbial community. They are primarily absorbed in the small intestine and the intestinal microflora influences the bioavailability of minerals. The interrelation between the microbiota and minerals in healthy people is rarely investigated. The present state of knowledge related to the interactions between the gut microbiome and some of the most important minerals (Fe, Zn, Ca, Mg, and Cu) is reviewed. Scientific studies that evaluated the effect of microminerals on the composition of the gut microbiome using *in vitro*, *in vivo* models and human clinical trials are assessed. The 'special effects' most important minerals have on the human microbial flora are described together with the clarification of the functions of these minerals in relation to intestinal microbiota activity. Currently available evidence indicates that supplementation of Ca, Zn, and Mg stimulates the growth and colonization of beneficial gut bacteria *Lactobacillus, Bifidobacterium,* and *Ruminococcus*. Supplementation with Fe can have detrimental effects and could lead to an increased abundance of pathogenic bacteria. A reduction in beneficial microbes during Fe supplementation was observed while this negative effect was not present when animals were fed with Fe biofortified products. The route of administration and chemical form of Fe are prominent factors in shaping gut microbiome composition. The role of Mg is still puzzling as Mg deficiency modulates beneficial gut bacteria. Zinc has a strong positive effect on gut bacterial composition and function. An enhanced understanding of the gut microbiota-mineral interactions could help in the development of new strategies for combating many diseases (i.e., cancers, autoimmune and non-communicable diseases). Further clinical trials should investigate the effect of micronutrients on the microbiome and the consequential influence of these interactions on the host health.

Keywords: Gut microbiome, Bacterial colonization, Trace elements, Iron, Zinc.

CAFFEINE CONSUMPTION HABITS AMONG UNIVERSITY STUDENTS IN SERBIA

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Caffeine is the most common purine alkaloid naturally present in the fruit, leaves, and beans of coffee, cacao, and guarana plants. It added to beverages and food supplements too. While a moderate daily intake of caffeine could exert beneficial effects, including increased alertness and combating fatigue, excessive caffeine consumption (> 400 mg/day) is associated with the risk of adverse health effects. This study aimed to estimate the caffeine intake among Serbian university students based on a caffeine consumption habits survey. Out of the 867 undergraduate students (708 females and 157 males), who completed the online survey, 99.7% reported that they regularly consumed caffeine in any form. The prevalence of caffeine-containing products consumption was the highest among students with irregular meals and smokers. The mean daily caffeine intake was 195 mg (3.1 mg/kg/day), with no significant difference between genders and nutritional status. However, a significant correlation between the total caffeine intake and student's age ($r=0.095$, $p<0.001$) was observed. Brewed coffee, followed by instant coffee, energy drinks, and teas, contributed most to daily caffeine intake. The most common motivations for caffeine consumption were sensory effects, daily routine, and desire for alertness. 12.8% of students have consumed more than 400 mg caffeine/day, while more than half (55.5%) reported experiencing at least one adverse effect related to caffeine intakes, such as tachycardia, tremor, insomnia, and an increase in the frequency of urination. Overall, the high prevalence of caffeine-containing product consumption requires further research to estimate the total caffeine intake among university students and appropriate interventions on awareness of caffeine-containing food and drinks impact on health.

Keywords: caffeine, students, coffee, drinks, adverse effects

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EVALUATION OF CYTOTOXIC POTENTIAL OF TAXIFOLIN AGAINST HUMAN REPRODUCTIVE SYSTEM CANCER CELL LINES

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There is accumulating evidence that flavonoids could exert antioxidant, anti-inflammatory, immunomodulatory and strong anticancer activities. Taxifolin (dihydroquercetin) is a member of the phytonutrient family that has shown antiproliferative effects and enhanced apoptosis in different multidrugresistant cancer cell lines. However, the potential of this flavanonol against human reproductive system cancer cell lines has not been explored yet. Thus, the aim of this research was to investigate the cytotoxic potential of taxifolin in a broad concentration range (1, 5, 10, 25, 50, 100, 150 µM) using different cell lines: two choriocarcinoma cell lines (JEG-3 and JAR) and HeLa human cervical carcinoma cell line. The control cell line was HTR-8/SVneo, the immortalized cells of the firsttrimester human extravillous trophoblast. The cytotoxicity of taxifolin was evaluated by using the 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. After 24 h incubation with taxifolin, it could be observed that normal trophoblast cells respond to treatment differently from the cells of the malignant phenotype. There was no significant reduction in cell viability in HTR-8/SVneo cells in any of the used concentrations. However, the highest concentrations (100 and 150 µM) significantly reduced cell numbers in all three cancer cell lines (JEG-3, JAR and HeLa). The most pronounced cytotoxic effect (30% viability reduction vs. control) was observed in JEG-3, cancer cell line with the highest metastatic potential out of those used in this research. These results provide first data on the anticancer potential of taxifolin in human reproductive system cancer cells and have to be verified through *in vivo* experimental approach as well as through elucidation of the molecular mechanisms involved.

Keywords: dihydroquercetin, choriocarcinoma, cell viability, trophoblast.

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ADEQUATE ZINC INTAKE HELPS IN THE PREVENTION AND MANAGEMENT OF CARDIOVASCULAR DISEASES

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The importance of Zn for cardiovascular health continuously gains recognition. Compromised Zn homeostasis and prolonged inflammation are common features in various cardiovascular diseases (CVDs). Zn biochemistry alters several vascular processes, and Zn status is an important feature of cardiovascular health. This paper presents a concise, thorough overview of the most recent data on the relation between Zn homeostasis and CVDs. The importance and potential suitability of Zn status to be used as a biomarker of CV health are discussed, highlighting present controversies and research gaps that entail further research. Zn deficiency could be a contributing factor to the development of cardiovascular diseases. Impaired Zn homeostasis is associated with common genomic and proteomic modifications that relate to CVDs. Lower serum Zn levels are associated with a higher risk of CVDs. On the contrary, higher serum Zn concentrations are related to a decline in relative risk of CVDs death. Zn interventions improve risk factors for CVDs. There is a direct association between serum Zn and metabolic risk factors for the development of CVDs, i.e., serum lipids, T2DM, and obesity. Zn supplementation could potentially increase the effectiveness of presently used therapeutic drugs for managing CVDs. The precise mechanisms of the role of Zn deficiency in the pathogenesis of CVDs are still not known. The biological properties of Zn, playing a role in the physiology and pathology of CVDs, should be examined further. Further community-based observational cohort studies may be useful for obtaining more precise and evidence-based conclusions. Additional long-term, well-designed studies, performed in various population groups, should be pursued to further clarify significant relationships between Zn and CVDs. Further research is necessary to examine the interaction between Zn intake and status data with present preventative schemes and currently employed treatment methods that could help in the prevention and management of many ensuing CVDs.

Keywords: Zinc, Cardiovascular Diseases, Obesity, Zind Deficiency, Cardiovascular Health

ANTIHYPERGLYCEMIC AND ANTIOXIDANT PROPERTIES OF PHENOLIC COMPOUNDS FROM MORINGA OLEIFERA LEAVES DURING IN VITRO DIGESTION

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Diabetes mellitus is one of the leading causes of morbidity and mortality worldwide. Because of this, much research focuses on the search for natural compounds and ingredients with antidiabetic activity. Moringa (*Moringa oleifera* Lam) is a plant that in recent years has caused great interest due to its significant content of phenolic compounds with potential beneficial effects on human health. Our aim was to characterize the phenolic profile of moringa leaf powder and analyze their antioxidant and antihyperglycemic properties during in vitro digestion. Free and bound phenol extracts were obtained from three moringa leaf powders using conventional and alkaline extractions. The powder with the highest total phenol content and antioxidant activity (ABTS, DPPH) was selected and digested following the harmonized Infogest in vitro digestion protocol. The phenolic profile was analyzed by targeted (UPLC-ESI-QqQ-MS/MS) metabolomics. The inhibition of α-amylase and α-glucosidase was evaluated by in vitro assays. Thirty phenolic compounds, mainly flavonoids, were identified and quantified, of which quercetin-3-glucoside and kaempferol-3-*O*-glucoside were the major compounds in all samples. Moringa leaf powder showed higher antioxidant activity after intestinal digestion. The highest inhibition of α-amylase was observed in the intestinal digests of the extract of bound phenols and the moringa leaf powder (IC_{50} = 3.84 ± 1.72 and 3.84 ± 1.29 mg/mL, respectively) y the extract of free phenols and the gastric digest of moringa leaf powder showed the highest inhibition of α-glucosidase (IC_{50} = 1.82 ± 1.40 and 3.24 ± 1.80 mg/mL, respectively) using the drug acarbose as a reference. Therefore, free and bound phenol extracts or moringa leaf powder could be used as nutraceuticals or functional ingredients to prevent and treat type 2 diabetes mellitus.

Keywords: Moringa oleifera, phenolic compounds, in vitro digestion, antioxidant activity, antihyperglycemic activity.

PROBIOTIC IMPACT ON GLICLAZIDE PERMEABILITY

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Interindividual differences in drug response sometimes occur due to the impact of the intestinal environment on drugs, mainly due to the effects of gut microflora. Given that gliclazide is a drug with wide interindividual variation in oral bioavailability, this study aimed to determine the effects of probiotic bacteria on its permeability. The permeability of gliclazide with and without probiotic bacteria was tested using in vitro PAMPA model at pH 7.4 for 6h. In order to study the potential accumulation of gliclazide in probiotic bacteria or biotransformation, the total mass was calculated as a sum of mass in the acceptor and donor compartment. Concentrations of gliclazide were determined by HPLC analysis at 229 m. Probiotic bacteria significantly increased the permeability of gliclazide across the PAMPA membrane (4.77±0.67 vs. 1.34±0,04) x 10^{-6} cm/s that may be explained by the metabolic activity of probiotic bacteria, i.e., the production of short-chain fatty acids, which lower the pH of the medium increases the amount of non-ionized molecular form of the drug. The total amount of gliclazide during incubation with bacteria, significantly decreased that could be a consequence of partial metabolism of the drug by enzymes of probiotic bacteria. Probiotic bacteria, naturally present as part of gut microflora and in the form of supplements, increase the permeability of gliclazide that may affect its absorption and bioavailability. This assumption might be addressed in future studies.

Keywords: probiotics, PAMPA, gliclazide, drug permeability, drug transport

FLAXSEED OIL AS A NEUROPROTECTIVE AGENT DURING TRIMETHYLTIN-INDUCED NEURODEGENERATION IN FEMALE RATS

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It is increasingly apparent that the prevention/treatment of disease is not only achieved through pharmacological therapy but also through the consumption of natural products. Flaxseed oil (or linseed oil, FSO) derived from the seeds of the flax (*Linum usitatissimum L.*) gained worldwide awareness as a functional food with potent neuroprotective properties. Trimethyltin (TMT) is an organotin neurotoxicant that selectively targets the limbic region, particularly the hippocampus, and induces selective and progressive hippocampal neurodegeneration and gliosis. In response to injury, cells massively release adenosine-5'-triphosphate (ATP) into the extracellular space, where it acts as a "danger signal" and promotes microglial chemotaxis and phagocytosis as well as the release of pro-inflammatory cytokines. The levels of ATP in the extracellular space are tightly controlled by ecto-nucleoside triphosphate diphosphohydrolases (NTPDases)/ecto-5'-nucleotidase (eN) enzyme chain, which acts together as an immune checkpoint since they degrade pro-inflammatory ATP and generate anti-inflammatory adenosine. Therefore, we sought to determine whether supplementation with FSO may prevent TMT-induced neurodegeneration and gliosis in female Wistar rats. Animals were continuously treated with FSO (1 ml/kg, orally) for two weeks, then received a single dose of TMT (8 mg/kg, i.p.), and application of FSO continued for twenty-one days. Data have convincingly shown that FSO continuous treatment ameliorated TMT-induced neuronal loss in the CA3 hippocampal region, ameliorated reactivation of astrocytes and microglia, and inhibited the increase in ATP/ADP hydrolysis rates. These findings support beneficial neuroprotective properties of FSO against TMT-induced neurotoxicity and hint at a promising preventive use of FSO in hippocampal degeneration and dysfunction.

Keywords: flaxseed oil, hippocampus, trimethyltin, neuroprotection, gliosis

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PHENOLIC PROFILE AND IN VITRO BIOLOGICAL ACTIVITIES OF BLACKTHORN FRUIT (*Prunus spinosa* L.)

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The blackthorn, *Prunus spinosa* L. (Rosaceae) is a continental shrub widespread in Europe. The fruit is used in traditional medicine in the treatment of respiratory disorders, as well as diuretic, spasmolytic and anti-inflammatory agent. The aim of this study was to investigate the phenolic profile and in vitro biological activities: antioxidant, anti-diabetic, anti-tyrosinase, and anti-acetylcholinesterase. Furthermore the effect of blackthorn fruit extract on probiotic microorganisms was also studied. The blackthorn fruits were collected from two localities in Serbia and extracted with water, methanol and ethanol (50% *V/V*) at room temperature. The total phenolic contents was analysed by the Folin-Ciocalteu assay and was the highest in methanol extracts (321.36±9.13 and 217.04±17.99 mg GAE/100 g). On the contrary ethanol extracts contained the highest total flavonoids content (67.88±1.05 and 39.70±3.19 mg CE/100 g). Hydroxycinnamic acid derivatives (caffeoylquinic acid, caffeoyl hexoside) and quercetin glycosides (rutin, quercetin pentosyl-hexoside, isoquercitrin, quercetin pentoside) were detected in methanol extracts of both samples by LC-MS. Blackthorn ethanol extracts showed pronounced ferric reducing activity in FRAP test (2.80±0.07 and 1.89±0.01 mmol Trolox /100 g) and highest ability to neutralise ABTS radical (41.02±0.77 and 45.84±1.01 mmol Trolox /100 g). On the other hand methanol extract of both samples exerted significant DPPH radical scavenging activity (3.38±0.1 and 2.61±0.17 mmol Trolox/100 g) and highest activity in β-carotene/linoleic acid bleaching test (20.89±2.28 and 21.16±2.43%). Methanol extract of both samples inhibited the enzimes: α-amylase (IC_{50} 2.05±0.05 and 1.26±0.04 mg/ml) α-glucosidase (IC_{50} 0.63±0.02 and 0.43±0.06 mg/ml), tyrosinase (IC_{50} 1.0±0.07 and 0.57±0.02 mg/ml), and acetylcholinesterase (IC_{50} 0.56±0.28 and 2.16±0.56 mg/ml). In addition the methanol extracts of both samples markedly stimulated the growth of investigated probiotic microorganisms.

The blackthorn fruit represents a rich source of biologically active compounds and although it is almost forgotten it should be used again in human nutrition to maintain health.

Keywords: Prunus spinosa, fruit, phenolics, antioxidant, enzyme inhibitory activity

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THE EFFECT OF *IN VITRO* DIGESTION ON PHENOLICS CONTENT AND ANTIOXIDANT CAPACITY OF BISCUITS PREPARED WITH WHEAT AND QUINOA FLOUR

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Nowadays, much attention is paid to phytochemicals as bioactive compounds of food. Phenolic compounds are abundant micronutrients in our diet for which it has been reported to have multiple biological effects, including antioxidant activity. Quinoa flour is increasingly used today because it increases the biological value of wheat bakery products by increasing the content of proteins, minerals and antioxidant compounds. The aim of this work was to determine the bioavailability and antioxidant properties of these biologically valuable compounds after simulated in vitro digestion. Total phenolics content and antioxidant potential (ABTS-test) of biscuits prepared with wheat (90%) and quinoa (10%) flour was investigated before and after in vitro digestion. After biscuits extraction in water, the content of total phenolics (44.63±0.23) was much lower than the content of total phenolics in the samples after in vitro digestion (202.19±2.21). Similar results were obtained for the analysis of ABTS radical cation scavenging activity. Namely, the antioxidant activity of biscuits water extracts is about 3 times higher after in vitro digestion (45.45±0.62 μg Trolox/ml) than before *in vitro* digestion (13.67±0.27 μg Trolox/ml). This shows the high bioavailability of the phenolic compounds of the biscuits prepared from wheat and quinoa flour after the activity of digestive enzymes. Also, *in vitro* simulated gastrointestinal digestion improved their antioxidant potential measured by ABTS-test.

Keywords: in vitro digestive of biscuits, wheat, quinoa, total phenolics, ABTS-test

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HEAT-INACTIVATED PROBIOTIC *LACTOBACILLUS CURVATUS* BGMK2-41 ACTIVATES *CAENORHABDITIS ELEGANS* HOST DEFENSE VIA p38 MAPK SIGNALING PATHWAY

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The host-microbiota cross-talk represents an important factor contributing to innate immune response and host resistance during infection. It has been shown that probiotic lactobacilli exhibit ability to modulate the immune system and enhance pathogen elimination. Therefore, the aim of this study was to test the potential of heat-inactivated probiotic *Lactobacillus curvatus* BGMK2-41 to stimulate immune response and resistance of the *Caenorhabditis (C.) elegans* against pathogens. The round worm *C. elegans* has become a useful model system for innate immunity studies in terms of pathogen-host-microbiota interactions. The *C. elegans* wild-type (WT) N2 strain together with *pmk-1* (KU25) immunocompromised mutant was used to decipher mechanisms elicited by heat-inactivated BGMK2-41. Activation of canonical immunity pathway was evaluated by qRT-PCR and western blot analysis in WT and *pmk-1* worms after overnight treatment with heat-inactivated BGMK2-41. Moreover, immune activation was tested by nematode killing assay by using *Staphylococcus aureus* ATCC25923 and *Pseudomonas aeruginosa* PA14 as pathogens. Results revealed that feeding worms with BGMK2-41 increased mRNA levels of the *tir-1*, *pmk-1* and *atf-7* genes and upregulated levels of phospho-p38 MAPK protein, suggest an activation of the canonical p38 MAPK immunity pathway. Further, we showed that BGMK2-41 upregulated p38 MAPK-dependent transcription of C-type lectins, lysozymes and tight junction protein CLC-1, which consequently contributed to prolong survival of the nematodes. In functional test, worms previously fed with BGMK2-41 showed increased survival rate against both pathogens. Finally, all above-mentioned effects were completely abrogated in the *pmk-1* mutant. The results of this study suggest that p38 MAPK-dependent immune regulation by BGMK2-41 is essential for probiotic-mediated *C. elegans* protection against gram-positive and -negative pathogenic bacteria and could be further explore for development of probiotics with potential to increase the resistance of the host towards pathogens.

Keywords: Lactobacillus curvatus, immunity, Caenorhabditis elegans, p38 MAPK, pathogenic bacteria

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KNOWLEDGE, ATTITUDES AND DIETARY PRACTICES OF PHARMACY STUDENTS REGARDING THE IMPORTANCE OF NUTS CONSUMPTION

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Nuts are nutrient-dense foods with complex matrices rich in unsaturated fatty acids and other bioactive compounds. Numerous reported health benefits of nuts may be attributed to their unique nutrient profiles and synergistic interaction of their constituents, including macronutrients, micronutrients and phytochemicals. The objective of this study was to explore pharmacy students' nuts consumption practice, as well as their attitudes and knowledge concerning nuts nutritional and health properties. A cross-sectional survey based on the online self-administered questionnaire, was conducted in September 2020. Among respondents (n=136, 90.4% female and 9.6% male, mean age 23.7±2.0 years), more than half agreed or strongly agreed that regular consumption of nuts might have beneficial effects on health, including reducing the risk of cardiovascular disease. Furthermore, the majority of participants perceived nuts as healthy sources of proteins and monounsaturated and polyunsaturated fats. The most common reason cited by respondents for eating nuts were taste (80.2%), followed by health effects (53.1%) and nutrient profiles (45.0%). The most commonly consumed nuts among pharmacy students were almonds (35.2%) and peanuts (28.4%), followed by hazelnuts (19.1%) and walnuts (15.6%). Based on self-reported data, students predominantly consume raw nuts (54.0%) as a snack between meals (78.5%). Nutrition-related courses within the faculty curriculum were the main sources of acquiring information regarding the beneficial effects nuts exert on health outcomes. Although certain gaps in knowledge were determined, in most senior students, the attitudes and dietary practices regarding the consumption of nuts were at an adequate level. Nevertheless, expanded efforts and additional educational programs may be useful in strengthening the capacities of these future health professionals for providing adequate nutrition advice and proactive advocacy of nuts health benefits.

Keywords: Nuts, Attitude, Knowledge, Dietary practice

THE BIOACTIVITY OF KOMBUCHA FERMENTED *CAMELLIA SINENSIS*, *COFFE ARABICA* AND *GANODERMA LUCIDUM* EXTRACTS

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The scientific interest in the medicinal properties of Kombucha beverages, a carbonated drink with live microorganisms, has increased recently. Hence, the aim of this study was to examine the antioxidant, antidiabetic, and antineurodegenerative potential of unfermented and also Kombucha fermented *Camellia sinensis* (green tea), *Coffea Arabica* (coffee) and *Ganoderma lucidum* (Reishi) extracts. The extracts were prepared as follows: the first (unfermented) set contained 1 L of water, 50 g of sucrose and 20 g of dried and ground green tea, coffee, or Reishi basidiocarp, while the second (fermented) set contained all of the aforementioned ingredients that were individually inoculated with Kombucha. The antioxidant activity was assessed by DPPH, β-carotene bleaching and total reducing power (TRP) assays. The inhibition of α-amylase and α-glucosidase activity was used to estimate the antidiabetic potential, while the level of inhibition of acetylcholinesterase and tyrosinase was used to evaluate the antineurodegenerative activity. The results suggested that the fermented extracts of green tea, coffee and Reishi showed significant antioxidant effects, however, they were lower compared to the unfermented extracts. The unfermented green tea extract exhibited the highest DPPH-scavenging activity (87.46%) as well as the highest preservation of β-carotene (92.41%), while the fermented coffee extract showed the highest TRP (120.14 mg AAE/g) at 10 mg/mL. The α-amylase inhibition was significantly lower than expected, however, the extracts were quite effective in inhibiting α-glucosidase, especially the unfermented Reishi extract, inhibiting 95.16% of α-glucosidase activity, which was higher than the positive control. The most effective acetylcholinesterase inhibitor was unfermented green tea extract (68.51%), while the fermented coffee extract inhibited 34.66% of tyrosinase activity at 10 mg/mL. Finally, the extracts showed rather a high bioactivity, while their more detailed mechanisms of action are expected to be revealed in future research.

Keywords: biological activities, coffee, green tea, Kombucha, Reishi mushroom

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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF SWEET MARJORAM (*Origanum majorana* L.) ESSENTIAL OIL

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Sweet marjoram (*Origanum majorana* L.) is a perennial herbaceous plant that grows in Egypt and the Eastern Mediterranean, especially in Cyprus. It is an important aromatic herb used worldwide as a spice. Sweet marjoram contains up to 3% of essential oil, which is used in the perfume industry, and due to its insecticidal, fungicidal, antibacterial and antioxidant properties in the pharmaceutical, cosmetic and food industry. The aim of this work was to isolate the essential oil of sweet marjoram, determine its qualitative and quantitative composition and examine the antimicrobial activity of the isolated oil. The essential oil was obtained by Clevenger-type hydrodistillation from the aerial parts of sweet marjoram (hydromodule 1:10 m/v, extraction time 120 min). The qualitative and quantitative composition of the essential oil was determined using gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detector (GC-FID) methods. The 17 compounds were identified, which represent 95.26% of the total essential oil content. The main identified compound was terpinen-4-ol (39.91%), followed by linalool (20.98%) and α-terpineol (8.23%). The antimicrobial activity of the essential oil was tested using disc diffusion method on Gram-positive, Gram-negative bacteria and fungi. The marjoram essential oil showed activity on the following microorganisms: *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus luteus*, *Escherichia coli* and *Proteus vulgaris*. The obtained results show that sweet marjoram essential oil is a good source of natural antimicrobial substances.

Keywords: sweet marjoram, essential oil, GC-MS, GC-FID, disc diffusion method

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VARIATION IN SOME ESSENTIAL ELEMENTS AND ANTIOXIDANTS CONTENT IN ORGANICALLY PRODUCED SPELT AND MAIZE GRAINS

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Frequent variations and extremes in meteorological factors, affect not just yield, but also grain composition of produced crops. Organic crop production has many challenges, particularly under the rain-fed conditions, when water supply is limited and crops could be additionally subjected to the harsh environment. Aim of the research was to follow variations in grain composition of organically produced spelt wheat (var. Nirvana) and red grain maize (var. Rumenka) during 2011-2017 period, which included meteorologically different seasons. Analysed grain components included essential elements: Ca, Mg, Zn, Fe and Mn (determined by ICP-OES), as well as antioxidants, such as polyphenols, yellow pigments (YP), total glutathione (GSH) and phytate (Phy) (determined spectrophotometrically). Results revealed that the lowest concentrations of polyphenols, YP and GSH and slightly reduced Phy in the grains of both, spelt and maize were present during the season with extremely high precipitation, as 2014 was. Nevertheless, drier seasons had positive impact on the accumulation of all examined antioxidants and the highest values were obtained during meteorologically optimal years. Based on the content of essential elements, 2014 was also important for reduced Mg, Fe, Zn and Mn accumulation in spelt grain. For maize, seasons with drought (2012 and 2013) were significant for reduced accumulation of all examined elements. The highest values of all essential elements were noticed in 2016 and 2017, seasons with relative uniform precipitation distribution and optimal temperature during grain filling periods of spelt and maize. Principal component analysis revealed that drier periods present during grain filling induced high variations of GSH, Phy and Mg concentrations in spelt grain, as well as high variations of YP, Mg, Fe, Mn and Zn concentrations in maize grain. It could be assumed that growing season is an important factor affecting nutritional quality of organically produced crops, such as spelt and maize.

Keywords: Essential minerals, Antioxidants, Organic agriculture, Temperature, Precipitation amount.

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PHYTOCHEMICAL PROFILING OF 18 STRAWBERRY CULTIVARS

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Strawberry (*Fragaria×ananassa* Duch.) fruits are an important source of a wide variety of nutritive compounds such as sugars, vitamins and minerals, as well as bioactive compounds among which phenolics stand out as being of crucial importance. The huge number of strawberry cultivars available on the market generally differs in specific nutritional characteristics. Therefore, fruits of 18 strawberry cultivars (′Roxana′, ′Arosa′, ′Joly′, ′Asia′, ′Alba′, ′Aprika′, ′Sibilla′, ′Garda′, ′Primy′, ′Jeny′, ′Laetitia′, ′Albion′, ′Capri′, ′Clery′, ′Premy′, ′Rumba′, ′Vivaldi′ and ′Irma′) were characterized in this study using HPLC in relation to the concentration of individual sugars, organic acids and phenolic compounds. ′Capri′, ′Rumba′ and ′Jeny′ were dominant strawberry cultivars in terms of glucose (4.84, 4.60 and 4.34 g/100 g FW, respectively), fructose (5.31, 4.79 and 4.68 g/100 g FW, respectively), malic (3.73, 3.58 and 2.93 mg/g FW) and shikimic (27.0, 15.35 and 21.55 □g/g FW) acid content, while ′Premy′, ′Aprika′ and ′Sibilla′ were distinguished among all other cultivars regarding sucrose content (0.82, 0.68 and 0.67 g/100 g FW, respectively). Furthermore, ′Laetitia′, ′Sibilla′, ′Rumba′ and ′Capri′ differed from other cultivars by its high content of citric acid (6.86, 6.24, 5.93 and 5.44 mg/g FW, respectively). Additionally, evaluation of individual phenolics showed that cultivars ′Joly′, ′Laetitia′, and ′Asia′ were distinguished from others as the richest concerning identified anthocyanins, almost all flavonoids, and phenolic acids. Their assessment was important for revealing new sources of natural antioxidants, so cultivars with higher phenolic content should be promoted and more frequently consumed. The quality of fruit is directly related to its metabolic profile constituted of individual primary and secondary metabolites. Considering that sugars and organic acids define the sensorial quality of strawberry fruit, their optimal ratio combined with phenolic compounds plays important roles in the fruit quality definition and characterization of strawberries as a functional food.

Keywords: fruit quality, HPLC, organic acids, phenolics, sugars.

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QUANTIFICATION OF POLYPHENOLS IN SOME AUTOCHTONOUS APPLE CULTIVARS FROM SERBIA

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Polyphenols are interesting as compounds that add nutritional value to foods, but also as indicators of the environmental stress effects and related to resistance toward diseases in higher plants. Therefore, the chemical examination of polyphenols is useful from the aspect of satisfying consumer needs and consideration of resistance to agroecological conditions. Autochthonous cultivars are characterised by good adaptability to the local environmental conditions and represent a valuable source of genetic variability. The subjects of this study were 17 samples of autochthonous apple varieties collected in two consecutive seasons (2018 and 2019). For comparison, 5 standard and 5 resistant apple cultivars were also collected. Before extraction of polyphenols with 0.1% hydrochloric acid in methanol, the mesocarp and the peel were separated. The polyphenolic profile was determined using ultra-high performance liquid chromatography (UHPLC) coupled with diode array detector and TSQ Quantum Access Max triple-quadrupole mass spectrometer. In total of 7 compounds were quantified in mesocarp, while 11 were quantified in peel. Most of analysed polyphenols were detected in higher amount in the samples from 2019 compared to 2018. Neochlorogenic acid was detected in the highest amount in mesocarp (503.12 mg/kg per fresh weight (FW) in autochthonous Šipina cultivar , 134.03 mg/kg in resistant Prima cultivar and 68.37 mg/kg in standard Idared cultivar). In the peel of all analysed cultivars, quercetin-3-*O*-glucoside and quercetin-3-*O*-rhamnoside, along with neochlorogenic acid, were found in higher concentrations compared to other compounds. Phlorizin and quercetin-3-*O*-glucoside were also detected in relatively high amount in the mesocarp of all samples. Higher amount of phlorizin in the mesocarp was generally detected in samples of autochthonous cultivars (1.76 – 55.55 mg/kg FW in autochthonous cultivars, 0.34 – 5.11 mg/kg in resistant cultivars and 0.46 – 8.81 mg/kg in conventional cultivars).

Keywords: Malus domestica, autochthonous cultivars, neochlorogenic acid, phlorizin, quercetin.

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PHYSICOCHEMICAL AND RELATIVE POLLEN CHARACTERISTICS OF STRAWBERRY TREE HONEY FROM ALBANIA

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This work investigated the characteristics of strawberry tree (*Arbutus unedo* L.) honey produced in Albania. In the last years, the typical bitter flavor and the positive medicinal properties attributed to this type of honey, has shaped the consumer behavior and the demand has been increased. The *arbutus* plant is widespread in Albania, with diffusion in coastal lowlands up to 700-800 m above the sea level, dividing Albania diagonally. The production of strawberry tree honey is typical in the central and southern parts of Albania, where large massives are found. Also, in the South-East of Albania, in the region of Përmet, the other species *Arbutus andrachne* is spread, known as Greek strawberry tree. The common names used to identify this plant in Albania are "*Maré*" and "*Koçimare*", depending on the region; and from the red fruits in some local areas is produced an alcoholic beverage called "*Raki"*.

To accomplish the aim of the study the physicochemical analyzes were performed. Also, antioxidant capacity was determined by total phenolic content (TPC) and radical scavenging activity (RSA) using DPPH. The results obtained are similar with other data published for this type of honey in the region, with some minor changes. It is evident that this type of honey possesses high water content 19.99±1.74%, which in some cases exceeds the limit of 20% established in honey standard. Also, this type of honey, compared with other unifloral honeys, exhibits high TPC 1017.38±149.73 mg GAE/kg and RSA 25.59±5.85%; which are linked with its medicinal properties and uses in traditional medicine. *Arbutus unedo* L. pollen is underrepresented in the sediment and a melissopalynological analysis shows specific pollen presence of 25-40%.

Thus, the characteristics of strawberry tree honey produced in Albania, are of great importance for this unifloral honey and further studies on its characterization will serve to promote and establish a market strategy.

Keywords: *Strawberry tree, Honey, Albania, Bitter, Health*.

UHPLC OrbiTrap MS ANALYSIS OF BUCKWHEAT HONEY, NECTAR AND POLLEN POLYPHENOLS

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There has been a great interest in the study of phytochemicals as natural antioxidants. Buckwheat (*Fagopyrum esculentum*) is recognized as a good source of nutrients and valuable phenolic compounds. In addition to being considered as functional food, buckwheat is also a reliable and high-yielding honey plant. By collecting nectar and pollen from buckwheat flowers, bees make high valued buckwheat honey. Identification of phenolic compounds in Serbian buckwheat honey, nectar and pollen, was carried out by using ultra-high performance liquid chromatography coupled with linear trap quadrupole OrbiTrap connected to mass spectrometer (UHPLC OrbiTrap MS). There were identified more phenolic acids and flavonoids in buckwheat honey and nectar, while (epi)catechin units, their galloylated derivatives and procyanidin dimers were found only in buckwheat pollen. Additionally, among all phenolic compounds, dominant presence of quercetin and its glycosides, rutin and quercitrin, in buckwheat pollen was noticed. The main contribution off all phenolic compounds in buckwheat honey and nectar was attributed to propolis-derived flavonoids. The obtained results point out similarities in polyphenolic profiles of the buckwheat nectar and honey and revealed the difference in pollen profile. Nevertheless, there was noticed a good correlation between phenolic profiles in analysed samples. Furthermore, presence of significant and widespread phenolic compounds was found in all buckwheat samples.

Keywords: Phenolic compounds, Buckwheat (Honey, Nectar, Pollen).

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ELEMENTAL PROFILE OF RECORDED AND UNRECORDED FRUIT SPIRITS AND HEALTH RISK ASSESSMENT

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Home production of fruit spirits has a very long tradition in Serbia. Such spirits are often not being taxed nor captured by official sale statistics, and therefore are termed “unrecorded alcohol”. Unrecorded alcohols also escape safety control but reach numerous consumers, thus raising health risk concerns.

A total of 153 samples of fruit spirits was collected during 2020 in Vojvodina, 26 with tax stamp (recorded), and 127 produced in private homes or small-scale distilleries and obtained mainly directly from producers (unrecorded). Profiles of 22 elements were provided by ICP-MS and a direct mercury analyser. Daily intake of elements was estimated at different consumption levels, considering the share of recorded and unrecorded alcohol consumption. Hazard quotient (HQ)/hazard index (HI) method was used to estimate health risk, as well as comparison with benchmark doses (BMDLs).

Only copper and lead concentrations exceeded toxicological thresholds proposed by the AMPHORA project (73.2 and 8.5% of all samples, respectively). The mean copper content of unrecorded spirits was substantially higher than that of recorded ones (8.2 vs 1.2 mg/l), probably due to the different materials used for processing equipment. Exposure assessed in average and regular drinker scenarios translated into HQs was below the limit level of 1 for both men and women. Furthermore, HI, a sum of individual elements' HQs for a sample, also remained low (maximum 0.4). In chronic heavy drinker scenarios, only if total alcohol consumption was considered attributable to unrecorded spirits HIs exceeded 1 for 15% of the samples for men and 9.4% for women. In the same scenario, exposure to lead at a high percentile exceeded the BMDL for nephrotoxicity, whereas arsenic exposure was below the BMDL in all exposure scenarios.

No legal limits for elemental contaminants in spirits have yet been set, although some of them can cause toxic effects even at low concentrations.

Keywords: Fruit spirits, Elements, Risk assessment, ICP-MS

QUALITY AND SAFETY CONTROL OF UNRECORDED FRUIT SPIRITS FROM SERBIA

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The established tradition of unrecorded, homemade fruit spirits consumption has been identified as a health risk source, especially in countries located at the Balkan Peninsula, Central and Eastern Europe. Hence, 100 unrecorded fruit spirit samples from Serbia, divided into 6 types - plum, apricot, pear, quince, apple and grape pomace were analyzed for the presence of volatiles - acetaldehyde, ethyl acetate, methanol and higher alcohols (n-propanol, iso-butanol, n-butanol, iso-amyl alcohol and n-amyl alcohol). The AMPHORA chemical testing limits guideline was used for the safety assessment of the volatiles. Acetaldehyde, which may contribute to the carcinogenicity of alcoholic beverages, exceeded the limit of 50 g/hl pa in plum (5.3%) and grape pomace (6.7%) samples. Ethyl acetate was below the AMPHORA limit (1000 g/hl pa) in all samples. Methanol, which has been described to be the most common cause for surrogate toxicity, was above the limit (1000 g/hl pa) in pear (30.8%), plum (23,7%) and apricot (15,8%) samples. Higher alcohols, which have been speculated as a cause for liver cirrhosis in Eastern Europe, were found to be above the limit (1000 g/hl pa) only in apricot samples (5.3%). The application of canonical discriminant analysis (CDA) showed that plum and apricot spirits are generally characterized by higher levels of methanol in comparison to the other examined samples, while acetaldehyde dominates in grape pomace spirits. Distribution of higher alcohols among analyzed spirits was described by the following pattern: *n*-propanol – plum, *iso*-butanol and *n*-butanol – apricot, *iso*-amyl alcohol – quince and grape pomace. The obtained results suggest that control measures should be included in order to maintain the quality of homemade spirits and minimize the potential adverse health effects.

Keywords: Unrecorded fruit spirits, volatiles,quality and safety control, HSS-GC-FID

CHEMICAL COMPOSITION OF COMMERCIALLY AVAILABLE BILBERRY-BASED JUICES

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The consumption of superfruit juices has risen globally. Namely, the consumption of fresh raw and commercially available bilberry juices is becoming a part of a healthy lifestyle due to potential health benefits derived from biological effects of naturally occurring phenolics contained in berries. Moreover, assessing the quality and safety of food products, especially the ones considered as health beneficial is of great importance.

The aim of this study was to examine total phenolic content (TPC), anthocyanin content, antioxidant activities as well as the content of elements, hydroxymethylfurfural (HMF) and patulin of eight commercially available bilberry-based juices. TPC was determined using Folin-Ciocalteu method, total monomeric anthocyanin content by the pH differential method while *in vitro* antioxidant activity by DPPH radical assay. The HPLC methods were employed in quantification of HMF and patulin. Fifteen elements were analyzed using ICP-MS method (nutritionally important minerals were not included).

TPC was up to 2861.5 mg GAE/L while total monomeric anthocyanin content was up to 452.27 mg/L cyanidin-3-glucoside equivalents in the analyzed samples. High *in vitro* antioxidant capacities were confirmed in the samples with high TPC and total monomeric anthocyanins with a statistically significant correlation observed between TPC and antioxidant activity. The amount of HMF, a quality indicator, ranged between 0.4 mg/L and 17.9 mg/L in the analyzed juices, meeting the regulation. A mycotoxin patulin was quantified in only one sample, with the content below maximum regulated level (50 µg/kg, European Commission) indicating the use of good quality raw material in juice manufacture. Analysis showed that low amounts of lead were found in two juice samples, but below allowable level. Aluminum and barium were quantified in all samples, boron and cobalt were determined in three samples, while ten elements were not detected.

The results of this study are important to estimate the overall benefits and risks of consumption of commercially available bilberry-based juices.

Keywords: bilberry, juice, phenolic compounds, quality

DETERMINATION OF NITRITE IN PROCESSED MEAT PRODUCTS, PRODUCED IN THE ALBANIA, BY SPECTROPHOTOMETRIC METHOD AND RAPID TESTS

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In Albania, there is an increase in the daily amount of meat consumption and in particular salami and sausages, that are even cheaper compared to meat, especially those produced in the country. Sodium nitrate and sodium nitrite are approved additives found in many processed meat products (salami, sausages, ham etc.), considered the most important curing ingredients for preserving meat products. Unfortunately, recent research has demonstrated some negative effects because sodium nitrite reacts with stomach acid and produces nitrosamines, that are associated with some types of cancer prevalence. For this reason, the European Union has determined the maximum levels at which nitrates and nitrites can be used in meat products.

The study was based only on salami and sausages obtained from Albanian companies. These samples were collected at some of the biggest markets in Tirana. Samples were analyzed to determine the nitrite content by two methods: spectrophotometric method and rapid tests. The spectrophotometric method was used based on diazo-coupling reaction and absorption measured at 540 nm. The linearity of the calibration curve was good for nitrite content (R^2=0.99) in the concentration range 0.2-1.2 mg/l.

The nitrite concentration range for the spectrophotometric method was 0.188-60.590 mg/kg in salami samples and 1.201-16.950 mg/kg in sausages samples. The results obtained showed that none of the analyzed samples exceeds the limits allowed by Regulation (EC) No. 1333/2008, the maximum amount of nitrite that can be added to meat products it is 150 mg/kg.

However, some of the samples have the presence of nitrites in levels very close to limit that indicates that it is important to evaluate meat products for considered it safe. Also, from the results obtained from both applied methods (the rapid tests and the spectrophotometric method) it can be concluded that very similar values for nitrite content were obtained.

Keywords: nitrite, salami, sausages, spectrophotometric method

BIOLOGICAL ACTIVITY OF *Satureja montana* L. ETHANOLIC EXTRACTS AND THEIR EFFECT ON OXIDATIVE STABILITY OF COLD PRESSED SUNFLOWER OIL IN LONG-TERM STORAGE CONDITIONS

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Medicinal plants represent a rich source of phytochemicals, many of which possess diverse biological activities, including antioxidants. The objectives of this study were to assess antioxidant, antibacterial and anticancer activities of savory (Satureja montana L.) ethanolic extracts and to monitor the changes of oxidative status during long-term storage conditions of the cold pressed sunflower oil (CPSU) with the addition of extracts at various concentrations. Extracts were obtained by Soxhlet extraction and ultrasound-assisted maceration, using 70% and 96% ethanol. Antioxidant activity was evaluated by DPPH and β-carotene bleaching assays. In both assays, extracts showed remarkable antioxidative properties which were equal or stronger than those of BHT and BHA. Also, regardless of the extraction method and assay applied, the 70% ethanol extracts proved to be more potent than 96% ethanol ones. For antibacterial activity assay, minimal inhibitory concentrations (MIC) were obtained by the microdilution method. Tested Gram-negative bacteria (E. coli and S. typhimurium) were more resistant than Gram-positive (S. aureus and L. monocytogenes) with MIC=1,25–10 μg/mL and 0,0781–0,3125 μg/mL, respectively. As results obtained by MTT test, the extracts didn't show anticancer activity (IC50>100 μg/L) against tested tumor cell lines: PC-3 (prostate), HT-29 (colon) and HeLa (cervix) in the applied concentration range. For investigation of oxidative stability of CPSU, only 70% ethanol extracts were used. Samples of CPSU (with addition of 250, 500 and 1000 ppm of extracts), as well as control samples (with addition of 200 ppm of BHT and CPSU without any additives), were stored at room temperature for six months. In order to determine the level of oxidative changes, samples were analyzed for peroxide, anisidine value and conjugated dienes and trienes. Based on obtained results, it can be concluded that savory extracts are powerful natural antioxidants that can delay the oxidative deterioration of CPSU and whose effectiveness is comparable to the synthetic antioxidant BHT.

Keywords: savory, ethanolic extracts, biological activity, cold pressed sunflower oil, oxidative stability

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DETERMINATION OF HEAVY METAL LEVELS IN SARDINE SAMPLES

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The purpose of this study is to quantify the level of contamination by traces of heavy metals of Sardina fish samples using atomic absorption spectrometry (AAS). The sample digestion was carried out using ETHOS 1 - Advanced Microwave Digestion Labstation. After digestion, the concentrations of Pb and Cd were measured by graphite furnace method (Perkin Elmer 900T spectrometer with Zeeman AAS background corrector) with an auto sampler. Hg was analyzed by the cold vapor technique on Perkin Elmer flow injection system FIAS 100. $NaBH_4$ was used as a reducing agent, and 2% HCl was used as a carrier in FIAS analysis. Graphite furnace atomic absorption spectrometry (GFAAS) is one of the suitable methods for the determination of trace metals in food and biological samples because of its speed, minimum need for sample preparation, the possibility of automation, good sensitivity and low detection limit. The linearity of the analytical response across the studied range of concentrations were excellent, obtaining correlation coefficients higher than 0.99. The precision values associated with the analytical method, expressed as RSD values, were less than 3% for certain elements. The limit of quantification was 0.020 mg kg^{-1} for Pb and Hg and 0,005 mg kg^{-1} for Cd. Analysis revealed the presence of metal trace elements in the sardine samples at concentrations below the thresholds established by national and international regulations. The mean value for cadmium, lead and mercury was 0,008 mg kg^{-1}, 0,065 mg $kg^{-1}$and 0,057 mg kg^{-1}, respectively.

Keywords: heavy metals, sardines, atomic absorption spectrometry, GFAAS, FIAS

TOTAL PHENOLIC AND FLAVONOID CONTENT AND ANTIOXIDANT POTENTIAL OF FRUIT METHANOLIC EXTRACTS OF THREE TRADITIONAL PEAR VARIETIES FROM SERBIA

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Serbia is one of the centers of agrobiodiversity, with large number of traditional *Pyrus communis* L. varieties. Jagodarka is almost disappeared variety. The ripening period is in late May and early June. The fruit is very small (15-20 g), elongated-pear-shaped. The peel is thick, yellow-green, the fruit is juicy and rots rapidly. Ilinjača ripens in mid-July. The fruits are small to medium-sized (20-30 g), pear-shaped, with greenish-yellow peel. The flesh is white, medium sweet. Kaluđerka ripens in October. The fruit is large (up to 250 g), elongated asymmetrical pear-shaped, covered with thick, dry skin. The fruit is tasty. Pear fruits were collected in central Serbia. The whole fruits were stored at -20°C, and extracted with methanol. Total phenolic and flavonoid content of methanolic extracts at concentration of 25 µg/mL were determined spectrophotometrically, and antioxidant activity was evaluated by using $DPPH^{\cdot}$, $ABTS^{\cdot+}$ and FRAPS assays. Kaluđerka showed higher total phenolic content (116.68 mg GAE/g) compared to Jagodarka (97.77 mg GAE/g) and Ilinjača (91.22 mg GAE/g). Ilinjača displayed higher flavonoid content (21.61 mg QE/g) compared to Kaluđerka and Jagodarka (18.61 mg QE/g and 12.28 mg QE/g, respectively). The extract of Kaluđerka exhibited higher antioxidant capacity in $DPPH^{\cdot}$ assay (IC_{50} = 15.45 µg/mL) than Jagodarka and Ilinjača (IC_{50} =24.78 µg/mL, and 45.95 µg/mL respectively). In $ABTS^{\cdot+}$ assay higher activity of Ilinjača was recorded (2.11 mg AAE/g), compared to Kaluđerka (1.41 mg AAE/g) and Jagodarka (1.23 mg AAE/g). In the FRAP assay the highest value was obtained for Kaluđerka (823.00 µmol/Fe(II)/g), compared to Ilinjača (743.33 µmol/Fe(II)/g) and Jagodarka (564.67 µmol/Fe(II)/g). The values obtained by $DPPH^{\cdot}$ assay were strongly correlated to total phenolic content, while those obtained by $ABTS^{\cdot+}$ and FRAP assays showed strong correlation to total flavonoid content. The results indicated that examined varieties can be used as potential source of phenolic bioactive compounds.

Keywords: pear; variety; total phenolic content; total flavonoid content; antioxidant potential

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC PROFILING OF CAROTENOIDS IN SWEET CORN GENOTYPES

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Sweet corn is one of the most popular vegetables, being consumed all year round both fresh and preserved. It is highly appreciated not only for its particular taste, delicate flavor and sweetness but also for the nutritional properties, being a good source of carbohydrates, proteins, fiber, carotenoids and potassium. Among the biologically-active compounds it contains, carotenoids have multiple beneficial effects for human health (free radical scavengers, antioxidants, positive effects in prevention of certain types of cancer (lung, breast, colon and prostate cancer)/ UV-induced skin damage/ coronary heart disease, cataracts and macular degeneration; besides, carotenoids with β-ring end groups taken from the diet act as precursors for the production of retinoids in animal cells. Hence, the main aim of this work is to characterize the carotenoid content from several commercial sweet corn genotypes. Plant material was produced by the Research and Development Station for Agriculture (RDSA) Turda (Romania). Sweet corn kernels in milk stage were extracted with ethanol and acetone, the resulting suspension being filtrated under vacuum, then subjected to liquid-liquid extraction with diethyl ether-water (1 : 4) / evaporation to dryness in a rotary evaporator/ dissolution in ethyl acetate/ membrane filtration. Total carotenoids were determined by UV/VIS spectrophotometry using a T80+ UV-VIS spectrophotometer (PG Instruments Ltd, UK), while particular carotenoids were determined by reverse-phased high performance liquid chromatography (HPLC). HPLC analysis was accomplished using a Flexar system (Perkin Elmer, USA), consisting in two UHPLC pumps, a solvent degasser, an autoinjector, an UV-VIS detector, a controller and a computer running Chromera software. Separations were monitored at 450 nm, using a Nucleosil 5-C18 column (Macherey Nagel), the quantitative determinations being accomplished by the external standard method. HPLC analysis revealed as major carotenoids lutein, zeaxanthin and β-cryptoxanthin, while β-carotene was a minor carotenoid, the HPLC fingerprints of carotenoids in the studied genotypes showing significant differences. Provitamin A carotenoids (β-cryptoxanthin and β-carotene) are present in low quantities, the recorded provitamin A values are rather modest; however, certain genotypes proved to contain significant amounts of lutein and zeaxanthin. The obtained results are relevant for future nutrition studies, this work being in the meantime a valuable tool in assisting the breeding activity for improvement the nutritional quality of sweet corn.

Keywords: carotenoids, sweet corn, chromatography, HPLC, fingerprinting

LEAD (Pb) LEVELS IN BABY FOODS – CURRENT REGULATIONS AND FUTURE PERSPECTIVES

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Lead (Pb) is a harmful toxic metal found as a contaminant in food, air, consumer products, etc. The harmful effects of prolonged exposure to low doses of Pb have raised concerns all over the world, indicating that there is no safe blood Pb level (BLL). Babies and young children represent a highly sensitive population to Pb toxic effects, having in mind that they are in a growing period. Additionally, absorption of Pb in the digestive tract of the younger population is extremely higher than in adults. All these facts imply that strict regulations are needed for babies' and children's food. Current regulations in the European Union, as well as in Serbia, have set the maximum Pb level in infant formula at 20 ppb. The World Health Organisation provides 10 ppb as a provisional guideline. According to a growing agreement among health experts, Pb levels should not exceed 1 ppb in foods consumed by babies. In the U.S.A, the Academy of Pediatrics, the Environmental Defense Fund, and Consumer Reports have all advocated a 1 ppb limit in foods and beverages consumed by infants and children. Based on scientific data and current regulations, in April 2021, the Food and Drug Administration released an action plan called "Close to Zero" that aims to reduce exposure to Pb and other toxic metals in babies and young children's foods to the lowest possible levels. Future trends and needs for Pb levels in baby foods globally should be action to decline Pb levels as low as possible.

Keywords: Pb, regulation, EU, U.S.A., young children, food

THE CONTENT OF PIGMENTS, POLYPHENOLS AND FLAVONOIDS IN AVOCADO (*Persea Americana*) SAMPLES

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In the 21st century, the popularity of avocado fruit (*Persea Americana*) has increased as well as its use. Many studies have shown that both the peel and meat of this fruit are rich in bioactive components that positively affect human health. The aim of the present study was to determine the total amount of pigments, free polyphenols and flavonoids in avocado (*Persea Americana*) fruit. Extraction of the peel and meat of fruit was performed with 80% methanol. The prepared samples were stored in cold and dark place until further analysis. The content of selected bioactive compounds was examined by standard spectrophotometric methods. The obtained results have shown that the concentration of investigated bioactive components was significantly higher in the peel sample in comparison to the meat sample. The results for pigments exhibited a high concentration of chlorophyll *a* and *b* in the peel (19.39 and 11.12 µg/g, respectively), while in the meat only chlorophyll *a* was detected (1.34 µg/g of dry weight). It was observed that the content of total carotenoids was higher in peel compared to meat sample (3.78 µg/g and 0.96 µg/g, respectively). The content of free polyphenols in peel was 10 times higher than in meat (10.76 ± 0.74 mg/g GAE and 1.23 ± 0.04 mg/g GAE, respectively), while flavonoids were detected only in peel sample (2.98 ± 1.18 mg/g QE). According to obtained results, there is a significant difference in the content of bioactive compounds of the peel and the meat of the avocado, however, obtained values indicate that the usage of this fruit in the human diet may have a positive effect on human health.

Keywords: Avocado, Pigments, Polyphenols, Flavonoids, Bioactive compounds

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HUMAN NOROVIRUS REDUCTION ON STAINLESS STEEL AND PLASTIC SURFACES WITH COMMERCIAL DISINFECTANTS

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Human norovirus is considered the most common cause of acute nonbacterial gastroenteritis in the world. They are spread primarily by fecal-oral route, either by consumption of contaminated food or water, direct person-to-person spread, or environmental and fomite contamination. Their persistence in the environment and resistance to disinfectants considerably contribute to their transmission. Since norovirus can survive on surfaces for weeks, cleaning and decontaminating surfaces is critical. One of the effectiveness tests for validation of a compound to be registered as a virucide, indicates that it is acceptable if a minimum 4 log10 reduction of virus particles is demonstrated compared to control (Environmental Protection Agency, 2017). The aim of this study was to test the efficacy of some commercially available disinfectants for norovirus inactivation on stainless steel and plastic surfaces. Human NoV suspension (concentration 5 log genome equivalents, (GE) was applied on representative surfaces and treated with hypochlorite based or chlorine-free disinfectants, with 5 and 15-minute contact time, with/without post rinsing the surfaces. Samples were collected by the swab method. Extraction of norovirus RNA was done by Trizol method and RT-qPCR was completed (Norovirus Real-TM, Sacace Biotechnologies, Italy). On stainless steel reduction of norovirus particles was higher with hypochlorite based disinfectant after 15 min contact time and post rinsing and amounted to 2.23 ± 0.03 log GE while chlorine-free disinfectant had no effect on norovirus reduction without post rinsing. On the plastic board tested disinfectants had a similar effect regardless of the contact time. Better reduction at the level 2.55 ± 0.08 log GE was obtained by rinsing surfaces after treatments with hypochlorite based and chlorine-free disinfectants. Results indicated that different factors, such as disinfectant type, application methods, type of material, should be considered when choosing measures for prevention and control environmental contamination by noroviruses.

Keywords: human norovirus, commercial disinfectants

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SELECTED BIOACTIVE COMPOUNDS CONTENT OF CINNAMON SPICE

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Cinnamon spice is obtained by peeling the inner bark of an evergreen tree *Cinnamomum zeylanicum* (fam. Lauraceae), native to Sri Lanka. It is one of the earliest known spices on the Asian continent. Today, it is highly valued and widely used all around the world, as a whole (bark sticks), ground spice or essential oil. Cinnamon has a long tradition of use in cooking (as flavoring agent), aromatherapy (as fragrant agent), traditional medicine (for treatment toothache, dental problems, bad breath, diabetes, rheumatism), and is also recognized in official medicine (show antioxidant, antidiabetic, antimicrobial, anti-inflammatory activities). The biological activity of cinnamon is attributed to a number of bioactive compounds, and as the most important stood out cinnamaldehyde and *trans*-cinnamaldehyde (Cin). In this study, dry, ground cinnamon was analysed to determine the content of: total phenolics (TPC), total flavonoids (TFC), total hydroxycinnamic acid derivatives (HCAs) and total carotenoids (TCC). Bioactive compounds were extracted by solvent extraction (SE) and ultrasound assisted extraction (USE) in 80% acetone and warm water (50 °C). The content of bioactive compounds were determined spectrophotometrically. The TPC in acetone extracts prepared by UAE was the highest (29.83 ± 0.02 mg GAE/g of DW), but did not show a significant difference in comparison to TPC achieved in acetone extract obtained by SE (29.79 ± 0.11 mg GAE/g of DW). The highest TFC (1.41 ± 0.17 mg CE/g of DW) and HCAs (14.59 ± 0.79 mg CGAE/g of DW) were observed in acetone extract prepared by SE, while the highest TCC (277.10 ± 4.82 μg/g) was acquired in acetone extract prepared by UAE. Also, the content of tested bioactive compounds of cinnamon in water extracts was higher compared to some spices described in recent studies, which places cinnamon as a good source of bioactive compounds with potential health benefits.

Keywords: bioactive compounds, cinnamon, spice.

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ANTIOXIDANT ACTIVITY OF SELECTED *ALLIUM* SPECIES GROWN IN SERBIA

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Species of the genus *Allium* are used for different purposes: as food (vegetables, spices, flavor agent in the bakery industry), medical agent in folk medicine, and as decorative perennials. Effect of onion, garlic and leek on human health have been described in many studies. The presence of compounds with potential antioxidant activity are considered responsible for their health effect. Other species of this genus have been intensively studied in recent years in order to estimate their nutritional value and healing effect. In this paper, the antioxidant activity of edible parts of two *Allium* species (*Allium nutans* and *A. odorum*) was determined, by DPPH· and $ABTS^{\cdot+}$ scavenging assays. The tested species were grown in Serbia, in open field conditions and were foliar treated with selenium fertilizer (Na_2SeO_4) in four doses (0, 10, 20 and 30 g per ha), in the phase of intensive growth. Antioxidant activity was determined spectrophotometrically, by reading the absorbances of the samples at 515 nm in the case DPPH· assay and at 734 nm in the $ABTS^{\cdot+}$ assay. The obtained results were expressed as a percentage of inhibition of DPPH radicals and ABTS radical cations caused by the action of the extract of the tested *Allium* species. *A. nutans* had the highest antioxidant activity in control variant – without Se treatment (23.77% ± 0.19) in DPPH· assay, and in variant II - 10 g of selenium fertilizer per ha (69.05 ± 6.06) in $ABTS^{\cdot+}$ assay. The highest antioxidant activity of *A. odorum* was detected in variant IV - 30 g of selenium fertilizer per ha (25.75 ± 0.28) in DPPH assay. In *A. odorum* there was no significant difference in antioxidant activity in the control variant (33.41 ± 0.38) and variant III - 20 g of selenium per ha (33.79 ± 0.46), in ABTS assay.

Keywords: $ABTS^{\cdot+}$, Allium species, antioxidant activity, DPPH·.

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PHYTOCHEMICAL COMPOSITION AND *IN VITRO* BIOLOGICAL ACTIVITIES OF GOJI BERRY CULTIVATED IN SERBIA

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Due to the presence of many nutrients and bioactive compounds, fruits of *Lycium* species (*Fructus Lycii*, goji berries) are recognized as a "superfood," which regular consumption might exert many beneficial health effects. Therefore, in addition to China and other Asian countries, the cultivation of *Lycium* species has become widespread throughout Europe. This study investigated the total phenolics (TPC), total flavonoids (TFC) and polysaccharides content, as well as biological properties of goji berry (**Lycium barbarum** L.) cultivated in the Belgrade region, Serbia. The HPLC method was used for the identification of individual phenolic compounds (rutin, quercetin-diglucuronide, chlorogenic acid and caffeic acid). Antioxidant properties of goji berry extracts with different solvent were evaluated for radical scavenging (DPPH, ABTS), reducing power (CUPRAC, FRAP), and β-carotene/linoleic acid bleaching inhibition capacity. Additionally, goji berry extracts were screened for their anti-diabetic (α-amylase, α-glucosidase), anti-tyrosinase, and anti-acetylcholinesterase activities. Methanol was identified as the most effective solvent for the extraction, resulting in the highest contents of TPC (3.76 mg GAE/g dry weight), TFC (0.65 mg CE/g dry weight), as well as superior antioxidant activity evaluated by all assays. Also, the methanol extract of goji berry inhibited all tested enzymes in concentration-dependent manners. The highest enzyme inhibitory activity was shown for tyrosinase (IC_{50} of 1.4 mg ± 0.01 mg/mL). The IC_{50} values were 4.66 ± 0.25 mg/mL, 10.68 ± 0.07 mg/mL, 7.07 ± 0.1 mg/mL for α-amylase, α-glucosidase and acetylcholinesterase inhibition activity, respectively. Overall, obtained results suggest that goji berry cultivated in Serbia is a valuable source of bioactive compounds that can be use in the food, nutraceutical, and cosmetic industries.

Keywords: goji berry, bioactive compounds, antioxidant, enzyme inhibitory activity

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ASSESSMENT OF THE ANTIOXIDANT POTENTIAL OF WHITE, RED AND BLACK CURRANT (*Ribes*) SAMPLES

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In recent years, oxidative stress is one of the biggest causes of various pathological conditions like inflammation, cardiovascular and some neurological diseases. Numerous studies have shown that berry fruits which include white, red and black currants are a good source of bioactive compounds such as phenolic acids, tannins, anthocyanidins,flavan-3-ols, etc. These chemical compounds are also known as potentantioxidants and play a key role in quenching free radicals and protect cellsfrom oxidative stress. In this study, the antioxidant activity of white, red and black currant fruits has been evaluated. For extraction, 80% acetone and acidified (0.1% HCl) methanol were used.The assessment of the antioxidant activity of samples was performed by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. According to obtained results, the samples of white, red and black currant extracted with acidified (0.1% HCl) methanol had similarability to scavenge DPPH free radicals (86.22% ± 0.28, 86.90% ± 0.19 and 84.34% ± 1.05of DPPH radical inhibition,respectively) while the ability of inhibition for samples extracted with 80% acetone varies significantly(86.09% ± 0.47, 76.61% ± 0.76 and 12.5% ± 4.37 of DPPH radical inhibition, respectively). Based on the high values of antioxidant activity of the samples it can be concluded that the consumption of currant fruits may potentially have a beneficial effect on human health and protecting cells from oxidative stress.

Keywords: Currant, Antioxidant activity, DPPH radical

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ANTIOXIDANT SCORES COMPARISON OF AVOCADO AND OLIVES

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Dietary habits of individuals worldwide appear to be undergoing a transition toward higher consumption of plant foods, especially plant foods rich in bioactive ingredients. This changing pattern involves, among other, the increasing popularity of foods such as avocado and olives, used as a salad, oil, or as a spread. Both olives and avocado are high in calories and have the same macronutrient energy ratio (4:19:77 for protein, carbohydrates and fat, respectively) that makes them interesting as rich sources of beneficial unsaturated fatty acids. Beside macro- and micro-nutrients, olives and avocado contain various phytochemicals, of which polyphenols are the most interesting ones. This study was aimed to assess and compare total polyphenol content as well as antioxidant activity of avocado (Hass and Fuerte varieties) and olive (Amfissa and Kalamata varieties) fruit extracts. Pitted samples were dried to constant mass, defatted and then extraction was conducted by 70% ethanol using ultrasonic bath. Total phenolic content (TPC) was carried out by Folin-Ciocalteu colorimetric assay. Four different spectrophotometric methods ($DPPH^{\cdot}$, $ABTS^{\cdot+}$, FRAP, and CUPRAC) were used for antioxidant capacity assessment. Values of antioxidant potentials and phenolic content (X) were transformed into a T_{score} by means of the formula T_{score}=(X-min)/(max-min), where min and max, respectively, represent the lowest and the highest values of variable X. For each sample, the five T_{score} were summed up, yielding a global antioxidant score (GAS) ranging from 0 to 5. The results of this investigation indicated that the variety was one of the important factors that influenced the type and amount of extracted biomolecules. Although the calculated GAS value for olives was even 12-fold lower on average than that determined for avocados, a statistically significant difference between these foods was absent due to a very small sample size. Therefore, future experiments should include much more samples in order to confirm these preliminary results.

Keywords: avocado, olive, extract, GAS

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CHEMICAL COMPOSITION OF NETTLE (*Urtica Dioica* L.) SEEDS AND FATTY ACID COMPOSITION OF NETTLE SEED OIL

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Stinging nettle (*Urtica dioica* L.) is a perennial flowering plant belonging to the family Urticaceae. It is a good source of minerals, especially iron, vitamin C and pro-vitamin A, but it is also rich in protein, carbohydrates, and oil. Research results indicate a high nutritive value for nettle and confirm their extensive health-promoting properties. This paper is deals with the chemical composition (moisture, ash, fiber, oil, protein, starch, and soluble sugars content) of nettle seed ("Jeligor", Svrljig, Serbia) and fatty acids composition of nettle seed oil. The ash content was determined by annealing the sample in a furnace at 850 °C. The oil was obtained by extraction by using reflux and trichloroethylene as solvent and evaporation of the solvent under vacuum. The composition of fatty acids was determined by using the GC-MS method. The protein content was determined according to the standard Kjeldahl procedure (Nx5.7). The content of soluble sugars and starch was determined by the anthrone colorimetric assay using glucose as standard. The results showed that most of the chemical composition consists of oil with the content of 20.1%, followed by proteins with a content of 17.5%. The content of starch and soluble sugars also accounts for a significant proportion of the total chemical composition of nettle seeds and was 10.3% and 3.12%, respectively. The ash and fibber content was 13.06% and 7.92%, respectively, while the moisture content was 5.15%. Among the fatty acids, linoleic acid was the most abundant (86.05%), followed by oleic acid (12.03%). The results demonstrated the nettle seeds could be a good supplement in bakery products based on cereals to increase the nutritional value.

Keywords: Urtica dioica L., seeds, chemical composition, fatty acids, oils

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CHARACTERIZATION OF MALTESE HONEY HARVESTED IN DIFFERENT SEASONS BY USING PHYSICOCHEMICAL PARAMETERS AND APPLIED MULTIVARIATE DATA ANALYSIS

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Malta, a country renowned for its honey, possesses high variability of flora during the seasons. As the collection period affects honey content and quality, the significance of this study refers to the obtained data of honey samples collected during autumn, spring and summer seasons. The physicochemical parameters that were analyzed were moisture and Brix content, electrical conductivity, pH, free acidity, diastase activity, proline and hydroxymethylfurfal (HMF) concentration. It was noted that all samples showed low values of diastase activity and HMF, which is well known for honey with low natural enzyme content. Higher values of pH and moisture content were noted in autumn honey samples. The principal component analysis (PCA) performed on the physicochemical parameters provided a more obvious classification of Maltese honey collected at different seasons. It was noted a grouping of summer honey by free acidity, diastase activity and proline content. Differentiation of autumn honey was based on electrical conductivity, moisture and HMF content, while spring samples have the lowest value of all physicochemical parameters. In addition, it was noted the agreement between the established season variability of honey samples from Malta obtained by the physicochemical analysis and the applied statistical analysis.

Keywords: Malta, Honey, Seasons, Physicochemical analysis, PCA.

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EFFECT OF *COTA TINCTORIA* ON THE SURVIVAL AND BIOFILM FORMATION OF *LISTERIA MONOCYTOGENES*

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Listeria monocytogenes is a pathogenic bacterium, with human disease and infection linked to dairy products, processed meat, as well as products that are kept refrigerated for a long time since this bacterium *L. monocytogenes* can survive and grow at low temperatures. Plants are an excellent source of compounds in the search for natural products that can be used in the control of pathogens in the food industry. *Cota tinctoria* (L.) J. Gay ex Guss., yellow chamomile, is used in the food industry for the production of diary and butchery products and this study aimed to investigate its effect on the growth of common food contaminant *L. monocytogenes*. Five different dry extracts were extracted from aerial parts, using various solvents ethanol/water solution (100%, 70%, 50% and 30% v/v) and decoction. Three food isolates and two ATCC strains of *L. monocytogenes* were used. Obtained extracts exhibited promising antimicrobial potential (minimal inhibitory concentration range 0.56-4.50 mg/mL and minimal bactericidal concentration 1.12-9.00 mg/mL) as determined by microdilution assay. On the other hand, their antibiofilm capacity did not exceed 50% inhibition of 48h *L. monocytogenes* biofilms as observed in crystal violet assay. The 70% ethanol extract was selected due to its low MIC values and studied for the dynamic of inhibition of bacterial growth at 4°C and 37°C. Application of *C. tinctoria* extract slowed down the growth of *L. monocytogenes* at both temperature conditions to a moderate extent. *C. tinctoria* has shown the promising capability to reduce the growth of *L. monocytogenes*, one of the most common bacterial food contaminants, and should be further explored in this manner.

Keywords: Cota tinctoria, Listeria monocytogenes, Biofilm, Antimicrobial.

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EXAMINATION OF VITAMIN C AND TOTAL PHENOLIC CONTENT IN RAW QUINCE AND ITS PRODUCTS

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Quince (*Cydonia oblonga* Mill.) belonging to the family Rosaceae, originates from the Caucasian area. Quince has many positive biological activities such are antimicrobial, antioxidant, antiallergic and wound healing. It is used in traditional medicine to cure cough, sore throat, canker sores, diarrhea, high fever, dysentery and gum problems. The main objective of this study is the determination of ascorbic acid and total phenolics content in raw quince fruit, fruit juice, compote and jam in order to get the information of their potential use as a source of biologically active compounds. Vitamin C was determined by direct titration with iodine in prepared samples (water extracts of raw quince fruit, jam, compote and quince juice). The same samples were used for the colorimetric determination of total phenolic content with the Folin-Ciocalteu reagent. The content of total phenolics and vitamin C decreases from raw quince fruit, juice, compote to jam. Raw quince fruit contains 15.75 mg of vitamin C in 100 g; quince juice contains 10.05 mg in 100 mL, while quince compote and jam contain 8.3 and 4.98 mg vitamin C in 100 g respectively. Total phenolic content found in samples analysed was 48.88 mg gallic acid equivalents (GAE) in 100g for raw quince fruit, 45.74 mg GAE in 100 mL for quince juice, and 40.65 and 38.57 mg GAE for quince compote and jam, respectively. Content of vitamin C in raw quince fruit is highest and decreases with the duration of thermic processing of quince products. Total phenolics content in unpeeled quince fruit is relatively high while processing does not decrease their concentration significantly.

Keywords: quince juice; quince compote; quince jam; vitamin C; phenolics

CHEMICAL AND PHENOLIC COMPOSITION OF FRUITS OF RASPBERRY AND BLACKBERRY PROPAGATED BY STANDARD AND *IN VITRO* TECHNIQUES

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Berry fruits are a valuable source of phytochemicals, primarily sugars, organic acids and phenolic compounds. Since sugars and organic acids are the most abundant soluble solids, they greatly determine fruit's taste, which is one of the most important parameters for consumers' acceptance. Due to the high content of naturally occurring antioxidants, particularly flavonoids, phenolic acids and anthocyanins, berries are considered as an important source of health-promoting compounds. The Republic of Serbia is one of the leading producers of raspberry and blackberry in Europe. Although high yield and satisfactory fruit quality largely depend on agroecological conditions, they cannot be reached without high-quality planting material. Raspberry and blackberry are mostly propagated vegetatively by conventional methods. However, micropropagation enables a year-round supply of physiologically uniform, true-to-type and disease-free planting material. Thus, we established the orchard using planting material propagated by standard technique (ST) and by *in vitro* tissue culture (TC). Three-year study evaluated the fruit quality of raspberry 'Meeker' and blackberry 'Čačanska Bestrna', considering the different origins of the planting material. Several chemical parameters of fruit quality (dry matter, content of sugars, total acids content, pH, total pectins) were analysed. High-performance liquid chromatography was used for the determination of phenolic acids (protocatechuic, 4-hydroxybenzoic, ellagic, gallic, *p*-coumaric, caffeic and ferulic acid), flavonols (quercetin) and anthocyanins (cyanidin and pelargonidin). No significant differences were observed between berries from the ST and TC plants in both fruit species regarding any analysed chemical parameter. As expected, the most abundant phenolic acid in all berry samples was ellagic acid. With exception of 4-hydroxybenzoic acid in blackberry, the type of planting material did not significantly affect the content of bioactive compounds in both blackberry and raspberry. On the other side, experimental year significantly influenced the abovementioned compounds, excluding the content of ellagic acid, 4-hydroxybenzoic acid and gallic acid in raspberry.

Keywords: Rubus, 'Meeker', 'Čačanska Bestrna', bioactive compounds, ellagic acid.

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SUGAR PROFILE OF ORGANIC AND CONVENTIONAL MAIZE GRAINS

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There are plenty of research articles which emphasised divergences in nutrients content between organic and conventional food products. In order to determine possible differences between maize grains grown under organic (OM) and conventional (CM) production systems, the sugar profile was determined by HPLC-RI analytical technique. For that purpose, two samples of the Rumenka maize (*Zea mays*) variety were used. Samples were grown and collected at the experimental field of Maize Research Institute (Zemun Polje, Serbia) during three seasons (2015-2017). The obtained results (the content of pentoses, hexoses, non-reducing and reducing disaccharides) are expressed as % of total soluble sugars. The content of total individual sugar was in the range from 3.91% (OM-17) to 5.18% (OM-15). The lowest content of pentoses was recorded in organic maize from 2017 (1.08%) while the highest content was in conventionally grown grain (1.48%) from the same year. Hexoses content was in range from 0.84% (OM-17) to 1.84% (CM-16). Regarding disaccharides content it was observed that non-reducing disaccharides (1.17 (CM-17) - 1.67% (OM-16)) were more represented compared to reducing disaccharides (0.38 (CM-17) - 0.76% (CM-16)). The content of hexoses significantly differs ($p < 0.05$) between grains from applied production systems during all seasons as well as the pentoses content from 2017. In both cases, it was higher in conventional grains. Organic maize was a better source of non-reducing disaccharides (seasons 2016 and 2017) compared to conventional samples. Additionally, seasonal variation (2016/2017) in the content of reducing disaccharides was observed in conventional maize grain.

Keywords: maize, organic seed, conventional seed, sugars

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POLYPHENOLS CONTENT OF BERRY LEAVES AND CALLUSES

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Secondary plant metabolites are sinthetised as stress-responding compounds and provide selective advantages to plants. This is a hudge group of compounds with heterogeneous structures. One of the biggest class of these compounds are polyphenols. Most of them also act as antioxidants, providing benefits for human health. Consequently, food scientists explore possibilities for the isolation and application of these bioactive compounds in food industry. In order to obtain polyphenols, which are present in the plant in low quantities, a conventional plant cultivation is not efficient enough. *In vitro* plant tissue culture techniques, in highly controlled conditions and using appropriate medium, allow, in shorter period of time, production of the whole plant or the plant tissue with ability to synthesize desired compounds, overcoming this problem.

In order to determine whether *in vitro* tissues can be a good source of polyphenols, in this paper, *in vitro* shoot and callus cultures were established. It was done with two berry fruit genotypes – blackberry (*Rubus* subg. *Rubus* Watson 'Čačanska Bestrna') and blueberry (*Vaccinium corumbosum* L. 'Toro'). Extraction and quantification of total phenolics (TPC) and total flavonoid content (TFC) were done both from callus and leaves tissues obtained from *in vitro* plants, as well as from field-grown plants. TPC and TFC were the highest in leaves of blueberry (13.47–14.06 mg GAE/g DW, for *in vitro* and field-grown plants, respectively) whereas low TPC and TFC values (0,78–2,39 mgGAE/g DW) for both callus cultures were obtained.

Results show that *in vitro* tissues could be a good source of polyphenols. Considering the cultivation time reduction and the process simplification, tissues are rentable sources of polyphenols.

Keywords: plant tissue culture, berry fruits, polyphenols, flavonoids

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CONTENT OF MACRO- AND MICROELEMENTS AS A TOOL TO ASSESS THE BOTANICAL ORIGIN AND CULTIVATION SYSTEMS OF POTATO

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Minerals are macro and micronutrients necessary for the growth and proper functioning of the human body. Humans require at least 22 mineral elements for their wellbeing, and potato (*Solanum tuberosum* L.) is their excellent source. The mineral content of potato crops is influenced by several factors, including soil type, climatic conditions, production systems, and variety choices. There is currently little data relating to the metal content of potato tubers with various agricultural systems in which they are grown. Comparative studies rarely control for these factors and they do not repeat the experiments over several growing seasons. On the other hand, available experiments show contradictory results, often due to the limited number of samples analyzed, varieties of potato, short time between the experiments and/or geographical/ecological variability. Therefore, the main objective of this work was to identify specific chemical markers that can serve as indicators of the type of production, botanical origin, and ripening time. The paper compares the content of 16 macro- and microelements of a statistically significant number of samples - 48 bulk and 48 peel - of four varieties of potatoes (two red and two yellow varieties with various ripening times) from three years and three types of production (conventional, integral and organic). Inductively coupled plasma - optical emission spectrometry (ICP-OES) was used to analyze macroelements, while the microelements were determined with ICP- quadrupole mass spectrometry (ICP-QMS). Multivariate analysis of variance (MANOVA) was conducted to determine the source of variation among the types of production, parts of potato, and varieties. The results indicate that Ca, Mg, and K may be considered as indicators of the type of production, botanical origin, and ripening time. Additionally, microelements such as Cd, Co, Cr, Mn, Ni, Pb, Cu, Zn including Fe and Na can distinguish between production types and botanical origin of potato.

Keywords: Potato, multielemental analysis, type of production, MANOVA

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PESTICIDE RESIDUES IN CHICKEN EGGS - A SAMPLE PREPARATION METHODOLOGY FOR ANALYSIS BY GAS CHROMATOGRAPHY / MASS SPECTROMETRY

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The aim of this research was to adapt the QuEChERS method for routine pesticide multiresidue analysis in chicken eggs samples using gas chromatography coupled to mass spectrometry (GC/MS). Pesticide residues were extracted using the modified QuEChERS technique with acetonitrile and then purified using the dispersive solid phase extraction (d-SPE) clean-up. Also, for comparison ethyl acetate was used as a solvent for the extraction of non-polar and polar analytes. A sample preparation method was developed for the analyses and validation of 57 pesticide residues. Several clean-up approaches were tested: d-SPE with Enhanced Matrix Removal-Lipid (EMR-Lipid), combination of primary secondary amine (PSA) and C18 sorbents, and for the third preparation used florisil cartridge (tubes, 170 µm, 80 Å). Compared to the currently established EN 1528 method, this validated method uses much less solvent, it is quick and easy to prepare a sample for analysis. Matrix-matched solutions were also prepared by serially diluting the intermediate solution with blank eggs sample extracts containing none of the tested analytes to perform matrix-matched calibration with the same concentrations as in the solvent. The linearity of the analytical response across the studied range of concentrations (0.010 - 0.10 mg kg^{-1}) was excellent, obtaining correlation coefficients higher than 0.99. The average recoveries of the pesticide ranged from 72% to 115%, for fortification levels of 0.01; 0.02 and 0.1 mg kg^{-1}. The precision values associated with the analytical method, expressed as RSD values, were less than 20% for the pesticide in the egg matrices. Internal standard calibration was established in order to compensate for recovery losses of certain pesticides and possible matrix effects. Triphenyl phosphate was used as an internal standard and its retention time was 27.40 min. Limit of quantification was 0.01 mg kg^{-1} for the majority of the pesticides.

Keywords: pesticides, eggs, QuEChERS method, GC/MS, matrix effect

GAS CHROMATOGRAPHY – MASS SPECTROMETRY BASED METABOLITE PROFILING OF SEEDS FROM CULTIVATED BERRY FRUITS

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Berry seed oils are a good source of essential fatty acids and bioactive compounds that could be potentially used as functional ingredients in food, cosmetic and pharmaceutical industry. Fruit processing produces large amounts of by-products in the form of seeds and their better utilization is promising and economically attractive field. Therefore, more information about seeds and their composition is required.

The object of this work is characterization of 37 different genuine Serbian strawberry, raspberry, blueberry, black currant, blackberry and gooseberry cultivars and determination of its authenticity regards their botanical origin by fatty acid profiling. Gas chromatography - mass spectrometry (GS-MS) analysis was applied for the separation and identification of the fatty acid methyl esters obtained from analysed seed oils. A set of chromatographic signals as unique multivariate fingerprint was used for sample characterization. Resulted fatty acid profiles within the same fruit species showed specific pattern, only intensity of the signals differ among cultivars. The most abundant fatty acid in berry seed oils was linoleic acid, followed by oleic, α-linolenic and palmitic acids. Pattern recognition methods applied for classification of cultivated berries cultivars confirmed their botanical origin. This kind of investigation can contribute to the inter-cultivar discrimination and enhancing the possibilities of acquiring an important authenticity factor.

Keywords: Fatty acid profile, Fruit seeds, Authenticity, Botanical origin.

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NITRATES AND NITRITES IN MEAT PROCESSED PRODUCTS: REGULATIONS AND OCCURRENCE

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Use of nitrite and nitrate as common food additives is a controversial issue. Both represent a class of preservatives mostly used during meat processing. Nitrite is an approved additive considered a foremost curing ingredient for the preservation of meat products, nitrate as well. Except for that as nitrate is frequently encountered in the environment, it is considered as a natural constituent of the human diet that raises concern for its potential harmfulness related to the human health conditions and environmental contamination. Nitrite is commonly used in cured meat products while nitrate is used mainly in long shelf life raw fermented meat products. The use of both chemicals is regulated by Albania Directive ”Food Additives other than colorants and sweeteners”.

The authors of this paper investigated the occurrence of these chemicals in two selected cured meat products because of an ongoing discussion and rumour in local media for presence of nitrate in cured meat products though even well below the allowed maximum level. As all the producers sustain they do not use nitrate potassium/sodium in their cured meat product, the authors analysed two kinds of sausages in different national and foreign laboratories to confirm the presence or not of nitrate and more important, trying to explain the route of nitrate entering in these products. The results of nitrate findings in both products were inconsistent showing fluctuations between labs though all results were well below the maximum limit. The authors tried to explain the cause of this presence by analysing raw material and processing water or considering other ingredients where nitrate would be present as a contaminant. The respective analysis confirmed that nitrate originated from these sources contributed at only a detectable presence of nitrate in cured meat products leading to the conclusion that the persistent but fluctuating nitrate presence is mainly consequence of the chemical biological conversion of initial nitrite to nitrate. Potential factors influencing this conversion should further be studied.

Keywords: food preservatives, meat processed products, nitrites, nitrates

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CHARACTERISTICS OF *PSEUDOMONAS* SPP. ISOLATES FROM FOOD OF ANIMAL ORIGIN

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Pseudomonas spp.as ubiquitous microorganism is often found in environmental raw materials as a contaminant. *P.* aeruginosa and P. fluorescens but also P. putida, P. fragi and P. cochorii may be isolated from milk and meet. From the view point of food hygiene synthesis of thermostable lipolytic and proteolytic enzymes in the cold chain of food production, can cause the spoilage of final product. As a causative agent of nosocomial infections, Pseudomonas spp. are often resistant to a large number of antimicrobial substances. Due to their ubiquity and ability to acquire resistance represents a potential risk to human health. The aims of the study were to assess antimicrobial susceptibility of Pseudomonas spp. isolated from food and to evaluate their proteolytic and lipolytic activity. A total of 40 isolates (20 from raw milk; 20 from carcasses of slaughtered pigs) were examined. All of the isolates were oxidase and, catalase positive, produced a pigment on Tryptone Soy Agar and had a characteristic odour. They also showed haemolysis on Blood agar, lipolysis on Tributyrin and proteolysis on Casein agar. Antimicrobial susceptibility testing was performed by disk diffusion test on piperacillin/ tazobactam, ticarcillin, imipenem, meropenem, aztreonam, amikacin, gentamicin, levofloxacin and ceftazidime. The sensitivity of milk isolates was 100%; 65%; 100%; 100%; 25%; 75%; 30%; 65% and 100%, whereas the susceptibility of carcass-derived isolates was 95%; 55%; 95%; 95%, 0%; 95%, 10%, 25% and 100%. Susceptibility of milk and carcass-derived isolates to piperacillin/tazobactam, ticarcillin, imipenem, meropenem, aztreonam, amikacin, gentamicin, levofloxacin and ceftazidime was 100%, 65%, 100%, 100%, 25%, 75%, 30%, 65%, 100% and 95%, 55%, 95%, 95%, 0%, 95%, 10%, 25%, 100%, respectively. There was no resistance to ceftazidime indicating no ESBL strains. MDR strains resistant to three or more antibiotics were 2 (10%) from milk and 4 (20%) from meat, namely ticarcillin, gentamicin, levofloxacin in milk isolates and ticarcillin, gentamicin, levofloxacin in three and piperacillin / tazobactam, ticarcillin, gentamicin, levofloxacin of one carcase isolate. In the present study, four MDR isolates were detected, 2 (10%) being isolated from milk and 4 (20%) from meat. Their resistance patterns were as follows: resistance to ticarcillin, gentamicin, levofloxacin (milk: n=2); resistance to ticarcillin, gentamicin, levofloxacin (carcase: n=3), resistance to piperacillin/tazobactam, ticarcillin, gentamicin, levofloxacin (carcase: n=1). In conclusion, pseudomonads might contaminate raw food of animal origin consequently leading to spoilage and considered as a reservoir of *Pseudomonas* spp. resistome.

Keywords: Pseudomonas spp., *food, spoilage, antibiotic, susceptibility*

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INHIBITORY ACTIVITY OF AUTOCHTHONOUS LACTOCOCCI ON *Listeria monocytogenes* DURING THE KAJMAK STORAGE

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Some of autochthonous lactic acid bacteria have antilisterial effect which enables them to be used as the protective culture. In order to exame the antilisterial effect of autochthonous lactic acid bacteria, the following variants of kajmak were prepared at different concentrations of Listeria monocytogenes ATCC19111 (103, 104, 105 cfu/ml): A1- inoculated with Listeria monocytogenes ATCC19111; A2- inoculated with L. monocytogenes with the addition of autochthonous Lactococcus lactis BGBU1-4; A3- inoculated with L. monocytogenes with the addition of autochthonous Lactococcus lactis spp. cremoris 565. The number of L. monocytogenes was monitored on Palcam agar in 0., 7., 14., 21. and 28. day. The results showed that in variant with L. monocytogenes (A1) the number was maintained, while in variants with added autochthonous strains (A2, A3)., number slightly decreased during 7 days. However, from 14th to 28th day, amount of L. monocytogenes was significantly reduced in variants with added autochthonous strains. Among these variants better results were detected at concentrations 103 and 104cfu/ml of L. monocytogenes and the number was reduced to 1.54-1.38log and 2-1.55log, respectively. In the variants at concentration of 105 cfu/ml of L. monocytogenes, added autochthonous strains slower decreased the number achiving 2.38-2.77log. It is concluded that the addition of autochthonous strains showed a great antilisterial effect, especially in the lower concentrations of *L. monocytogenes* and in the later period of kajmak storage.

Keywords: autochthonous lactic acid bacteria, inhibitory activity, *Listeria monocytogenes*

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ANTIMICROBIAL ACTIVITY OF ALLYL THIOSULFINATE (ALLICIN), ITS INCLUSION COMPLEX WITH β-CYCLODEXTRIN AND AJOENES AND VINYLDITHIINS DERIVATES

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The main carriers of the pharmacological activity of garlic (*Allium sativum* L.) are organosulfur compounds. The most important among them is allyl thiosulfinate (allicin), which is very unstable. Allicin is produced upon tissue damage, as a secondary metabolite from alliin in a reaction catalyzed by the enzyme alliinase. Allicin has antibacterial, antifungal and antiviral activity. The most important pharmacologically active allicin derivatives are ajoenes and vinyldithiins. They take part in the inhibition of thrombocyte aggregation, regulation of blood pressure, and reducing triglyceride and phospholipid levels. The aim of this work was to examine and compare the antimicrobial activity of allicin, its derivatives ajoenes and vinyldithiins, as well as its inclusion complex with β-cyclodextrin, in the moment of synthesis and after two months. The synthesis of allicin was performed by oxidation of allyl disulfide using acidic hydrogen peroxide at +4 °C for 4 h. The reaction mixture was neutralized while cooling and obtained allicin was isolated by extraction using diethyl ether. The inclusion complex was prepared by mixing the allicin and β-cyclodextrin in a molar ratio of 1:1 at 10 °C, using kneading method. Ajoenes and vinyldithiins were synthesized from allicin using acetone (80 °C for 5 h) and *n*-hexane (45 °C for 90 minutes), respectively. The antimicrobial activity of allicin, inclusion complex, ajoenes and vinyldithiins was tested using disc diffusion assay in different time intervals (0, 8, 23, 40, 52 and 62 days). The following microorganisms were used: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*. The results show that antimicrobial activity was reduced in the following order: ajoenes, inclusion complex, vinyldithiins, allicin. Inhibition zones for ajoenes and vinyldithiins remained unchanged after two months, while allicin antimicrobial activity declined rapidly with time. The allicin activity and stability are increased by its incorporation in the inclusion complex.

Keywords: allyl thiosulfinate, ajoenes, vinyldithiins, inclusion complex, antimicrobial activity

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MICROBIOLOGICAL CONTAMINATIONS OF FOODSTUFFS

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Availability of a sufficient amount of safe food is a basic human right and precondition of healthy eating and of the population's health preservation. Unsafe food causes many acute and life-long diseases, ranging from diarrhoeal diseases to various forms of cancer. The regular public health control of harmful substances in food enables prompt recognition of risk in food, since their presence in food even in legally allowed concentration increases the risk of health damage in people, especially in sensitive population groups such as pregnant women, infants, little children, the elderly and immunodeficient people. Foodborne illness can be caused by microbiological, chemical or physical hazards. Microbiological and chemical contaminations of foodstuffs represent a risk for the occurrence of food born transmissive diseases.

The data on chemical and microbiological food safety is taken from the annual report on the health condition of the population of the Republic of Srpska (in 2017), prepared by the Public Health Institute of the Republic of Srpska.

Of the total number (5356) of foods inspected for physical and chemical safety, 51,1% (2736) of samples come from imports 10,8% (578) trade, and 38,1% (2040) from production. Results of laboratory inspection of food show that of the total number of samples inspected for chemical and physical safety 3,5 % of samples were non-compliant. Of the total number of foods (16900) inspected for microbiological safety, 9,5% (1601) of samples come from imports, 16,5% (2780) trade, and 74,1% (12521) from production. Results of laboratory inspection of food show that of the total number of samples inspected for microbiological safety 0,59% (100 samples) of samples were non-compliant.

Microbiological and chemical contaminations of foodstuffs represent a risk for occurrence of food born transmissive diseases. In developing countries, microbiological contamination of foodstuffs represents one of the leading causes of illness and death in up to five-year old children.

Keywords: food, contaminations, microbiological and chemical

RAMAN SPECTROSCOPY FOR CHARACTERIZATION OF PLANT BIOACTIVE COMPONENTS USED AS NUTRACEUTICALS

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Infrared (IR) and Raman spectroscopy are complementary vibrational spectroscopy techniques, which may provide important composition-related information of complex plant/food samples. Generally, vibrational measurements can be performed directly on plant tissues or on samples isolated from the plant material by distillation or extraction. Evaluation of biological tissues without extraction, which can lead to degradation of the bioactive components (ex., antioxidants), short time of analysis, a high degree of precision, use in order to perform fast quality checks of raw materials or continuous controlling of the production, are advances of application of Raman spectroscopy to analysis of nutraceutical compounds. This technique allows to obtain spectra (Raman fingerprints) which present characteristic key Raman bands of individual bioactive components. These bands provide information about the chemical composition of the investigated samples as primary (proteins and amino acids, lipids and fatty acids, carbohydrates) and secondary metabolites (flavonoids, polyphenols and other phenolic substances, terpenoids (mono-, sesqu-, and tetraterpenes), alkaloids, nitrile compounds, iridoids) present. The ability for rapid monitoring of various plant bioactive components makes Raman spectroscopy one of the techniques with future more wide application in the nutraceutical field. As the existed demand to solve complex issues of nutraceuticals is increased recently, by using multidisciplinary approach, Raman spectroscopy can play important role in it.

Keywords: Raman Spectroscopy, plant bioactive components, nutraceuticals

CHEMICAL PROFILING OF LEAVES OF DIFFERENT SPECIES OF THE GENUS *SALIX* L.

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Medicinal properties of willow bark (*Salix* L., Salicaceae) are well documented. It is traditionally used to relieve pain, treat fever and inflammatory conditions. In contrast, leaves of *Salix* species are usually considered as waste after bark collection and are mainly not studied. Nowadays, waste products from plant processing are gaining increasing interest as promising sources of bioactive compounds. Therefore, the aim of this study was to characterize the chemical composition of leaves of five different willow species, namely *S. alba*, *S. amplexicaulis*, *S. babylonica*, *S. eleagnos*, *S. triandra*.

Leaf extracts were obtained by microwave-assisted extraction using water as solvent. Chemical composition was determined by High Performance Liquid Chromatography.

In the analyzed samples presence of 11 phenolic compounds was confirmed with 6 phenolic acids (gallic, chlorogenic, *p*-hydroxybenzoic, syringic, *p*-coumaric and *trans*-cinnamic acid), 4 flavonoids (epicatechin, rutin, quercetin and naringenin), and the salicylic glycoside salicin. The amount of these compounds varied between species. Rutin (1.2-21.9 mg/g), salicin (2-17 mg/g) and chlorogenic acid (2.18-5.14 mg/g) were the most abundant components. Leaves of *S. amplexicaulis* were the richest in salicin, *p*-hydroxybenzoic, *p*-coumaric acid and quercetin. *S. alba* leaves were characterized by the highest amounts of gallic, *trans*-cinnamic acid and epicatehin, *S. eleagnos* by syringic acid, rutin and naringenin, while *S. triandra* by chlorogenic acid. The lowest concentrations of most of the analyzed components were found in leaves of *S. babylonica*.

The obtained results indicate that leaves of *Salix* species contain significant amounts of health beneficial phenolic compounds and have potential to be utilized as sources of these important phytochemicals.

Keywords: Salix, willow leaves, phenolics

AMINO ACIDS COMPOSITION IN SELECTED NUTS

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In recent years, we are witnessed that a nuts very popular food and is recommended as part of a diet because it contains numerous bioactive compounds. Nuts contain a high quantity of protein, i.e. amino acids that are known to be necessary for important processes such as tissue growth, energy production, immune function and nutrient absorption. In order to check the amount of individual amino acids found in the favorite samples of nuts of our citizens, we determined total protein and amino acid profile (17 amino acids) in nine samples of nuts (peanut, almond, hazelnut, walnut, Brazil nut, cashew, pecan, pistachio, and pine nuts). Total amino acids composition was determined by ion chromatograph with electrochemical detector, manufactured by Thermo, model ICS-5000, with silver reference electrode (Ag / AgCl) and gold (Au) working electrode and chromatographic column AminoPac PA10 and pre-column AminoPac PA10 guard. Analyzed nuts samples contained 11.7-29.2 g/100 g total proteins. Pecan contained the least protein, while peanut was the richest in total protein. Among the amino acids, glutamic acid, aspartic acid and arginine contents were generally high, whereas methionine, cysteine, histidine and tyrosine contents were low. Peanut was the most important source for essential amino acids (lysine 1.08 g/100 g; histidine 0.71 g/100 g; threonine 0.87 g/100 g; leucine 1.97 g/100 g; isoleucine 1.25 g/100 g; valine 1.07 g/100 g; phenylalanine 1,38 g/100 g; and methionine 0.4 g/100 g). Cashew was the biggest source of essential amino acids, 37% (essential/total amino acids), while the smallest source was almond, 27%. Almond was the biggest source of non-essential amino acids, 73%. The obtained results indicate that the nuts are incomplete in the content of amino acids. Knowledge of the amino acid composition of nuts is essential for combination with other food groups when formulating a balanced diet.

Keywords: Nuts, Diets, Proteins, Amino acids.

USE OF PROBIOTIC LACTIC ACID BACTERIA (LAB) IN THE CONTROL OF FOODBORNE PATHOGENS

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Food safety and quality and their associated risks pose a major concern worldwide regarding not only the relative economical losses but also the potential danger to consumer's health. Foodborne illness is a serious public health concern. According to World Health Organization (WHO) 600 million, almost 1 in 10 people in the world, fall ill after eating contaminated food and 420 000 die every year. Unsafe food poses global health threats, endangering everyone. Infants, young children, pregnant women, the elderly and those with an underlying illness are particularly vulnerable. Several commonly used approaches to control foodborne pathogens include antibiotics, natural antimicrobials, bacteriophages, bacteriocins, ionizing radiations, and heat. Lactic acid bacteria (LAB) are a diverse group of microorganisms consisting of Gram-positive, aerotolerant, acid-tolerant, usually nonsporulating and nonrespiring rod or cocci microorganisms, and play an important role in the process of fermentation of food by inhibiting spoilage/pathogenic bacteria and by producing excellent flavor, aroma, and texture of fermented foods. Probiotics are live microorganisms that are intended to have health benefits when consumed or applied to the body. They can be found in yogurt and other fermented foods, dietary supplements, and beauty products.

The aim of this work was to evaluate the use of probiotic lactic acid bacteria in the control of some food pathogens.

The study was conducted with six strains of lactic acid bacteria isolated from Macedonian cheese against three food pathogens, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella typhimurium*, using diffusion method.

Results showed that most of the isolates demonstrated antibacterial activity against tested bacteria, and should be consider as a potential strategy for the prevention and control of foodborne pathogens and foodborne infections. Our results show that probiotic lactic acid bacteria strains from foods can be excellent candidates in the control of foodborne pathogens.

Keywords: Probiotic, Foodborne pathogens, Food safety

STUDY OF TOTAL AFLATOXINS IN WHEAT, CORN, CEREALS REGARDING TO CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS

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The study was undertaken to assess food safety for the parameter of total aflatoxins in cereal samples, through measurements of chemical and microbiological characteristics to see their level in accordance with the norms established by European legislation. In total, 10 samples were analyzed taken from the market randomly or different cities of Albania. Cereal samples were subjected to chemical analysis such as moisture and Aw, as well as ELISA (immunological analysis) for the determination of total aflatoxins and were analyzed too by microbiological part for total microflora and fungi. Based on the chemical analyzes performed, the samples mainly turned out to be within the norms set by the European legislation. Aflatoxins are derivatives of difuranocoumarins. They are among the most poisonous mycotoxins. The main naturally produced aflatoxins based on their natural fluorescence (blue or green) are named B1, B2, G1 and G2. They are unstable under the influence of ultraviolet light, in the presence of oxygen, at pH extremes (<3, >10) and to oxidizing agents. Aflatoxins are produced only from a closely related group of Aspergillus: Aspergillus flavus, A. parasiticus and A. nomius strains. Other species such as A. bombycis, A. ochraceoroseus and A. pseudotamari are also species that produce aflatoxin, but they are found less frequently. Aflatoxins pose a problem in relation to many food products (nuts, spices), however, in terms of cereals they are especially problematic in the case of corn.

Experimental evidence showed that the level of contamination with microorganisms was low. It turned out that the samples were at the right level of humidity. This means that storage conditions were good. The highest moisture values resulted in sample S10 (Corn) and the lowest value in sample S2 (Corn flakes with gluten-free honey-Nestle). Samples S1, S2 and S3 did not have water activity above the norm which would affect their crocanticity. Corn grain, respectively samples S4 and S10 had lower values than the optimal one, (<0.87). Wheat grain S8 and S9 also had lower values, Au <0.80. Wheat flour S6 and S7 were within normal. The highest Au turned out to have samples S10 (corn) and the lowest samples S2 (Corn flakes with gluten-free honey-Nestle). The sample which had the highest level of total aflatoxins was sample S9 (87.07 ng/kg). Meanwhile, other samples have a level <50 ng/kg, so no value was detected within the range of concentrations used to construct the calibration curve. The maximum level of total microflora resulted in sample S8 (Wheat). In terms of mold contamination, the sample which resulted in a high level of contamination was sample S10. The sample S9 showed a low level while the other samples were not contaminated with mold.

This study was achieved by correlating with the measurements of humidity, water activity and fungal microbial load, which were below the value of legislation, indicate good storage conditions of samples which had not developed fungi and had not produced aflatoxins.

Keywords: aflatoxin, ELISA, moisture, Aw, microbial load etc.

TRANSCRIPTIONAL PROFILING OF HUMAN INTESTINAL EPITHELIAL CACO-2 CELLS INFECTED WITH *BACILLUS CYTOTOXICUS*

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Bacillus cytotoxicus is a highly cytotoxic species identified as a causal agent of severe necrotic enteritis. Specifically, this species produces the CytK-1 variant of the Cytotoxin K, which is highly toxic to human intestinal Caco-2 and Vero cells. To better characterize the pathogenesis of intestinal epithelial Caco-2 cells after infection with *B. cytotoxicus*, their transcriptome was analyzed by Affymetrix GeneAtlas microarray system (Affymetrix/Thermo Fisher Scientific Waltham, MA, USA). Differential gene expression analysis was conducted on 48226 genes and compared with non-infected cells pre-incubated with culture medium that mimics intestinal growth conditions. Most of the induced genes appeared to be involved in nucleic acid metabolism, transcription, protein synthesis, cell modifications, as well as regulation of surface protein production. After 60 minutes, *B. cytotoxicus* induced ca. 6500 genes, among which 50.3 and 49.7% were upregulated and downregulated, respectively. In this study, alterations were noted for mucin family genes, from which mucin 16 (*MUC16*), associated with cell surface integration, was the highest upregulated gene. Gene encoding fibrillin-1 (*FBN1*), a structural component of microfibrils of the extracellular matrix, significantly induces upon exposure to *B. cytotoxicus*. Further, the macrophage receptor MARCO-like, which has an important role in the removal of pathogens from the body was highly affected, as well as serine peptidase inhibitors Kazal types 13 and 6 (*SPINK13*, *SPINK6*) and carbamoyl-phosphate synthetase 1 (*CPS1*). Myosin ID (*MYO1D*), required for normal planar cell polarity of some cells, also upregulated. On the other hand, downregulation was noted for epidermal growth factors, such as epiregulin (*EREG*) and amphiregulin (*AREG*), epithelial cell adhesion molecule (*EpCAM*), and fibrinogen beta chain (*FGB*). Significant alterations in the expression levels of APO family members *APOA4*, *APOC3*, *APOD*, and *APOH* were observed. The present study demonstrates transcriptomic profiles of infected intestinal epithelial Caco-2 cells providing novel insights into molecular events underlying *B. cytotoxicus* pathogenesis.

Keywords: Caco-2, Bacillus cytotoxicus, transcriptional profiling, microarray

POSTBIOTIC EFFECTS OF *ENTEROCOCCUS FAECIUM* BGZLM1-5 ON REDUCTION OF *LISTERIA MONOCYTOGENES* ATCC19111 IN MILK

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The genus *Enterococcus* belongs to lactic acid bacteria and inhabits various habitats such as soil, water, plant, and gastrointestinal tracts of a wide variety of animals, and are well adapted to living and surviving in adverse conditions. Enterococci can be found in a variety of fermented foods contributing to the ripening and aroma development of certain cheeses, as well as probiotics to improve human or animal health. In addition, enterococci have the ability to produce various antimicrobial compounds such as lactic acid, hydrogen peroxide, and bacteriocins with broad-spectrum activity against spoilage and pathogenic microorganisms and might be useful as natural preservatives in food products. Many strains of *Enterococcus faecium* produce bacteriocins inhibiting the growth of *Listeria monocytogenes*, one of the most important foodborne pathogens in public health.

Bacteriocin-producing *E. faecium* BGZLM1-5 was isolated from raw cow milk sampled in a rural household at the Zlatar Mountain in the Republic of Serbia. Since live cells of BGZLM1-5 possess strong antilisterial effects, we examined the possibility of BGZLM1-5 postbiotic as a safe and controllable replacement for live bacteria to reduce *L. monocytogenes* ATCC19111. The results showed that the bacteriocin produced by BGZLM1-5 had a good temperature (in the range of 50°C to 100°C) and acid and alkali (stable at pH 3 to 11) resistance. After 40% ammonium sulfate precipitation and desalination, the size of the bacteriocin is approximately 3.5 kDa. In addition, we showed that 5% (v/v) of the cell-free supernatant and 0.5% (v/v) of the partially purified bacteriocin reduced the viable number of *L. monocytogenes* ATCC19111 after three days of milk storage from $7.6x10^5$ CFU/ml to $2.7x10^5$ CFU/ml and from $7.6x10^5$ CFU/ml to $3.7x10^5$ CFU/ml, respectively. According to these results, *E. faecium* BGZLM1-5 has the potential to reduce the number of *L. monocytogenes* ATCC19111 and could be used as a food preservative.

Keywords: Enterococcus faecium, Listeria monocytogenes, postbiotic, bacteriocin, milk

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PRESERVATIVES IN LIQUID HERBAL DIETARY SUPLEMENTS AND EXPOSURE ASSESSMENT

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Dietary supplements could contain preservatives, chemicals widely used to prolong shelf life of sensitive products, and thus present potential health risk. An investigation of actual usage levels of preservatives in dietary supplements was conducted to provide concentration data for exposure assessment, further combined with product usage instructions declared by the manufacturers.

Eighty eight herbal dietary supplements purchased in pharmacies in Novi Sad in 2018 (36) and 2021 (52) were liquids with either sorbates or benzoates on ingredients list. The method of analysis was HPLC-UV. Daily intake of sorbic and benzoic acid was expressed as percentage of their respective acceptable daily intakes (ADI) of 11 and 5 mg/kg body weight/day.

Conformity evaluation revealed three supplements containing benzoic acid in excess of the maximum permitted level of 2000mg/L (4244-6811mg/L). Benzoic acid ADI was exceeded by three supplements, one intended for adults (148%), one for adolescents (132%) and children (206%), one if used by preschool children (156%) and toddlers (300%). No supplements exceeded sorbic acid ADI by any of the population groups. Mean exposure of adults, adolescents, children and preschool children ranged from 23 to 31% of ADI for benzoic acid, and from 5 to 10% of ADI for sorbic acid, whereas exposure of toddlers was substantially higher (77 and 14%, respectively). High exposure level (95th percentile) reached 84% (benzoic) and 23% (sorbic acid) in population of preschool children. Mean exposure from 31 samples intended for toddlers, excluding nine samples demanding pediatrician's recommendation for dosing, corresponded to 77% of ADI for benzoic and 14% for sorbic acid. Only three supplements were intended for infants, one of which reached 70% of benzoic acid ADI.

However, more realistic approach considering usually short period of supplements' usage showed significantly lower exposure and no health concern.

Keywords: Herbal dietary supplements, Preservatives, Exposure assessment, HPLC

DETECTION OF CHOKEBERRY ADULTERATION BY HPTLC-BASED METABOLOMICS

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Chokeberry is a plant belonging to the family Rosaceae which has a long history of edible and medicinal use. The aims of this study are: i) to investigate of HPTLC profile of berries samples using HPTLC technique based metabolomics approach, ii) to determine the botanical origin of investigated chokeberry adulterations iii) to identify the most important botanical markers responsible for classification.

A simple, rapid, and high-throughput planar chromatography (HPTLC) method was applied in combination with multivariate techniques for differentiating chokeberry samples and identification of the main metabolites of chokeberry and four common adulterants. Images of HPTLC chromatograms obtained using two different detections i.e. visible light and derivatization with natural products reagent were used as a dataset for multivariate analysis.

Principal component analysis and Orthogonal Partial Least Squares Discriminant Analysis confirmed the discrimination of five botanically different samples and recognized their main markers such as responsible for differences between chokeberry and four common adulterants.

The obtained results showed that the HPTLC-based metabolomics approach can be a very reliable technique for the detection of chokeberry adulteration.

Keywords: Chokeberry, High-performance thin-layer chromatography, Adulteration, Metabolomics.

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COMPARISON OF CHEMICAL COMPOSITION AND ANTIMICROBIAL PROPERTIES OF *SALVIA OFFICINALIS* L. AND *MENTHA PIPERITA* L. ESSENTIAL OIL

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Salvia officinalis L. and *Mentha piperita* L., both belonging to the Lamiaceae family, are some of the world's oldest and most popular herbs. Essential oils, usually displaying prominent antimicrobial properties, are an attractive alternative to synthetic preservatives used in the food industry.

In this work, antimicrobial properties of essential oils obtained from aerial parts of *Salvia officinalis* L. and *Mentha piperita* L., were investigated against some common food-borne pathogens, seven G (+) bacteria, eight G (-) bacteria strains, and one yeast. The minimum inhibitory concentration (MIC), as well as minimum lethal concentration (MLC) of the plant essential oils (EO), were determined by broth microdilution assay on the concentrations in the range of 7.50 mg/mL – 0.47 mg/mL. Both *Salvia officinalis* and *Mentha piperita* essential oils exhibited stronger antibacterial activity against Gram (+) than Gram (-) bacteria, with MIC and MLC values ranging from < 0.47 mg/mL to ≥ 7.50 mg/mL. Inhibitory and lethal concentrations for both essential oils against Gram (-) bacteria were in the same range of concentrations, from 3.75 mg/mL to ≥ 7.50 mg/mL. *M. piperita* essential oil showed stronger activity against the yeast *Candida albicans* (MIC= 0.94 mg/mL and MLC = 1.87 mg/mL) in comparison to *Salvia officinalis* essential oil (MIC= 1.87 mg/mL and MLC = 3.75mg/mL).

Volatile components of both essential oils were characterized by GC-FID and GC-MS. *Salvia officinalis* essential oil was the most abundant in α-thujone (22.28 %), camphor (21.72 %) and 1,8-cineole (9.71 %), whereas L-menthone (30.51 %), L-menthol (26.29 %) and isomenthone (10.44 %) were predominant components of the *M. piperita* essential oil. These terpenes are probably responsible for the observed antimicrobial activity of the essential oils. Therefore, *Salvia officinalis* and *Mentha piperita* essential oils could be considered as valuable sources of natural antimicrobial agents, potentially applicable in the food industry.

Keywords: antimicrobial, medicinal plant, essential oil, volatile components

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TOTAL PHENOLICS, VITAMIN C CONTENT AND ANTIOXIDANT POTENTIAL OF *ROSA TOMENTOSA* HIPS

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Species of the genus *Rosa* are traditionally used medicinal plants in Serbia to treat a variety of ordinary diseases, for cosmetic preparations and as food products. Fresh or dried, rose hips are used for the production of tea, jam, juice and dietary supplements. Products prepared from air-dried rose hips present a rich source of ascorbic acid and phenolic compounds which are effective against infectious and urinary diseases, diarrhea, rheumatism and arthritis. This study was designed to provide data about the content of total phenolics (TPC), total flavonoids (TFC) and ascorbic acid values and to determine the antioxidant activity of extracts obtained from *R. tomentosa* hips (pericarp and nuts). Spectrophotometric methods were used for the quantification of phenolic compounds and the determination of the antioxidant capacity of extracts. Quantification of the vitamin C (ascorbic acid) was done using high-performance liquid chromatography (HPLC) with a photodiode array detector (DAD). The highest TPC and TFC were determined in pericarp extract (168.62 mg GAE/g and 24.54 mg QE/g of dry extract), while extracts of nuts showed the lower values (143.80 mg GAE/g and 19.11 mg QE/g of dry extract, respectively). The results of HPLC analysis showed notable vitamin C content of 418.95 µg/g dry weight in *R. tomentosa* hips pericarp. Antioxidant activity was evaluated by DPPH radical, ABTS radical cation and β-carotene assays. All results were expressed as IC_{50}. In the DPPH radical and ABTS radical cation tests extracts obtained from pericarp possessed higher antioxidant potential (IC_{50} 0.38 mg/ml and IC_{50} 0.71 mg/ml, respectively) than those from nuts (IC_{50} 0.41 mg/ml and IC_{50} 0.81 mg/ml, respectively). Additionally, in the β-carotene test, the highest value of antioxidant activity was found for nuts extract (IC_{50} 1.76 mg/ml). The study suggests that the hips of *R. tomentosa* are a rich source of natural antioxidant compounds and should be considered as a novel functional food product.

Keywords: Rosa, Ascorbic acid, DPPH, ABTS, β-carotene

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MINERAL COMPOSITION OF SELECTED EDIBLE NUT SEEDS

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Numerous epidemiological studies have confirmed that lifestyle and diet can be very effective in preventing and treating many non-communicable diseases. Replacement of unhealthy food intake between the main meals, i.e. snacks, with foods rich in nutrients such as nuts make a significant contribution to the prevention and treatment of some types of cancer, diabetes mellitus type 2 and cardiovascular diseases. Among the essential bioactive components contained in nuts are healthy minerals, such as calcium, magnesium, potassium, phosphorus, zinc, ferrum. The objective of this study was to identify the mineral composition of several varieties of nuts, which are part of everyday diet. The concentrations of 7 elements (Na, K, Ca, Mg, Fe, Zn, and P) were determined in nut samples (peanut, almond, hazelnut, walnut, Brazil nut, cashew, pecan, pistachio and pine nuts). The mineral contents were analyzed by inductively coupled plasma optical emission spectrometry using a Agilend 5110 dual view, ICP-OES, after microwave-assisted acid digestion. The contents of investigated elements in nut samples were determined as mg/kg in the range from 21.4 to 63.0 for Zn; 25.9 to 107.0 for Fe; 84.2 to 1995 for Ca; 981 to 3724 for Mg; 2295 to 4414 for K and 2814 to 7244 for P. The minimum contents of Na were below the detection limit of the method used. The obtained experimental data are another confirmation that nuts, and above all almond and Brazil nut, are important sources of bioactive components i.e. healthy minerals. Knowing the effect on human health, it is necessary the nuts to be recommended and included in the usual daily diet.

Keywords: Nuts, Healthy diets, Non-communicable diseases, Minerals.

HONEY FRAUDS ON CROATIAN MARKET

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Honey market in Croatia has been seriously influenced by COVID-19 epidemics. Its' impact was initially felt when general limitations of mobility were enforced having influenced the normal beekeepers' production doings. Furthermore, it also inflicted supply chains and raised transportation costs what significantly influenced the prise of honey. Climate changes as well as unfavourable human activities (in agriculture, industry, urbanization, etc.) additionally diminished beekeeping outputs and consequently led to the raise of the honey prices. Old human saying says "where there is demand there will be supply". Croatian honey market is not an exception. It reflects the situation on wider market of European Union, where great portion of honey on offer, derives from so called "external sources".

With the courtesy of Croatian Beekeepers' Association, a study of Croatian food market was conducted in order to find what are the numbers of imported and entered honey, their origin, worth and selling prices. Furthermore, these numbers were confronted to their Croatian counterparts in order to obtain first measurable indications of the honey market trends. Significant efforts were undertaken in the analysis of the samples taken from the most attractive selling locations along the Croatian Adriatic coast (60+) during tourist seasons.

The results showed that large portion of these samples were significantly altered or were not honeys at al. It also showed that they were deliberately intended mostly for specific population – foreign tourists, unknowing to quality and characteristic sensory properties of most popular Croatian honeys (sage, lavender, rosemary, tangerine, etc.). It also revealed that national regulatory and control authorities were not up to the task, nor in prevention, nor in sanction of these occurrences.

This paper is going into the deeper elaboration of the results of this study that has been designed as consumer protection project that also dealt with considerable safety issues.

Keywords: Honey, Frauds, Adulteration, Safety, Quality

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INFLUENCE OF RASPBERRY VARIETY ON THE AROMATIC PROFILE OF RASPBERRY GEIST

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Serbia is the world's leading producer of raspberries which are mainly exported as frozen fruits. Changes in the world market's demands resulted in new ways of use and processing of raspberries. As a consequence of these new trends, the production of raspberry spirit drinks has become more important. The quality of raspberry spirit drinks depends on the raw material (variety) and the processing way. Due to the specific and delicate aroma, raspberries are a very interesting raw material for the production of spirit drink obtained by maceration of unfermented berries (Geist). According to the EU Regulation, Raspberry Geist is obtained exclusively by maceration of unfermented fruits and distillation. Therefore, it does not contain volatiles formed during fermentation, but exclusively those originating from the fruits, and to a lesser extent those formed during distillation. By analysis of three monovarietal Raspberry Geists, produced from three raspberry varieties (Willamette, Meeker, Tulameen), 133 volatile compounds classified into 12 classes were identified and quantified. The highest concentrations had ingredients originating from fruits (C13-norisoprenoids, aldehydes, terpenes, ketones, lactones). Among them, α-ionone and β-ionone had the highest concentration in all varietal Geists. Varietal Geists of Willamette and Meeker contained more α-ionone than the Geist of Tulameen. The opposite trend was found in terms of the content of β-ionone. The Willamette Geist had the highest concentrations of nonanal, decanal, α-terpineol, 2-heptanone, and 2 lactones (δ-decalactone and γ-dodecalactone). Tulameen Geist had the highest shares of 2 sesquiterpens (β-caryophyllene and α-caryophyllene) in total aromatic substances. Various aromatic profiles of varietal Geists affected the existence of fine differences in sensory characteristics of the products. All varietal Geists had a characteristic raspberry flavor. However, Geist of Willamette had an extra creamy note, Geist of Meeker had piney, terpenic character, while Geist of Tulameen was very fruity and most resembled raspberries.

Keywords: Rubus idaeus, raspberry Geist, volatile compounds, GC/MS

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INFLUENCE OF IMMUNE ACTIVITY OF COR A 9 FROM RAW AND ROASTED HAZELNUTS AFTER GASTRIC DIGESTION

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Cor a 9 is one of the most common hazelnut allergen, a non-glycosylated protein, consisting of two subunits, an acidic (ranging between 35-40 kDa) and a basic subunit (ranging between 20-25 kDa). Very important fact is that the acid chain carries the immunoreactivity, according to literature. The survival of large fragments of Cor a 9 is necessary for its ability to sensitize individual. The aim of this study was to investigate Cor a 9, and to compare the digestive stability and allergenicity of large and small peptides released after pepsin digestion of whole raw and roasted hazelnut grains in standardized and physiologically relevant *in vitro* conditions, after heat treatment (roasting as the most abundant type of heat treatment). *In vitro* simulated phases of oral and gastric digestion were performed with ground raw and roasted hazelnut kernels according to the 1.0 INFOGEST protocol. After digestion proteins were extracted from the digestion mixture and analysed by 1D and 2D SDS-PAGE, while their IgE test was examined in the sera of allergic patients using ELISA and 2D immunoblot. The focus of the research was on the analysis of the 2DE map by Image 2D Master Platinum 7.0 software, comparing region of acid and basic Cor a 9 from raw and roasted hazelnut. Cor a 9 peptides are resistant to gastric digestion, and are able to bind IgE patients. Roasted hazelnuts are more prone to digestion in the stomach than the raw sample and cause a milder IgE response in patients. The gastric digestion phase of raw and roasted hazelnut grains resulted in partial extraction and digestion of Cor a 9 into digestion-resistant peptides with preserved IgE-binding epitopes. These results show significant resistance of Cor a 9 raw and roasted hazelnuts to digestion in the stomach, as they remained mostly intact after 2 hours of gastric (pepsin) phase and retained their allergenicity.

Keywords: in vitro gastric digestion, Cor a 9, food matrix, IgE binding

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DOES CASTRATION AFFECT THE QUALITY OF MUSCLE TISSUE IN MORAVKA PIG BREED?

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The aim of this research was to evaluate the castration effect on chemical and fatty-acid compositions of *longissimus dorsi* muscle in Moravka, an autochthonous pig breed of combined production abilities. The trial conducted included 16 pigs: 7 entire males (EM) and 9 surgical castrates (SC). The animals were bred in farm conditions in separate, group pens (110 m^2 open and 40 m^2 covered part). Pigs were fed *ad libitum* with complete feed mixtures: I (25-60 kg, 15% of crude protein, and 13.6 MJ ME/kg) and II (60-120 kg, 13% of crude protein and 13.5 MJ ME/kg). Muscle tissue chemical composition was determined by means of standard methods in an accredited laboratory. Fatty acids as methyl esters were determined using the capillary gas chromatography with a flame ionization detector. The effect evaluation was determined by means of a General Linear Model procedure in SAS 9.1.3 software, and besides castration treatment, the model included also a linear regression effect of body weight at slaughter (BW). An average pig BW was 121 kg at the age of 333 days. The increase of BW for 1 kg resulted in the increase of water content in muscle by 0.06% ($p=0.040$). The EM group had higher water content (+2.3%, $p=0.047$) and lower content of cholesterol (−15 mg/100 g, $p=0.031$). The same group had lower content of saturated (−3.6%, $p=0.003$) and mono-unsaturated (−5.6%, $p<0.001$), and a higher share of poly-unsaturated (+9.0%, $p<0.001$) fatty acids compared to SC. From a nutritional aspect, the ratio of omega-6 and omega-3 essential fatty acids was more favorable in SC compared to the EM group (3.9 *vs.* 18.5, $p<0.001$). Castration had an effect on the quality of muscle tissue but regardless of some benefits determined in the EM group a main restricting factor for more massive fattening of non-castrated animals is a boar taint in the meat.

Keywords: pigs, longissimus dorsi, fatty acids

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THYMUS SERPYLLUM L. EXTRACT LOADED LIPOSOMES PRODUCED BY PROLIPOSOME METHOD

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Polyphenols, as secondary metabolites from plants, are used as food complements or ingredients within the pharmaceutical or cosmetic formulations. However, their use is rather limited, due to their low bioavailability, integrity, permeability, and solubility. Namely, polyphenols' sensitivity to environmental factors during food processing, distribution, or storage, or in the gastrointestinal tract, also limit their activity and the potential health benefits. Thus, the encapsulation of polyphenol extracts represents an appropriate way to overcome the mentioned disadvantages. *Thymus serpyllum* L. ethanol extract obtained in heat-assisted extraction (80°C), rich in polyphenol compounds, was encapsulated into liposomes produced using phospholipids and proliposome method. Total polyphenol content in extract and encapsulation efficiency were determined using the Folin-Ciocalteu procedure. Particle size, polydispersity index, and zeta potential of empty and extract loaded liposomes were measured during 28 days using photon correlation spectroscopy. Total polyphenol content of the extract was amounted to 2.08±0.14 mg of gallic acid equivalents/mL, whereas encapsulation efficiency was 89.4±0.8%. During the 28-days stability study, the particle size of empty liposomes varied between 420.6±4.3 and 581.6±3.4 nm with polydispersity index from 0.109±0.067 to 0.295±0.009, while the size of extract loaded liposomes was between 278.7±1.5 and 456.4±9.3 nm with polydispersity index 0.179±0.094 to 0.284±0.005. Zeta potential of empty liposomes varied from -17.1±0.2 to -27.3±0.5 mV, whereas the zeta potential of extract loaded liposomes was between -13.6±0.3 and -25.4±1.4 mV. The aim of this study is to provide evidence for food manufacturers and food scientists to make broader use of *T. serpyllum* loaded liposomes that can add value and improve the quality of existing food, pharmaceutical and cosmetic products.

Keywords: encapsulation efficiency, liposomes, proliposome method, Thymus serpyllum.

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ZETA-POTENTIAL AND PARTICLE SIZE OF FUNCTIONAL ADDITIVES BASED ON GOAT MILK PROTEINS AND *Agaricus blazei* Murill EXTRACTS

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In recent years, the manufacturing of functional food has gained a lot of attention. The most recent studies indicate that bovine milk proteins are suitable carriers for a whole range of bioactive substances, mainly because of their micellar structure.

The aim of our study was to examine the possibility of goat milk proteins (RM - raw skimmed milk and CN - casein fraction) to deliver *A. blazei* extracts (VA - hot water extract and GA - glucan extract) in order to use all the potential health benefits of this mushroom. Furthermore, the effect of thermal treatment (90^0C, 10 min; TRM – thermally treated RM and TCN - thermally treated CN) on carrier properties was also evaluated. The mixture of different goat milk proteins and *A. blazei* extracts were prepared and zeta-potential and particle size distribution were determined by a laser light-scattering particle size analyzer, using distilled water as a dispersant.

The best results were obtained with the RM and TRM mixtures. It can be concluded that changes in particle size and electric charge of the micellar surface occurring in goat milk after heating have a positive impact on carrier properties. The particle size of TRM mixtures increased by 13.3 to 15.7% compared to the size of TRM particles, whereas the zeta-potential of TRM/VA increased for 6.6% compared to that of TRM. TCNs did not follow this trend. The measurement of CNs particle size distribution showed the presence of significantly different sizes (coefficient of variation >30%), whereas the zeta-potential measurements were stable. These results imply that the measurements of zeta-potential cannot be interpreted without the particle size data.

Keywords: goat milk proteins; Agaricus blazei Murill; thermal treatment; zeta-potential; particle size

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RECOVERY OF NUTRITIVE AND FUNCTIONAL COMPOUNDS FROM BLACK SOYBEAN AND SWEET MAIZE BY-PRODUCTS THROUGH THE SPREAD. COMPARISON WITH FRUIT JAMS AND SPREADS

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The valorization of by-products from widely cultivated plants has become an international priority that should respond to the sustainability, environmental and economic challenges. In this context, sweet maize cob (SMC) and black soybean seed coat (BSC) were used to prepare a sweet spread according to the original procedure. Two spreads, different in BSC content, were prepared (3.2% SMC/BSC and 2.2% SMC/BSC containing spreads). The results of developed spreads chemical composition, their rheological and sensory characteristics were compared with that of commercial plum and raspberry jams and spreads. Plum and raspberry jams with 56 and 69% of fruits according to producer declaration, respectively, as well as plum spread with 82% of fruit were bought in a supermarket. Spreads made from SMC and BSC had the highest content of proteins, total fibers, hemicellulose, and cellulose. In relation to fruit jams, they are distinguished by the presence and content of *p*-coumaric, ferulic and 3,4-dihydroxybenzoic acids, as well as of flavonoids such as catechin and quercetin. SMC spread with 3.2% of BSC had the highest content of total anthocyanins (666.75 mg CGE/kg). Cyanidin-3-glucoside (Cy-3-Glu) was the most abundant anthocyanins in SMC/BSC containing spreads while in a raspberry jam that was cyanidin-3-sophoroside (Cy-3-Sop). Investigated products had a content of total anthocyanins with the following ascending order: plum jam > plum spread > SMC spread with 2.2% of BSC > raspberry jam > SMC spread with 3.2% of BSC. According to their rheological properties, SMC/BSC containing spreads were similar to commercial plum spread. The obtained results show that sweet maize cob and black soybean seed coat represent the substrates for the recapture of functional compounds and the development of functional foods. In addition, as a source of sugars and bioactive compounds, SMC and BSC could be a replacement for expensive berries in jams and spreads.

Keywords: Sweet maize cob, Black soybean seed coat, Sweet spread and jam.

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CHANGES IN LEAF AND FRUIT NUTRIENT CONCENTRATION OF NORTHERN HIGHBUSH BLUEBERRY ASSOCIATED WITH DIFFERENT NUTRIENT MANAGEMENT IN SOILLESS GROWING SYSTEM

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The initial focus in growing blueberries as soilless culture is on balanced and precise fertilizer applications which can improve the nutrient status of the plants. Hence, we examined the effect of different nutrient management practices on the content of macro- and micro- elements in the leaf and fruit of 'Bluecrop' highbush blueberry associated with analyses of pH, electrical conductivity (EC), and nutrient content of the substrate. A field study was carried out during the 2017 season on 5-year-old nursery plants grown in 50 L polypropylene pots at a commercial orchard near Belgrade. Each pot filled with the mix of pine sawdust (60%), white peat (30%) and perlite (10%) and placed at an in-row spacing of 0.8 and a between-row spacing of 3.0 m (4,170 plants ha^{-1}). The nutrient treatments were as follows: (Org) organic fertilizers application (64 kg N ha^{-1}, 42 kg P ha^{-1}, 52 kg K ha^{-1}); (Min) mineral fertilizers application (85 kg N ha^{-1}, 45 kg P ha^{-1}, 64 kg K ha^{-1}); and (Org-Min) combined application of organic and mineral fertilizers (72 kg N ha^{-1}, 48 kg P ha^{-1}, 68 kg K ha^{-1}). The trial was set up in a completely randomized design with 3 replications and 10 bushes/pots per replication for each fertilizer treatment. The leaf and fruit samples were collected at the beginning of July 2017 for inorganic nutrient analysis. Fertilizer treatments had a significant effect on leaf nitrogen (N), phosphorus (P), and potassium (K) concentrations which tended to increase in Min and Org-Min treatments. N accounted for the largest proportion of nutrients in the fruit with the highest concentration recorded in Min treatment. No differences in other fruit macro-nutrients were detected among the fertilizer treatments. As opposed to this, fruit micro- nutrients showed the highest concentrations of boron (B), copper (Cu), iron (Fe), and zinc (Zn) in Org treatment.

Keywords: V. corymbosum, substrate, fertilizers, macro- and micro- nutrients

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THE EFFECT OF ULTRASOUND AND COOKING TREATMENTS ON *IN VITRO* DIGESTIBILITY AND ANTIOXIDANT PROPERTIES OF WHOLEGRAIN WHEAT FLOURS

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The majority of wheat-based products are made from refined flour which lacks some essential nutrients that are lost during the milling process due to the removal of bran and germ. The abundance of dietary fibers, as well as antioxidant properties of polyphenols present in the wholegrain wheat flour, are the key health-promoting contributors associated with the amelioration of cancers, cardiovascular diseases, as well metabolic disorders such as type-2 diabetes and obesity. The aim of this study was to investigate the effect of ultrasound and cooking treatments on *in vitro* digestibility with multienzyme method, the content of total phenolic compounds, and antioxidant capacity by examining wholegrain wheat flours of six genotypes differing in amylose content and dietary fibers composition. Wholegrain wheat samples were mixed with distilled water at hydromodule 1:3, and subjected to ultrasound treatment at frequency 30kHz, the temperature of 40˚C for 10 minutes. Hydromodule 1:6 was used for the cooking treatment on a preheated hot plate (t=200˚C) with magnetic stirring for 3 minutes after reaching the boiling point. The mixtures were dried in a ventilation oven at 40˚C overnight, and ground in a laboratory mill afterward. The highest amylose-amylopectin ratio, an indicator of low glycemic index, was determined in the high-amylose genotype Titan SBE I (0.57). Results showed that cooking treatment positively influenced the digestibility of the wholegrain wheat flours. Waxy wheat genotypes showed a higher digestibility increase after cooking treatment than the high amylose wheat flours, which can be explained by amylose retrogradation and resistant starch formation. Ultrasound treatment positively affected total phenolic compounds content in all samples except waxy wheat flour. Furthermore, the applied cooking treatment had an overall negative effect on the antioxidant capacity. The findings obtained in this study can serve as valuable guidelines in the formulation and production of new wholegrain wheat foods.

Keywords: wholegrain wheat flour, ultrasound, cooking treatment, in vitro digestibility, antioxidants.

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POTENTIAL UTILIZATION OF CITRUS PEEL AS A RICH SOURCE OF ANTIOXIDANTS

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Thanks to their pleasant flavour and aroma, as well as the presence of vitamins, minerals, dietary fiber and antioxidants, citrus fruits are widely consumed all over the world. While the fruit is mostly consumed in the form of food or juice, the peel is often considered a by-product in food industry and generates large amounts of ecological waste. The aim of this study was to compare the chemical composition of the pulp and peel extracts and peel essential oil of grapefruit, orange, mandarin and lemon in order to evaluate the potential utilization of citrus peels in different industries. Quantitative LC-MS/MS analysis detected 19 phenolic compounds of which ellagic, ferulic and sinapic acids and rutin were most abundant, along with abscisic acid – a plant hormone, which has a potential application as an anti-inflammatory and antidiabetic drug. Vitamin C was determined spectrophotometrically. Interestingly, higher amounts of almost all analyzed compounds were found in peel extracts. Orange peel was rich in phenolic acids, grapefruit peel in coumarins, while lemon peel stood out with *o*-coumaric and abscisic acids. Orange pulp contained the highest amount of vitamin C. Essential oil composition was determined with GC-MS and highlighted limonene as the main compound. Obtained results support a more effective utilization of citrus peels as they are a rich and inexpensive source of neutraceuticals, antioxidants and aromatic compounds, that could be of great benefit to food, cosmetic and pharmaceutical industries.

Keywords: citrus peel, citrus pulp, polyphenolic compounds, essential oil

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SUGAR CONTENT OF SWEET MAIZE KERNEL UNDER DROUGHT CONDITION

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Small water-soluble sugars such as glucose and sucrose, and enzymes involved in their metabolism are recognized as crucial compounds in plant responses under oxidative stresses. Recent studies showed that raffinose and maltose play a role in plant protection from oxidative damage caused by drought. The aim of this study was to examine the sugar content in the kernel of two sweet maize hybrids (ZP355*su* and ZP553*su*), under different vegetation seasons (2016 and 2017). High-performance anion-exchange chromatography with pulsed amperometric detection was used for fructose, glucose, sucrose, maltose, and raffinose quantification. Meteorological conditions (precipitation, sum and average temperatures) during the maize growing season in 2016 were favorable, while 2017 (drought present) was a less favorable season for maize cultivation. In stressful 2017 the content of all tested sugars, except sucrose, in hybrid ZP553*su* was higher in comparison to 2016. In hybrid ZP355*su* content of fructose, glucose, and sucrose in 2017 was reduced compared to 2016. The opposite trend was found for the raffinose and maltose content in 2017. Obtained results from this study pointed out that an increase in sugar content may indicate potentially hybrid tolerance to drought conditions. Although a small number of genotypes were tested, the obtained results indicate that sugar content, especially of glucose and raffinose, can be used for an assessment of sweet maize genotype tolerance.

Keywords: Zea mays L. Saccharata, *Raffinose, Sucrose, Abiotic stress, HPAEC-PAD.*

Acknowledgements: This research was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Grant no. 451-03-68/2020-14/200040 (Maize Research Institute 'Zemun Polje', Belgrade-Zemun) and 451-03-9/2021-14/200168 (University of Belgrade - Faculty of Chemistry) .

THE EFFECT OF PHYTOHORMONES APPLICATION ON MORPHOLOGICAL AND BIOLOGICAL PROPERTIES OF *THYMUS PANNONICUS* ALL.

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This study has explored the effect of three phytohormones application, based on α-naphthyl acetic acid, named INCIT 1, INCIT 8 and INCIT K, on the root system growing potential and rooting stimulation of *Thymus pannonicus* All. cuttings. This plant is highly reputable not only in a folk medicine, but also recognized in the business sector, due to a multiple uses and beneficial effects on human health.

The research was located in Veliko Središte, Vršac (Serbia) and conducted in May, 2021. The cuttings were dipped in a powdered formulation of phytohormones and placed in plastic containers pre-filled with commercial substrate. The phytohormone-free variant was taken as a control. After 2 months, the cuttings were removed and the morphological and biological properties (polyphenols content and antioxidant activity by DPPH radical and FRAP method) were measured.

Based on the morphological results, all of treated samples showed satisfactory behaviour, whereby the most suitable phytohormone to stimulate the growth of cuttings was INCIT 8, in which the average weight of the aboveground vegetative part of the plant and root mass were higher for more than 60% in relation to the control; the number of roots were about 25% higher, whereby the mass of the whole plant was almost 3 times increased. Considering the biological activities in a 5% aqueous infusions of the samples, it was found that the polyphenols content and the antioxidant activity were lowered in treated samples, with relation to the control, excluding the sample INCIT 8 where the FRAP value was slightly increased, while the inhibition of DPPH radicals was higher by around 15%.

The applied phytohormone treatment may ensure economically viable yields of standard or even improved plant quality. In the following period, it is expected that all of treated adult plants will have a higher content of biologically active substances.

Keywords: Thymus pannonicus, α-naphthyl-acetic acid, Seedling morphology, Polyphenols, Antioxidant activity

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PROTEINS OF TOFU WHEY AS A WASTE IN TOFU PRODUCTION

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In food processing, it is a common industrial practice to discard by-products, leading to economic loss and environmental problems. In recent years, great attention in the world has been focused on finding opportunities for alternative use of these by-products and the application of the principles of circular/sustainable economy. Tofu whey is a pale-yellowish liquid that remains as a by-product after tofu squeezing; it is highly perishable due to its high water content and high content of nutritious substances for bacterial growth and represents an environmental problem. The aim of this study was to assess the protein content of fresh tofu whey, obtained during tofu production. Tofu was made on the pilot-plant scale from three soybean genotypes, by the method that includes hydrothermal cooking (short time/high temperature/under pressure) and the application of the chymosin-pepsin as a coagulating agent. Total protein content in samples was calculated after determining total nitrogen content (N, N×6.25) by the micro-Kjeldahl method. All investigated tofu whey samples were characterized by high contents of total protein (23.67-28.00%) which indicated significant nutritional value of this by-product. The high protein content in tofu whey samples suggests that they can be potentially useful for application as a cheap, protein supplement in food preparation and can enable circular/sustainable production through recycling of waste.

Keywords: by-product, tofu whey, protein supplement, recycling of waste, sustainable production

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SUSTAINABLE APPROACH FOR FOOD SHELF LIFE PROLONGATION TROUGH BY-PRODUCTS RECYCLING

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In recent years one of the main focuses of the food sector is the exploitation of the potential of fruit and vegetable by-products recycling, as a sustainable approach to reduce food waste. These by-products contain valuable compounds, especially phenolic substances, and therefore can be used to fortify food or to prolong the shelf life. In this context, we propose two case studies where dried olive paste, as by-products of oil industrial process, and pomegranate peel powder as by-products of fruit, were adopted as breading of ready-to-cook cod sticks. In both cases, shelf-life tests were carried out on all breaded cod sticks, during a proper refrigerated storage period at 4 °C. To verify the effectiveness of selected by-products on fish quality, microbiological and sensory properties were monitored, in addition to pH measurements. Chemical quality of both control and active samples was also assessed in terms of total phenols, flavonoids and antioxidant activity.

Results confirmed that all active samples showed higher content of phenols and flavonoids and greater antioxidant activity than the control fish, thus suggesting that both the addition of olive paste and pomegranate peel powder were responsible for a significant increase in cod stick nutritional quality. Furthermore, both types of active breading led to delay microbial growth, without affecting the sensory properties; rather, the presence of by-products as fish breading helped to slow down the sensory decline during the refrigerated storage. A total of 3 days of shelf life prolongation was recorded with olive paste, whereas more than 7 days was the prolongation recorded with the fruit peel powder. Therefore, data suggested that these by-products are valid ingredients to extend fish shelf life, as well as important nutritional sources.

Keywords: sustainable approach, fruit and vegetable by-products, fish shelf life

TREATMENT WITH SELECTED PSEUDOMONAS STRAINS PROVOKES GENOTYPE-DEPENDANT RESPONSE OF SIX SWEET PEPPER CULTIVARS TO DROUGHT

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The effect of 11 *Pseudomonas* spp. strains isolated from rhizosphere (eight strains) and phyllosphere (three) on the response of six (261, 268, 274, Una, Matica, and Kurtovska Kapija) pepper genotypes to water deficiency was examined. PCR-based analysis of *phcA* (phenazine), *prnD* (pyrrolnitrin) and *pltC* (pyoluteorin) genes revealed their presence in all selected strains, while more than one gene was detected in *P. chlororaphis* Bo, *P. synxantha* P4_16/1, *P. orientalis* R3_16/1, *P. fluorescens* ČL5, *P. putida* P2, and *P. vranovensis* P3 strains. Pepper seeds with (treated) or without (controls) bacterial strains were planted in pots. After four weeks of growth, drought was imposed by completely withholding water for one week. The relative water content (RWC), H_2O_2 production, lipid peroxidation, and proline production were determined. The Una genotype showed increased stress intensity in treatment with all selected strains, which indicates the exceptional sensitivity of this genotype to drought. It was observed that strains P4_16/1, R3_16/1, and ČL5, isolates from the phyllosphere provoke a decrease in the intensity of lipid peroxidation, RWC and proline production, respectively in most genotypes, compared to other strains analyzed here. This could be in correlation with the presence of *phcA*, *prnD* and *pltC* genes and possibly the synthesis of secondary metabolites encoded with these genes. Genotype 274 showed an increase in the concentration of proline after treatment with three phyllosphere isolates, which indicates its natural resistance to drought compared to other strains tested here. Strain specific response of 274 pepper genotypes was observed after treatment with strain Bo rhizobacteria, which decrease RWC, H_2O_2 production, and lipid peroxidation under drought conditions. According to obtained results, we could conclude that there is a relationship between plant response to an abiotic factor (drought) and biotic factor (bacteria).

Keywords: Pepper genotypes, Pseudomonas, Drought, Antioxidant enzyme, Abiotic factors

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FOOD PRODUCTION, PROCESSING, SUSTAINABILITY, ADDED-VALUE FOOD

IN SITU PRODUCTION OF XYLOOLIGOSACCHARIDES BY *ASPERGILLUS TUBIGENSIS* FROM CORN COB

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Xylooligosaccharides (XOS) are prebiotic, functional food ingredients, with biological benefits such as immunomodulatory and anti-inflammatory properties, anticancer and antioxidant activity. Usually, XOS are produced from xylan by the combination of pre-treatment, enzymatic, chemical and/or auto-hydrolytic methods. Fungal xylanases are the most suitable for XOS production. Xylan rich agro-industrial wastes (corn cob is one of them) are used as a substrate for fungal xylanase production and as start material for xylan extraction. *In situ* XOS production by fungal growth on xylan rich medium represents an attractive and advantageous approach but insufficient described till now. This method has many advantages over the others because bypasses the extraction and purification of xylan and enzymes, it is environmentally friendly, low cost and time-consuming. This study demonstrated *A. tubingensis* FAT35 has a great capacity for the synthesis of XOS using corn cob as a substrate in solid state fermentation (SSF). Obtained XOS, during the fungal growth, were characterized by TLC and HPLC. Significant antioxidant potential was shown by antioxidant tests (ORAC, DPPH and FRAP). The obtained XOS are suitable to be a functional food additive and are obtained in the simplest way that is both environmentally and economically suitable.

Keywords: prebiotic, XOS, functional food, fungi, Aspergillus

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CHEMICAL PROFILE OF DIFFERENT TYPES OF MEAD - NMR METABOLOMICS APPLIED TO THE OLDEST ALCOHOLIC BEVERAGE

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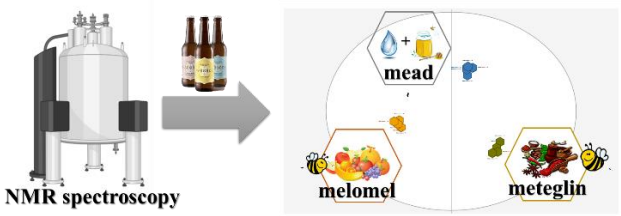
Mead (honey wine) is the oldest alcoholic beverage known for its antioxidant and therapeutic properties. Traditional mead is made from honey and water via fermentation with yeast. Different types of honey wine can be obtained using fruits, herbs and spices during or after the fermentation. Difficulties of the production process caused by differences in the quality and type of honey trigger the replacement of mead by other alcoholic beverages, like wine and beer. In the last decade the consumption of mead in Europe and America is steadily increasing, but only scarce information on the chemical composition of honey wines is available.



In the present study twenty meads from Bulgaria, Poland and Slovakia produced from linden, acacia, honeydew or polyfloral honey were analyzed using ^{1}H NMR spectroscopy. A number of compounds – amino and organic acids, alcohols, sugars and phenolic substances were identified and quantified. The chemical profiles of linden, acacia, honeydew and polyfloral honey were compared with the chemical profiles of the corresponding four types of mead using Nightingale's Diagrams. The most frequent chemometric methods (PLS-DA, OPLS-DA) were applied for determination of the components important for differentiation of meads according to geographical origin and variety of added ingredients - fruits (melomel) or spices (meteglin).

Keywords: mead, honey, NMR metabolomics

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PECTIN AS A CARRIER OF CHOKEBERRY POLYPHENOLS: INGREDIENTS WITH POTENTIAL HEALTH BENEFITS

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Chokeberry, one of the richest phenolics source, especially anthocyanins, represent a natural nutraceutical, due to its high antioxidant potential. Chokeberry juice processing generates a waste by-product, rich in polyphenolics, which can be extracted. Problem with polyphenols instability could be solved by spray drying, a microencapsulation technique for controlled production of small particles simultaneously increasing polyphenols bioavailability and stability. For microencapsulation of chokeberry extract (CE) and chokeberry waste extract (CWE) a pectin (P) (5% w/v) was used as a carrier. Microencapsulated systems, PCE and PCWE were examined (FTIR, SEM, particle size, zeta potential (ZP) and moisture content). *In vitro* simulated digestion method was used in order to determine changes in polyphenols content in gastrointestinal environment. The release profiles from microparticles were investigated by determining the total phenolic (TP) and total anthocyanins (TA) content, as well as individual phenolics using HPLC method.

Result showed that stability of polyphenols, especially anthocyanins could be improved using the spray drying. Obtained microparticles sizes were 7.36 and 11.65 μm, moisture content 4.84 and 5.32 %, for PCE and PCWE, respectively. SEM analysis showed that pectin provided less aggregated particles with expressed spherical shapes. FTIR showed several relevant picks, which indicate that extracts were successfully incorporated into biopolymer matrix. ZP was –38.52 and -39.94 mV, for PCE and PCWE, indicating favourable physical properties of particles. The amounts of TP (23.33 mg GAE/g for PCE, 35.90 mg GAE/g for PCWE) and TA (8.91 mg CyG/g for PCE, 12.85 mg CyG/g for PCWE) released from microencapsulated powders before, as well after digestion process (14.15 mg GAE/g for PCE, 19.82 mg GAE/g for PCWE) of TP and (6.22 mg CyG/g for PCE, 9.40 mg CyG/g for PCWE) of TA were achieved. Pectin showed significant effect on the polyphenol's degradation during digestion process, having the protective effect.

Keywords: Pectin, Chokeberry, Phenolics, Microencapsulation, Characterization

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THE EVALUATION OF THE ANTIOXIDANT POTENTIAL DURING THE OXIDATIVE POLYMERIZATION OF POLYPHENOL COMPOUNDS INDUCED BY THE LACCASE ENZYME

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The polymerization and crosslinking of various phenolic compounds induced by the laccase enzyme have been in recent years increasingly used in food industry, due to the emergence of products with improved properties, especially with increased antioxidant activity. In this regard, this study has explored the laccase-induced structural changes of two types of naturally occurring polyphenols, named gallic acid and epigallocatechin gallate, and their antioxidant activity. In the first part, the effect of the laccase of different origin - laccase of white rot fungi (*Ganoderma spp.*), previously grown on waste cereals and commercial laccase from Novozym® 51003, were investigated. Based on the results obtained after incubation (24 h, 50ºC), the sample containing a mixture of polyphenols, rather than individually, gave visible changes in the reading of the UV-Vis spectrum and increased antioxidant activity with small differences between commercial laccase and laccase of white rot fungi, which indicates that laccase obtained by growing fungi on waste cereals may be competitive for such a purpose. Moreover, the use of such an obtained laccase is more environmentally friendly and economically viable than use of commercial one. Further process optimization of polyphenols polymerization was performed within the method of multifactor statistical analysis (*Design Expert*), by using of commercial laccase, where by the effect of enzyme concentrations (0.1, 0.3 and 0.5 U/ml) and incubation time (4, 14 and 24 h) were monitored. The maximal antioxidant activity, measured by both DPPH (inhibition of 58.58% of radicals) and FRAP (176.57 mmol Fe^{2+}/ml) was achieved by a sample containing a mixture of polyphenols and 0.3 U/ml laccase enzymes, after 14 h, at 50˚C. The results of this study revealed that careful optimization of process variables during polyphenols polymerization is extremely important for obtaining the product with desirable value added properties, which may be implemented in food and pharmaceutical industry.

Keywords: laccase, oxidative polymerization, polyphenols, white rot fungi, agroindustrial waste

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MICROENCAPSULATION OF WILLOWHERB LEAVES EXTRACT WITH MALTODEXTRIN AND WHEY PROTEIN USING SPRAY DRYING

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Willowherb (*Epilobium angustifolium* L.) leaves are valuable source of polyphenolic compounds (phenolic acids, flavonoids and ellagitannins) that are mainly used in the treatment of benign prostatic hyperplasia. Spray drying as a fast, inexpensive, affordable, and flexible technique is the most commonly used method for encapsulating unstable compounds such as polyphenols on an industrial scale. Choosing the optimal carriers is one of the key steps in the development of the appropriate encapsulation process. In the presented research, the influences of maltodextrin and whey protein as carriers on the efficiency of spray drying and physicochemical properties of the obtained extract powders were investigated. The use of carriers significantly improved the drying yield (63.76% and 63.18% for extracts with maltodextrin and whey protein, compared to 47.75% for extract without carrier). Whey protein has shown significantly better encapsulation efficiency of polyphenolic compounds (92.02%) compared to maltodextrin (75.80%). Similar to phenols, higher encapsulation efficiency of total flavonoids was achieved in the case of whey protein (94.34%) compared to maltodextrin (93.00%) but this difference was not statistically significant. The moisture content (below 5%) and hygroscopicity (below 20%) of all dried extracts (without carrier, with maltodextrin and whey protein) were at an acceptable level to ensure microbiological stability. The time required to rehydrate the extracts in the aqueous medium was 30 seconds for both extracts with carriers, and 20 seconds for the extract without carriers. Relatively short rehydration time of dry extracts provides the possibility to use them in the form of instant teas. Therefore, willowherb leaves extract can be successfully encapsulated by spray drying using maltodextrin and whey protein as carriers.

Keywords: Epilobium angustifolium; Encapsulation; Carriers; Wall materials; Spray drying

Acknowledgements: Ministry of Education, Science and Technological Development of Republic of Serbia, contract number 451-03-9/2021-14/200003

COMBINED EFFECT OF ASCORBIC ACID AND SUGAR ON TEXTURE PROPERTIES OF MAIZE COMPOSITE BREAD

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The present study was carried out to demonstrate the combined effects of ascorbic acid and sugar on the texture properties of maize composite breads. The composite flour was prepared using 70% of wheat flour and 30% of flour obtained from grain of differently coloured maize - light blue, blue, red and yellow maize flour. A total of 12 breads were prepared, four of which were control composite breads, four breads with ascorbic acid, and four were breads with ascorbic acid and sugar. The results showed that the addition of ascorbic acid and sugar negatively affected the volume as well as the specific volume of composite breads. The highest loaf volume reduction of 8.7% was detected in red maize composite bread with the ascorbic acid+sugar addition. The texture analysis showed that the addition of ascorbic acid had no impact on springiness, cohesiveness and resilience of bread crumb, while it increased crumb hardness. Results showed that hardness was significantly higher when ascorbic acid and sugar were added to the light blue composite bread. The ascorbic acid addition increased crumb hardness by 11.09% and 4.5% in light blue and yellow composite breads, respectively. However, composite breads made with ascorbic acid and ascorbic acid+sugar showed a more compact structure, with a larger number of cells and smaller mean cell areas. Bread samples with ascorbic acid+sugar had the lowest springiness, which is indicative of brittleness and reflects the tendency of the bread to crumble when slicing.

Keywords: coloured maize, bread making, texture, ascorbic acid, sugar

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EVALUATION OF TECHNOLOGICAL CHARACTERISTICS OF WINE GRAPE VARIETALS IN THE VINEYARDS OF THE HOLY MONASTERY KOPORIN

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A field experiment was carried out during the year 2020 on grapevine varietals Riesling Rhine, Riesling Italian and Tamjanika, in Racansko winegrowing region (Smederevska palanka meteorological station), to study agrobiologic and technological characteristics of the varieties and the effect of the meteorological conditions on phenology, vigor, yield and technological characteristics. The experiment was carried out on 30 vines of each examined variety. Data revealed that meteorological parameters in the examined area had a big influence on phenology and yields of the grapevine. Average monthly temperatures in the last decade indicate an increase of 0.6-1.4℃. Average precipitation sum indicates that there is an increase and change in the distribution of precipitation in the last decade. There has been an increase of precipitation, by 55.2 mm, particularly in May. In year 2020, the precipitation sum for the site was 724.4 mm, with significant amounts in the following months, May 94.9, June 104.7, and July 142.4 mm, respectively. The amount and distribution of rainfall can have a major influence on yield, affecting the flowering, berry set and berry growth during the phenology, including the appearance of difficulties regarding pest control. The results have shown that there is a different response of varieties when it comes to pruning. The average number of shoots developed on the spur (2 buds) was 1.47 Riesling Rhine, 1.53 Riesling Italian, and 1.70 Tamjanika, and a number of developed shoots on the cane (8 buds) was 6.60, 6.43 and 6.37, respectively, which is 15% less than in the previous years. An average number of bunches per vine was Riesling Rhine 18.7, Riesling Italian 19.7, Tamjanika 11.0, and the average yield was 2.258, 2.046 and 1.930 kg per vine, respectively, 10% less than in the previous years. The conclusion would be that climate changes unfavorably affect agrobiologic and technological characteristics of wine grape varietals.

Keywords: Riesling Rhine, Riesling Italian, Tamjanika.

Acknowledgements: Monastery Koporin

IMPACT OF JUICING TECHNIQUES AND STORAGE TIME ON TOTAL PHENOLIC CONTENT OF SELECTED JUICES

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Escalating health consciousness among the consumers as well as rising demand for "on-the-go" beverages lead to a growing market offers of fruit and vegetable juices. Additionally, increasing consumers' interest in fresh juices reflects changes in preferences for beverages without disturbing preparation. Cold-pressed juices have gain attention and are claimed to have higher nutritional value compared to regular (normal) centrifuged ones.

Herein, we selected fruit (pomegranate) and vegetable (carrot and beetroot) species for juices preparation and aimed to evaluate total phenolic content (TPC) of freshly prepared unpasteurized juices obtained using three different household juicers: a cold-pressed juicer, a normal centrifugal juicer, and a citrus press. Moreover, the impact of home refrigeration-storage conditions on the TPC of analysed juices was investigated. Fresh fruit and vegetables, commercially available, both organic and conventional, were purchased from a local market in Belgrade, Serbia. TPC of analysed juices was investigated by Folin-Ciocalteu assay.

The initial TPC ranged from 115.9 mg/L GAE (conventionally grown beetroot, regular centrifuged juice) to 8272.7 mg/L GAE (cold-press pomegranate juice). Pomegranate juices had significantly higher TPC in comparison to selected vegetable juices ($p<0.01$). No significant differences were observed among the different types of juicing techniques regarding vegetable juices. Under simulated home-refrigerated storage conditions, all juices exhibited fluctuations in TPC values with a noticeable increase after 24 hours. On day 5 of refrigerated storage, TPC values remained almost unchanged in all juices, except pomegranate juice which exhibited a decreasing trend.

Comparison of juices processed using different extraction methods and coming from different (organic/conventional) grown produces, did not allow making conclusions about superiority of a particular product. The obtained results showed that introducing any of the juices to the consumers' diet could have an impact on the daily intake of health-promoting compounds.

Keywords: phenolic content, cold-press, juicing techniques, storage time

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DEVELOPMENT OF INNOVATIVE LIPOSOME-RELEASE SYSTEMS FOR ENCAPSULATION OF BIOLOGICALLY ACTIVE SOYBEAN PEPTIDES

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Bioactive peptides constituents of protein hydrolysates show great instability, poor absorption and bitterness when incorporated into food formulations. In addition, in the gastrointestinal system, there has been noted a loss of biological activity before desorption into the bloodstream and a lack of bioactivity at the target site in the body. The latest studies have focused on the field of encapsulation of proteins and peptides and for this purpose liposomes composed of natural phospholipids, molecules of cell membrane factors, are imposed as the most suitable choice. Therefore, the aim of this research is to develop new liposome-release systems suitable for encapsulation of soy protein hydrolysates. Multilamellar liposomes were prepared by forming a uniform thin film of phospholipids, cholesterol and soy protein hydrolysates, and by a sequential hydration process. For the purpose of obtaining unilamellar vesicles, the suspension of multilamellar vesicles was treated with high-intensity ultrasound waves. Unilamellar liposomes were characterized from the standpoint of size distribution, surface charge, degree of encapsulation, stability and retained antioxidant activity. A satisfactory percentage of soy protein hydrolysates encapsulation greater than 40% was achieved for all prepared suspensions, with a significant increase in encapsulation efficiency with increasing peptide mass. All liposome formulations were characterized by a negative zeta-potential value, with an increase in the negative charge above 30 mV in the case of suspensions with 60 and 100 mg of soybean peptides indicating greater stability compared to the suspension with 20 mg. The smallest particle size (191 nm) and more uniform distribution was observed in the suspension with the highest encapsulation efficiency (56%) and stability (-33.1 mV). Retention of antioxidant activity after the encapsulation procedure in liposomes was noted. In the simulated gastrointestinal system, liposome formulations were found to provide sustained release of antioxidant peptides derived from hydrolysates, indicating their potential application in food formulations.

Keywords: Soy protein hydrolysate, Hydrolysate encapsulation, Liposomal entrapment, Liposome-release system, Gastrointestinal in vitro model

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ACTIVE AND INTELLIGENT PACKAGING OF FOOD PRODUCTS

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Conventional packaging must be inert and represent only a passive barrier in the protection of food products from external environmental conditions. Active packaging is an innovative concept designed to incorporate appropriate active substances into a conventional packaging unit in various ways. The role of active substances is to react with certain components of the food or surrounding atmosphere (headspace), with the aim of extending the shelf life, while the initial quality of food remains unchanged. Active substances can be applied in the form of absorbers, emitters or other techniques. Oxygen scavengers, ethylene, liquid and moisture absorbers, flavor and odor absorbers or emitters, antimicrobials, etc. are the most commonly used systems of active packaging.

Intelligent packaging is created to monitor the condition of packaged food or the environment in order to provide information about its quality during transportation and storage. Intelligent packaging implies the use of different indicators, sensors and identification using RFID (Radio Frequency Identification Device - RFID) tags. The first task in designing an intelligent packaging system is to find a reliable interaction between the components of indicator and sensor with the packaged food product or headspace of packaging. They usually react with volatile compounds such as amines, ammonia, and ethanol and metabolites such as H_2S, CO_2, O_2, and ethylene that occur as a result of food decomposition and spoilage. These changes are most often reflected in the color change of intelligent systems, thus indicating, in real time, the quality and safety of packaged products. Intelligent packaging commonly includes time-temperature indicators, gas indicators, and freshness and ripening sensors. In addition, innovation and improvement of nanotechnology and nanomaterials will enable the development of better and new active and intelligent packaging.

Keywords: Active packaging, Intelligent packaging, Indicators, Sensors, Nanomaterials

Acknowledgements: This study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, III 46010 and III 46001.

EFFECTS OF MICROWAVE PASTEURIZATION ON THE THERMAL RESISTANCE OF *ZYGOSACCHAROMYCES ROUXII* IN ORGANIC INTERMEDIATE MOISTURE DATE

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In this study, it was aimed to determine the thermal resistance of the target microorganism (*Zygosacchoramyces rouxii*) in organic intermediate moisture dates during the pasteurization process. Different temperatures 60, 70 and 80°C at different time intervals were studied for determining the thermal resistance of microorganism in microwave and conventional pasteurization process. For both pasteurization methods, the come up times of the samples to the pasteurization temperature were determined. D and Z values of the date samples were calculated for both pasteurization methods. The D values of Z. *rouxii* were found as 7.19±0.18, 2.96±0.09, 2.02±0.03 minute for conventional pasteurization and 3.98±0.02, 2.01±0.01, 0.97 ±0.01 minute for microwave pasteurization at 60^{o}C 70^{o}C, 80^{o}C respectively. Theoretical and experimental F values were also calculated. Theoretical F^{37}_{85} values were determined as 7.35 minute for traditional pasteurization and 3.4 minute for microwave pasteurization. 5 logarithmic reduction in microbial load was achieved at 85 degrees with both heat treatment methods but lethal effect is provided faster by microwave.

F^{37}_{85} values calculated theoretically were confirmed experimentally for both processes. Microwave process decreased the heating time significantly.

Additionally, the effect of pasteurization methods on the quality properties of organic intermediate moisture date samples was investigated. Total soluble solid content, acidity, colour, water activity values were preserved by microwave treatment. In particular, the amount of HMF decreased significantly with microwave pasteurization. These findings demonstrated that microwave pasteurization can be alternatively instead of the conventional method, with the advantages such as less heating time and protecting the quality properties.

Keywords : Microwave, pasteurization, Z.rouxii, thermal resistance, date

MICROWAVE-ASSISTED EXTRACTION OF ESSENTIAL OIL FROM GINGER (*ZINGIBER OFFICINALE* ROSC.)

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Ginger rhizomes (*Zingiber officinale* Rosc., Zingiberaceae) are widely used as a popular spice, for food and beverages flavoring and as a medicinal raw material. The unique fragrance of ginger comes from essential oil. Traditional hydrodistillation of essential oils should be replaced by innovative environmentally friendly techniques due to its shortcomings (high energy and time consumption, increased risk of hydrolysis and thermal degradation for essential oil ingredients). The aim of this study was to compare conventional hydrodistillation and microwave-assisted hydrodistillation at different power levels in terms of qualitative and quantitative composition of ginger essential oil, energy and time requirements, and environmental impact. Microwave-assisted hydrodistillation achieved a higher extraction yield (1.70, 1.70, and 1.85% for power levels of 180, 360, and 600 W, respectively) compared to hydrodistillation (1.50%). The essential oil obtained by hydrodistillation contained 43 different compounds, while the essential oils obtained by microwave-assisted hydrodistillation at 180, 360, and 600 W contained 57, 58, and 51 compounds, respectively. The dominant compound in all samples was α-zingiberene followed by camphene, β-phellandrene, 1,8-cineole, neral, geranial, ar-curcumene, germacrene D, β-bisabolene, and β-sesquiphellandrene. The content of α-zingiberene in essential oils obtained by microwave-assisted hydrodistillation (34.12, 34.43, and 42% at 180, 360, and 600 W, respectively) was significant higher compared to essential oil obtained by hydrodistillation (29.89%). Distillation time and energy consumption were reduced by microwave-assisted hydrodistillation (37, 32, 27 min and 0.11, 0.19, 0.27 kVh for power levels of 180, 360 and 600 W, respectively, compared to 144 min and 1.44 kVh for conventional hydrodistillation). Therefore, microwave-assisted hydrodistillation is an applicable technique for the isolation of ginger essential oil that improves yield, reduces time and energy requirements, while maintaining oil quality.

Keywords: Ginger, Essential oil, Volatile compounds, Green Technology, Microwave-assisted hydrodistillation

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ENZYMATIC SYNTHESIS OF GALACTO-OLIGOSACCHARIDES FROM WHEY LACTOSE

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People's awareness of the beneficial effects of functional foods and the importance of prebiotics and probiotics for their health is constantly growing. Nowadays, more and more attention and time is devoted to finding new prebiotics with improved characteristics and optimizing their production.

Galacto-oligosaccharides (GOS) include a diverse group of indigestible carbohydrates that have a positive effect on human health, because they balance the intestinal microbiota, regulate cholesterol levels, help mineral resorption and vitamin synthesis. In previous studies, the transgalactosylation power of the enzyme β-galactosidase from Aspergillus oryzae was investigated, which enabled the production of valuable GOS products with a prebiotic effect from pure lactose.

Since whey, an environmentally harmful by-product of the dairy industry, is extremely rich in milk sugar (lactose) the possibility of its use in the synthesis of prebiotic products of galacto-oligosaccharides was investigated in this paper. The influence of different initial enzyme concentrations (0.1-1 g / l) and whey concentration in the range of 10-40% (w / v) on the total GOS yield and the composition of the obtained oligosaccharide mixture was examined. By dissolving whey powder in distilled water, solutions of up to 40% whey due to the physical state were prepared. During the experiments, the yield of galacto-oligosaccharides obtained in the reaction was compared, where whey powder (10-30%) was used as a substrate with the yields achieved in the reaction with pure lactose. It turned out that the yields of galacto-oligosaccharides in both cases were equal, which justifies the use of whey powder, because in this way significant savings in process costs are achieved. The maximum yield of galacto-oligosaccharides (about 70 g / L) was achieved at a whey concentration of 30% and an enzyme concentration of 0.2 g / L.

Keywords: whey, prebiotics, galacto-oligosaccharides, enzymatic synthesis, lactose

Acknowledgements: I owe a big thank you to the Ministry of Education, Science and Technological Development, whose scholarship I am a holder of

DEVELOPMENT OF MUSHROOM-BASED CEREAL FLOURS WITH IMPROVED NUTRITIONAL AND ANTIOXIDATIVE PROPERTIES

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Trametes versicolor, *Lentinus edodes* and *Pleurotus ostreatus* HK-35 mushrooms were used for the production of mushroom-based cereal flours. Three different cereals were used for this purpose: wheat (NS 40 S), rye (individual agricultural holding, Vojvodina province) and oat (Italico d.o.o). Sterilized grains (pH 6.0 – 6.5, $CaCO_3$) were seeded using mushroom's inoculum previously prepared on the malt agar, and incubated at 25 ± 2 °C for 20-30 days in the dark, until the grains were completely overgrown with mycelium. Control samples without seeding mushrooms' mycelium were prepared as well. The obtained grains were dried and milled into flours, in order to be used for the chemical characterization and antioxidative activity determination. All mushroom-based grain flours characteristics were compared with corresponding controls. The highest total carbohydrate content was determined for *T. versicolor* wheat (705.61±48.97 mg/g) and rye (749.15±42.09 mg/g) grain flours, with an increase of 26.39±1.09 % and 39.78±13.42 % compared to non-inoculated grains, respectively, while all inoculated flours had higher protein content compared to non-inoculated ones. A significant carbohydrate content increase was observed for *L. edodes* wheat grain flour (9.66±14.66 mg/g). Expand in total phenolic compounds content, between 7.72±0.39 and 217.74±54.65 %, was observed in eight out of nine tested samples, compared to the control. The highest values of phenolic compounds were noted for *T. versicolor* oat (30.94±0.07 mg/g) and rye (27.45±2.34 mg/g) grain flour. A significant increase ($p<0.05$) of chelating ability was observed for *P. ostreatus* and *L. edodes* wheat grain flours, when the very high chelating ability for both samples (93.62±3.01 %) was observed. Significantly higher reducing power ($p<0.05$) was detected in six out of nine tested samples, with the highest absorbance measured for *L. edodes* oat (1.88±0.08) and rye (1.15±0.04) grain flours. Obtained results revealed that the growth of different mushrooms on different cereals could be a promising method for enhancing the antioxidative potential of flours.

Key words: Mushrooms, Cereals, Flours, Phenolic Compounds, Antioxidative Activity

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EFFECT OF SOYBEAN/PROSO MILLET INTERCROPPING COMBINED WITH BIO-FERTILIZER ON ACCUMULATION OF ESSENTIAL ELEMENTS IN GRAIN

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Intercropping, as sustainable agricultural practice effectively uses environmental resources, affecting the nutritive quality of crops by modifying the concentration of macro- and micro-elements in grain. In order to improve the availability of nutrients from the soil, bio-fertilizers could also be used successfully in elements management. A field experiment was conducted with soybean (*var*. Selena) and proso millet (*var*. Biserka) to examine the integrated influence of intercropping and bio-fertilizer on Ca, Mg, S, Fe and Zn accumulation in grain. Mono-crops (T_1 - soybean, T_2 - millet), as well as tree planting patterns of intercrop, including alternating rows of soybean and proso millet (T_3), alternating strips of two rows of soybean and two rows of millet (T_4) and alternating strips of two rows of soybean and four rows of millet (T_5), were set up in 2018 and 2020. The same combinations, as subplots, were treated with bio-fertilizer Coveron (C) (containing mycorrhizal fungi, *Trichoderma* and plant growth-promoting rhizobacteria). After harvest, grains were dried and milled and preparation of samples was performed using wet digestion procedure with $HClO_4$ + HNO_3. The concentration of Ca, Mg and S were determined by ICP-OES, while Fe and Zn concentration was measured on ICP-MS. Results showed that Coveron reduced S concentration in soybean grain, while the status of other elements mainly remained unchanged. In terms of intercropping, soybean from T_5 combination had a significantly higher level of Fe in comparison to T_1 and T_3, and higher but not significantly than T_4, emphasizing alternating strips as more effective for accumulation of this element in grain. Combination T_3 + C provided the highest accumulation of Ca, while T_5 + C significantly raised Fe concentration. In terms of proso millet, bio-fertilizer expressed a greater impact on Ca, Fe and Zn by increasing their concentration. On the other side, all intercrop combinations expressed a positive impact on Fe concentration and simultaneously reduced Zn concentration. When mutual influence is considered, T_4 + C significantly increased Ca and Fe values, while T_2 + C increased Zn value, pointing positive effect of integrated and sustainable agricultural practices on an accumulation of essential elements in a grain of proso millet. In conclusion, different intercrop combinations combined with bio-fertilizer could be successfully used to improve the nutritional quality of soybean and millet grains in terms of sustainable food production, but further investigations are required to highlight other aspects of potentially enhanced nutritional quality.

Keywords: Nutritional quality, Sustainability, Macro-elements, Micro-elements

EVALUATION OF FUNCTIONAL PROPERTIES OF DEFATTED SEED CAKES AND FLOUR BLENDS

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The functional properties of flour are a consequence of complex interactions between different ingredients, the structure of their molecules, physicochemical properties. They are related to the natural environment in which they are located and measurements made and can be determined by the Solvent Retention Capacity (SRC) test. This test is based on the improved swelling behavior of individual polymer networks in selected individual diagnostic solvents - water, lactic acid (for glutenin), sodium carbonate (for damaged starch) and sucrose (for pentosans) which are used for predicting the functional contribution of each individual flour component. The gluten performance index (GPI), a new predictive SRC parameter, has been found to be an even better predictor of overall glutenin performance in flour in the environment of other flour polymer modulating networks.

By-products of fruit processing, such as pomace, pulp, peel or seeds, are a rich source of biologically valuable ingredients. They can be used to replace part of wheat flour in bakery and confectionery products, where they also have an impact on their functionality.

Plum and quince seeds, which remain after the industrial processing of this fruit, were used for the examination as a substitute for a portion of wheat flour. The oil was extracted from the seeds by the cold pressing process, and the remaining cake was ground to the flour particle size. The characterisation of defatted seed cakes, wheat flour, and mixtures was performed by examining the chemical composition and determining their technological and functional properties. Based on the results obtained by testing, it was determined that defatted cakes have a high nutritional value due to the high content of fat, protein and crude fiber. Variations in solvent retention capacity of flour blends depended primarily on the content of crude fiber and protein. This indicates the possibility of using defatted seed cakes as a source of functional ingredients to avoid syneresis and change the viscosity and texture of some formulated foods to develop low-calorie foods and foods with reduced gluten content.

Keywords: Functional properties, Solvent retention capacity, Defatted seed cake, Flour blends

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PUMPKIN SEED CAKE – ANTIOXIDANT AND NUTRITIONAL VALUE OF SELECTED SAMPLES

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Pumpkin seed cake, which remains after cold pressing oil extraction, is a nutritionally valuable but inexpensive raw material that is also considered ~~as~~ a potentially rich source of biologically active substances. Thus, the aim of this study was to measure total phenolics content (TPC) of four samples of pumpkin seed cake flour, as well as their antiradical potency by means of DPPH radical scavenging. In order to determine the nutritional value of each sample, moisture, ash, cellulose, lipids, proteins, carbohydrates, mineral content and fatty acid composition were also investigated using conventional methods. Concerning food safety issues the amounts of heavy metals and pesticides were also determined. The results obtained, pointed out differences between samples in their TPC, anti-DPPH activity and nutritional characteristics. TPC, determined as gallic acid equivalents (GA) using the spectrophotometric method with FC reagent, ranged from 24.9-194.1 mg GA/100 g. Correlated with TPC, observed anti-DPPH activity was modest with SC_{50} values ranged from 0.9-18.5 mg/ml, respectively. As for parameters of nutritional value, obtained results were in the line with previous findings, with protein content *ca*. 50%. Variations, mainly in the mineral (14.61-30.70 mg/100 g) and the carbohydrate content (9.38-21.86%), could be explained by the different geographical origin of the pumpkins. All tested samples complied with the approved health standards related to the content of heavy metals and pesticides. Nevertheless observed differences, it could be concluded that pumpkin seed cake could be considered as a naturally rich source of proteins, cellulose and minerals (Fe, Cu, Zn, Mg) with a reduced amount of oil, safe for human consumption. It is also a good source of polyphenols, thus its potential as a functional food ingredient should not be neglected.

Keywords: pumpkin seed, polyphenols, DPPH, nutritional value.

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GENTIAN ROOT: COMPARISON OF OPTIMIZED HEAT ASSISTED AND ULTRASOUND-ASSISTED EXTRACTION METHODS

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Gentiana lutea L. underground parts, also known as gentian root, are used for the preparation of dietary supplements, functional food, pharmaceutical, or cosmetic products. In our previous study, ultrasound-assisted extraction (UAE) and heat-assisted extraction (HAE) procedures were optimized to maximize the yield of the gentiopicroside, isogentisin, and total phenolic content (TPC) from gentian root. Therefore, the aim of this study was to compare the influence of those two extraction methods on the yield of gentiopicroside, isogentisin, and TPC. Gentian root extracts were prepared in five replications under the optimal conditions by UAE (80 °C, 31 min, solid-to-solvent ratio 1:42, ethanol concentration 49%) and by HAE (65 °C, 129.08 min, solid-to-solvent ratio 1:40, ethanol concentration 49.33%). Gentiopicroside and isogentisin content were analyzed by HPLC and expressed as mg/g of dry weight (dw). TPC was determined spectrophotometrically using the Folin–Ciocalteu method and expressed as mg of gallic acid equivalents (GAE)/g dw. Results are considered statistically equal, according to t-test, in the case of a P-value higher than 0.05. Gentiopicroside content in the extracts obtained by UAE was 15.78 ± 1.16 mg/g dw, while in the case of HAE content was 15.16 ± 1.10 mg/g dw, indicating that there was no significant difference (P = 0.40). Isogentisi content in the extract prepared by UAE was 7.87 ± 0.91 mg/g dw, whereas in the case of HAE content was slightly higher 8.58 ± 0.51 mg/g dw. Also, there was no statistical difference (P = 0.17) between these values. Moreover, there was no statistical difference (P = 0.6680) between TPC in the extracts obtained by UAE (11.29 ± 0.82 mg GAE/g dw) and HAE (11.46 ± 0.22 mg GAE/g dw). Therefore, both methods (optimized UAE and HAE) are effective and there was no significant difference between the extraction yield of gentian roots target compounds.

Keywords: Gentiopicroside, Isogentisin, Total phenolic content

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ANALYSIS OF THE IMPACT OF DIFFERENT FINING AGENTS ON THE PHENOLIC COMPOUNDS OF CABERNET SAUVIGNON WINES

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In this study impact of different fining agents (gelatine and albumin) on the catechin, epicatechin and *trans*-resveratrol content were investigated. Grape variety Cabernet Sauvignon was harvested in a state of technological maturity. Phytosanitary state: 100% health, sugar in the must 23% and total acid in the must 6.8 g/l. Alcohol fermentation with maceration was carried out by microvinification method at a temperature of 25°C using the pigeage system (punch-down twice a day). Free sulfur dioxide 5g / hl was added to the grape must. Yeast *Saccharomyces cerevisiae* (BDX, Lallemand, Canada) in the amount of 20g/ hl and enzyme preparation EXV (Lallemand, Canada) were used. Maceration has lasted 14 days and after that pomace was separated from the must and racket in pilot tanks to finish fermentation. The second operation was bottling and storage until the addition of fining agents. A control sample was wine without fining. In other four samples increasing concentration of gelatine and albumin (5 and 10 g/hl) was added. After two months all samples were analysed by Waters Acquity UHPLC H-Class with mass detector (Waters TQ (Tandem Quadrupole, WAT-176001263)). It was not observed a statistically significant difference between the control sample and wine with different doses of fining agents ($p \geq 0{,}05$). Content of catechin was decreased about 14% with a lower concentration and 28% with a higher concentration of gelatine. Albumin has affected the content of catechin about 14% reduction with both doses. Addition of gelatine and albumin had higher impact to epicatechin content and it was observed its reduction of about 30% for both doses of gelatine, and 24% for both concentrations of albumin. *Trans*-resveratrol was decreased up to 17% for all fining experiments.

Keywords: phenolic compounds, Cabernet Sauvignon, fining agents, gelatine, albumin

BLACK CHOKEBERRY-FRUIT FOR WINE PRODUCTION

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Black chokeberry (Aronia melanocarpa L.) possesses broad range of different compounds which show beneficial health effects on humans. It can be differently processed and one of final products include wine.

The analysed wine samples were produced by microvinification procedure. The control fermentation of black chokeberry must was conducted using selected yeast cultures. Enzyme and sucrose were added in part of the samples before fermentation, due to the increasing of phenolic compounds content of the final product. Total phenolic content (TPC) was spectrophotometrically determined by Folin-Ciocalteu method, while single polyphenolics were quantified using UPLC TQ-MS/MS. In addition, antioxidant properties were also estimated with Ferric Reducing Antioxidant Power (FRAP) assay and anti-DPPH radical activity.

The applied microvinification procedure significantly affected both polyphenol profiles and antioxidant potentials of the examined samples. Indeed, the wine enriched with phenolic compounds and with highest antioxidant activity was produced with addition of enzyme and sugar before start of fermentation. However, an opposite trend was observed for a control sample, produced without addition of enzyme and sugar. Among quantified phenolic compounds especially stood out following phenolic acids: chlorogenic (655.23-717.35), protocatechuic (575.77-645.27) and caffeic (77.72-97.21). The TPC and FRAP values were in ranges 2247.55-2457.23 mg GAE/L and 67.55-78.41 mmol/L Fe2+, respectively. On the other hand, anti-DPPH radical activity (expressed as an IC50 value) ranged from 1.41 to 1.67%.

In summary, black chokeberry wine may be considered as a rich natural source of phenolic acid derivatives that are, jointly with other active principles – both phenolic and non-phenolic, responsible for its high antioxidant potential.

Keywords: black chokeberry wine, microvinification procedure, phenolic compounds, antioxidant activity

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must is not verb in this sentence it is noun

APPLICATION OF PHYTOCOMPOUND-LOADED NANOEMULSIONS IN THE ACTIVE COATINGS AND FILMS FOR FOOD PACKAGING

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Nowadays, extended shelf life and the low environmental footprint of food products are some of the most important challenges that face food scientists. Active food packaging designed not only to protect and promote food products but also to maintain or even improve their quality and safety, prolong shelf life and diminish food losses is an interesting approach. In this context, the active materials for food packaging applications can be formulated and developed employing phytocompound-loaded nanoemulsions. This paper aims to give an overview of recent developments in the applications of phytocompound-loaded nanoemulsions in active coatings and films for food packaging. Various phytocompounds with antioxidative, antimicrobial, and nutraceutical properties such as essential oils, plant extracts, oil-resins, monoterpenes, aromatic aldehydes, flavonoids, vitamins can be employed to impart functional properties to the nanoemulsions-based coatings and films. Biopolymers, including polysaccharides (starch, cellulose, pectin, chitosan, gums, pullulan, alginate), proteins (corn zein, wheat gluten, soy protein, casein, gelatin, whey protein), and lipids (beeswax), are recognized as green and environmentally friendly materials for the development of innovative food packaging.

Phytocompound-loaded nanoemulsions with antioxidant and antimicrobial activity incorporated in the biopolymeric matrix are an interesting approach to prevent oxidation processes and microbial growth in food products. For example, higher thermal, UV-light, and storage stability were determined for β-carotene loaded within chitosan-coated nanoemulsion compared to free β-carotene. Rutin-loaded nanoemulsion incorporated within gelatin matrix triggered high free radical scavenging and reducing power ability of the films. Nanoemulsion loaded with plant oil in the pectin matrix provided an antimicrobial activity to the films against *Staphylococcus aureus* and *Escherichia coli*. Incorporation of nanoemulsion loaded with oregano essential oil in hydroxypropyl methylcellulose films impart antibacterial and antioxidant functionality. These findings highlighted the potential of phytocompound-loaded nanoemulsions to be used as functional constituents of active food packaging materials.

Keywords: Nanoemulsions, Phytocompounds, Coatings, Films, Food packaging

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ENCAPSULATION OF *ROSA CANINA* EXTRACT IN LIPOSOMES PRODUCED BY THIN FILM METHOD

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Rosa canina pseudo-fruit is rich in bioactive substances, such are carotenoids, ascorbic acid, polyphenols, fatty acids and minerals. Because of its diuretic, anti-inflammatory, anti-allergic and antioxidant properties it is frequently employed in traditional medicine. However, in order to protect mentioned bioactive compounds and to improve its bioavailability and controlled release, numerous encapsulation methods have been developed. Thus, *R. canina* ethanol extract obtained using percolation at ambient temperature (25°C), was encapsulated into Lipoid G liposomes produced in thin film procedure. Total polyphenol and flavonoid contents, as well as antioxidant potential of the extract and extract loaded liposomes were determined. Encapsulation efficiency, particle size, polydispersity index and zeta potential of the liposomes with extract were monitoring during 30 days. Total polyphenol and flavonoid contents of the extract were 103.8±1.9 mg of gallic acid equivalents/g of the extract and 64.2±2.4 mg of catechin equivalents/g of the extract, respectively; antioxidant potential of the extract was 15.3±2.1 mg of Trolox equivalents/g of the extract (ABTS assay) and 1.07±0.01 mg/mL of the extract (DPPH assay). Total polyphenol and flavonoid contents of the extract loaded liposomes were 0.316±0.023 mg of gallic acid equivalents/mg of lipids and 0.219±0.009 mg of catechin equivalents/mg of lipids, respectively; antioxidant potential of the sample was 0.134±0.004 mg of Trolox equivalents/mg of lipids (ABTS assay) and 270.6±10.5 mg/mL of liposomal suspension (DPPH assay). Encapsulation efficiency was the same during monitoring period and it was amounted 46.6±3.4%. Particle size and polydispersity index of the liposomes with extract were increased from 618.2±10.9 to 1698.0±104.2 nm and from 0.441±0.007 to 0.589±0.011, respectively, during 30 days. On the other hand, zeta potential of the extract loaded liposomes was decreased from -10.24±0.95 to -7.21±0.77 mV. *R. canina* extract loaded liposomes developed in this study have potential to be used in food, pharmacological and cosmetic industries due to beneficial health effects of *R. canina* active compounds encapsulated into liposomes.

Keywords: encapsulation efficiency, liposomes, Rosa canina, thin film method.

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FOOD PRODUCTION, PROCESSING, SUSTAINABILITY, ADDED-VALUE FOOD

HYDROLATS FROM SEVEN LAMIACEAE SPECIES AS POTENTIALLY USEFUL WASTE

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Essential oils, and hydrolats (floral waters) as distillation by-products, isolated from aromatic representatives of the Lamiaceae family have been widely used as flavouring agents in food and cosmetics. Considering the well-confirmed linkage among oxidative stress, diabetes mellitus and neurodegeneration, this research was aimed to examine the chemical composition, as well as antioxidant, antidiabetic and antineurodegenerative potential of hydrolats of seven Lamiaceae species (hyssop, lavender, basil, peppermint, savory, rosemary and sage) commercially grown on the fields of the Institute for Medicinal Plant Research "Dr Josif Pančić", Serbia. As results obtained by GC-FID and GC-MS analysis indicated, the major components of the hydrolats were *cis*-pinocamphone (hyssop), linalool and linalyl acetate (lavender), linalool and methyl chavicol (basil), menthone and isomenthol (peppermint), geraniol (savory), verbenone and camphor (rosemary) and 1,8-cineole (sage). The tested hydrolats performed slight antioxidant effects in DPPH and total reducing power assays with the highest values obtained for savory and sage, while in β-carotene bleaching assay lavender showed the strongest activity. Except peppermint hydrolate, all tested samples exhibited antidiabetic effects, efficiently inhibiting α-glucosidase activity (values ranged from 13.22-60.24%), with the highest value obtained for basil. However, there were no effects observed towards α-amylase. Regarding the evaluation of antineurodegenerative activity, the tested hydrolats showed great potential in inhibiting of acetylcholinesterase (27.27-74.39%) and tyrosinase (16.47-67.07%), with the highest values obtained for rosemary and lavender samples, respectively. Although antioxidant and antidiabetic effects of hydrolats were significantly weaker compared to positive controls, their antineurodegenerative effects were similar to those of galanthamine and kojic acid tested at 1 mg/mL. In conclusion, the examined aromatic plant's hydrolats should be considered as a source of components possessing antineurodegenerative effects, with potential utilization in food and pharmaceutical industries; hence they should not be discarded after distillation, as they usually are.

Keywords: hydrolats, chemical composition, antioxidant effects, antidiabetic effects, antineurodegenerative effects

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COMPARISON OF MICROWAVE-ASSISTED, SUBCRITICAL WATER, AND HIGH VOLTAGE ELECTRIC DISCHARGE EXTRACTION EFFICIENCY IN PRODUCTION OF DILL SEED EXTRACTS

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Anethum graveolens L. commonly known as dill is a plant native to the Mediterranean and West Asia. Dill is one of the most popular aromatic spices, but it is also cultivated throughout the world as a medicinal plant. Although the majority of the research is focused on essential oils, it has been proven that ethanolic and aqueous extracts of dill also possess significant pharmacological activity. It has been reported that the extracts of dill seeds exhibit diuretic, spasmolytic, antifungal, anti-inflammatory, antidiabetic, antioxidant, and anticancer activity.

Considering the highly significant biological potential of dill seed, there is a constant need for the improvement of extraction methods for obtaining dill extracts. Three green, innovative extraction methods were applied, and the efficiency of the methods was measured by the yield of the extraction, content of total phenols, total flavonoids and the antioxidant activity of the extracts. Microwave assisted extraction (MAE) was conducted on five different temperatures (40, 60, 80, 100 and 120°C) and two different times of extraction (5 and 10 min). Subcritical water extraction (SWE) was conducted also on five different temperatures (100, 125, 150, 175 and 200°C). Lastly, high voltage electric discharge (HVED) extraction was conducted on three frequencies (40, 70 and 100 Hz) and three extraction times (1, 5 and 15 min). The content of total phenols was in the range from 66.98 to 145.66 mg GAE/g DE (dry extract). The lowest yield was achieved by using HVED 66.98 mg GAE/g DE, whereas higher recovery was achieved with subcritical water (126.24 mg GAE/g DE). However, the most efficient extraction was MAE using temperature of 40°C for 10 min (145.66 mg GAE/g DE). It was established that using environmentally friendly solvents and relatively simple and inexpensive equipment can provide dill extracts rich in hydrophilic bioactive compounds in a short extraction time.

Keywords: Anethum graveolens, Microwave-assisted, Subcritical water, High voltage electric discharge extraction

HPLC ANALYSIS OF ASCORBIC ACID IN PRETREATED AND DRIED RED PEPPER (*CAPSICUM ANNUM*)

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Red pepper (*Capsicum annum*) fruits contain high amount of ascorbic acid (vitamin C), which is of great importance for human health. The aim of this study was to found how various pretreatments and drying methods influence on retention of ascorbic acid in dried pepper. The cultivar "Horgoška sweet 6", often used for the production of paprika, was selected for analysis. Five sets of experiments were performed to investigate the effect of the following parameters: pretreatment temperature (20 ^{o}C, 50 ^{o}C, 80 ^{o}C), pH value (3, 6.5, 10), additive (0.25% citric acid, 0.25 % potassium metabisulfite and 0.25 % citric acid + 0.25 % potassium metabisulfite), ultrasound (off and on) and drying method (hot air drying and freeze drying). The initial content of ascorbic acid in fresh pepper was 292 mg/100 g dry basis (d.b.) and it was reduced after all pretreatment and after drying of pretreated samples. Most of examined pre-drying treatments improve retention of ascorbic acid in final dried peppers, except treatments at 80 ^{o}C. Our results indicate that temperature and drying method were parameters that significantly influenced ascorbic acid content in dried peppers. Other parameters were not significant, but had a certain effect on retention of ascorbic acid. The best parameters were temperature 20 ^{o}C, pH 6.5, citric acid/potassium metabisulfite, without applying ultrasound including freeze drying method.

Keywords: citric acid, potassium metabisulfite, ultrasound, hot air drying, freeze drying

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THE INFLUENCE OF DIFFERENT HEAT TREATMENT ON THE VITAMIN C CONTENT IN PEPPER (CAPSICUM ANNUUM L.)

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The pepper (**Capsicum annuum** L.) is a vegetable commonly used in the human diet. Due to its exceptional sensory and nutritional properties, it is readily consumed both fresh and processed into various ready meals, pickling, salads, dried spices, etc. There are a large number of cultivars of peppers that differ in shape, size, color, aroma, degree of hotness, etc. The pepper is a rich source of carotenoids, vitamins, mineral matter, carbohydrates, organic acids and aromatic components. Numerous bioactive compounds found in pepper, such as vitamin C (ascorbic acid), contribute to its high antioxidant activity.

The aim of this study was to determine the vitamin C content after different heat treatments commonly used in the industrial processing of peppers, as well as in the household. The domestic cultivar "Elephant ear" was subjected to tree different thermal treatments: 1) cooked in a closed and opened dish, 2) roasting in microwave oven and on the hob, and 3) frying in sunflower oil, for 15 minutes. The content of vitamin C was determined using the indirect iodimetry method. The results were expressed in mg/100 g dry basis (d.b.) and compared with the fresh pepper used as a control. The content of vitamin C in the fresh sample was 1295.38 mg/100 g d.b., while in the peppers cooked in closed and opened dish its value was 1007.58 and 615.17 mg/100 g d.b., respectively. In the pepper treated in the microwave oven and on the hob, the content of vitamin C was 494.51 and 1201.40 mg/100 g d.b., respectively, while its value in the fried sample was 443.65 mg/100 g d.b. The highest loss of vitamin C was observed after frying treatment (65.75%), while the lowest loss was recorded in the sample roasting on the hob (7.26%), in relation to the initial amount in unprocessed pepper. Also, the results of vitamin C content indicate that its loss was higher when the thermal treatment was performed in an opened than in the closed dish probably due to the increased presence of oxygen that may intensify oxidation. However, the obtained quantity of vitamin C per mg/100 dry basis of the tested samples is more than enough to ensure daily intake of vitamin C and avoid its deficiency in the human diet.

Key words: Vitamin C, Pepper, Cooking, Roasting, Frying.

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EXAMINATION OF TECHNO-FUNCTIONAL PROPERTIES OF CHICORY (*Cichorium intybus* L) ROOT FLOUR AS A POTENCIAL INGREDIENT OF FUNCTIONAL FOOD

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Chicory (*Cichorium intybus* L.) has been used for hundreds of years as a herbal remedy for many diseases due to its health-promoting properties that include anti-inflammatory, anticarcinogenic, antimicrobial, antimutagenic, immune-stimulating, antihepatotoxic and antioxidative attributes. Although most plant parts have certain beneficial qualities, the root is known as the major source of inulin, dietary fiber with a prebiotic effect, and also contains other phytochemicals such as coumarins, flavonoids, sesquiterpene lactones, tannins, alkaloids, vitamins, minerals, and volatile oils. However, the use of chicory root is limited due to its bitter taste, which can be reduced by various treatments. The aim of this study was to examine the techno-functional properties (moisture content, water holding capacity, oil holding capacity, bulk density, solvent retention capacity) of chicory root flour, both treated and untreated, as well as those of composite flours made of its mixtures with wheat flour. Two samples of chicory root flour were used - raw flour obtained by grinding dried chopped root and flour treated to reduce bitterness. Treatment was performed by heating (140ºC for 30 min), and by heating and subsequent mixing with 10% honey and 10% sunflower oil. Composite flours made by combining both chicory root flours with wheat flour in the proportion of 1%, 2,5% and 5% were tested as well. Almost all investigated parameters were found to be more increased in chicory flour than in wheat flour, with exception of moisture content and bulk density. Also, the presence of chicory flour, treated and untreated, led to an increase in the values of all tested factors in composite flours. The obtained results can provide a general indication of the rheological and baking behavior of the dough from raw, treated or composite flours when a low degree of substitution is intended to be used to develop new products with added value.

Keywords: chicory root flour, techno-functional properties, bitterness reducing treatment, composite flour

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AGRO-CULTURE WASTE AS A SOURCE OF FUNCTIONAL FOOD INGREDIENTS

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One of the largest sector which produces highest amount of bio-waste is agriculture. Management of such waste is an important task not just from the economic point of view but also from the environmental aspect. Most of the bio-waste is disposed in landfills, in which it may transform into contaminants, pollute the surrounding environment, and produce the methane and carbon dioxide that cause global warming. On the other hand, agro-culture waste together with side streams from agri-food industry is valuable source of new bio-potent molecules that may possess valuable role in designing functional foods. Isolated ingredients from bio-waste may act as antioxidants, antimicrobials, food aromas and colorants and there is growing trend for utilization of agri-food waste as a source of such ingredients. Additionally, valorization of such waste is not just the important task for food industry but also it is in line with sustainability and environmental concerns. In line with that "green" technologies are gaining importance in agri-food sector due to the need to reduce pollution from toxic chemicals, make industrial processes safer and more sustainable, and offer 'cleanlabelled products' more and more required by the consumers.

In this research, poppy-cake, which represent waste in food industry, was extracted by subcritical water at different temperatures and pressures. Potential of obtained extracts to be used as functional ingredients in different functional products was investigated. Chemical analysis of their composition was determined by LC-MS analysis while battery of assays was applied for biological activity investigation. Simultaneously, other conventional and non-conventional extraction techniques were used in order to compare obtained results and to achieve a comprehensive insight into subcritical water potential as a green technique for functional ingredients isolation.

Keywords: Agri-culture waste, functional ingredients, functional foods, green extraction technologies.

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"GREEN" APPROACH FOR THE SMART AGRO-FOOD WASTE MANAGEMENT TO OBTAIN FUNCTIONAL FOOD ADDITIVES

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With the increase of world population, agricultural and food sectors are facing with the serious problem of generating large amounts of waste or by-products. It was estimated that the amount of agricultural waste reaches up to 3.5 billion tons annually, while in the food industry one third of the produced food is lost from the food chain in the form of waste. The amount of such waste is increasing daily, which not only leads to economic loss but also leads to environmental pollution. Inadequate management of such waste results in its conversion to various contaminants (such as methane and carbon dioxide) that pollute the environmental and contribute to global warming. Thus, there is a justifiable need to properly manage such bio-waste, and one of the strategies is to use it as cheap resources to obtain high-value compounds by technologies that are acceptable regarding modern environmental and health standards. . On this background, "Smart waste management" is considered to be an essential element of any country's long-term economic and environmental plan.

In the frame of this work, the bio-waste produced in food industry was used for the isolation of bioactive ingredients, in the first place phenols and flavonoids, by applying high pressure extraction technology and by using green solvents (water and ethanol). During the extraction process the operating temperatures were 120 and 150℃ while the pressure was constant (100 bar). Obtained extracts were analyzed spectrophotometrically and content of total phenols and flavonoids was determined. Additionally, bioactivity in terms of antioxidant capacity was measured by using battery of assays. Obtained results showed high extraction yields of observed components as well as high biological activity of the extracts.

Keywords: Green extraction technologies, subcritical water, high-pressure extraction, polyphenols.

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INFLUENCE OF ORGANIC BIOSTIMULATORS ON SEED MATURATION OF MEDICINAL AND SPICE PLANT SPECIES *CORIANDRUM SATIVUM* L.

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In an attempt to obtain faster and more uniform maturation of *C. sativum* seeds, an investigation was set up in March 2021, at the experimental field of the Institute for Medicinal Plant Research in Pančevo, Serbia. The aim was to determine the effect of biostimulators on *C. sativum* seeds ripening time and release. Prior to sowing, the seeds were immersed for 10 minutes in various liquid organic biostimulators: 1%, 3% and 5% Ecobuster1, and SlavolS, while those in control were treated with distilled water. The seeds were then sown in styrofoam containers filled with a mixture of peat moss. There were 30 plants per each treatment and they all grew in a Grow box, under the influence of artificial lighting and at a mean temperature of 23°C. With the appearance of the first true leaf pair, the plants were transplanted into 0.8L pots filled with the same substrate and transferred to a plastic house to grew under drip irrigation, at a mean temperature of 25 °C and daily ventilation. When more than 2/3 of plants formed seeds, the measurements on each individual plant took place. There was no significant difference between the treatments in the plants′ heights and weights, while there was in their seed maturation time. The highest percentage of mature seeds (95%) in the shortest ripening period was obtained in SlavolS treatment, which significantly differed from all other treatments, particularly the control (30% of released seeds during the same period). Similar to SlavolS but still significantly lower results were obtained with 5% Ecobuster1 (65% of released seeds during the same period). The obtained results suggest that the biostimulators SlavolS and 5% Ecobuster1 could be successfully applied when faster and more uniform maturation of *C. sativum* seeds want to be achieved.

Keywords: Ecobuster, SlavolS, seed treatment.

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FROM STARTER-ASSISTED TO FERMENTOME-DRIVEN: A PARADIGM SHIFT IN SOURDOUGH FERMENTATION

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The application of omics techniques helps to further unravel sourdough fermentation potential. Meta-genomic, culturomic, metabolomics and meta-transcriptomics analyses of eight sourdoughs representative from different countries in the world were performed. Cultivable bacteria and yeast species identified by the culture dependent methods were also identified by meta-genomic approach. Metagenomics analysis described the sourdough metagenome, including dominant bacterial and fungi strains and subdominant population. The metabolic functions identified by Kyoto Encyclopedia of Genes and Genomes (KEGG) strongly support the evidence of sourdough fermentation. Multi-copies genes encoding for enzymes involved in key sourdough metabolisms were identified in sourdoughs. Meta-transcriptomic profiles of the different sourdoughs confirmed the expression of core genes encoding for the biosynthesis or catabolism of amino acids by sourdough lactic acid bacteria. From the comparison of all omics data, emerged a clear picture of the potential metabolic background vs. metabolisms expressed under sourdough conditions. The ecological fundaments retrieved will ensure the resilience sourdough-fermented doughs to various causes of disturbance. The results of this study will allow the industrial development of the most stable and performing mixture of microbes to drive the sourdough fermentation.

Keywords: Sourdough, omics, Lactic acid bacteria, yeasts

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ACE INHIBITOR ACTIVITY AND ANTIOXIDANT ACTIVITY OF MOUNTAINOUS AND PLAIN EWE'S MILK CHEESES AND THE INFLUENCE OF DIFFERENT HERBS ON THE VOLATILE COMPOUNDS

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This study aimed to investigate the comparison of mountainous and plain areas on the composition, angiotensin-converting enzyme inhibitor (ACE-I) activity, peptide profile and antioxidant activity of Macedonian traditional cheeses. In this study, white brined and beaten cheese, were produced from ewe's milk from mountain (MW, MB) and plain areas (PW, PB) in almost the same traditional manufacturing steps. The cheeses (MW, MB, PW, PB) have been analysed for antioxidant activity and ACE-I activity after 60 days of ripening. The levels of antioxidant and ACE-i activity between the cheeses were found to be the lowest with 98.2 and 82.8 % in the plain white cheese (PW) and highest with 107.6 and 95.5% in mountain white cheeses (MW), respectively. The second part of this study compared the volatile profiles during ripening of white cheese (MW) and herbs containing cheeses (*Mentha longifolia* - WM, *Alium sativum* - WG, *Petroselinum crispum* - WP), made by using ewes' milk. The profiles of volatile compounds of cheeses were analyzed by GC/MS using a solid-phase microextraction (SPME). In the volatile fraction of the herbal cheeses, the volatile components consisted of 7 ketones, 9 acids, 16 esters, 19 alcohols, 8 hydrocarbons, 16 terpenes, 5 aldehydes and 11 others. The main chemical class among the herbal cheeses was acids and esters (68 % and 18 % w/w of all volatile components, respectively). A significantly (P<0.05) higher concentration of the volatile compounds was found in the White cheese with Garlic and White cheese with Menta with 41 % and 27 % of total volatile compounds, respectively. These differences in the volatile profiles of cheeses can be explained by the differences in raw materials, spices and production conditions.

Keywords: SPME/GC-MS, Herbs, plant enzymes, functional ingredients

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Poster presentation within sections
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EFFECT OF DRYING METHODS ON THE RETENTION OF ANTIOXIDATIVE PROPERTIES OF SOUR CHERRY

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Drying is largely used to preserve fruits and offers dehydrated products that can have an extended life of a year. Sour cherry (*Prunus cerasus L.)* is known as a rich source of bioactive compounds which possess high antioxidant activity and beneficial effect on health. The objective of the present study was to evaluate the effect of different drying methods on antioxidant properties, vitamin C, total phenolics and anthocyanins content of the sour cherry. Whole sour cherry fruits were dried convectively (air-drying), osmotically and by freeze-drying. The samples were analyzed for total phenolics content by Folin-Ciocalteau method and for total anthocyanins content by pH differential method. DPPH assay was applied for determining antioxidant activity of the fresh and dried sour cherry fruits and it was expressed as Ec_{50} value. The sour cherry preserved by freeze-drying demonstrated statistically significant better retention of the antioxidant activity, vitamin C, total phenolic and anthocyanins content compared to other drying methods. The air-drying has caused significant ($p < 0.05$) changes of vitamin C and total anthocyanins content while the osmotic dehydration significantly reduced all the investigated parameters compared to the fresh sour cherries. Antioxidant activity of freeze-dried samples decreased up to 6,62 % in relation to the control sample, while after air-drying and osmotic dehydration its reduction was up to 21,43 % and 38,56 %, respectively. Generally, anthocyanins and vitamin C of sour cherries were found to be more sensitive to drying than phenolic compounds, and the highest antioxidant properties were retained after freeze-drying treatment.

Keywords: drying, sour cherry, antioxidant property, freeze-drying

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SOLID WASTE OBTAINED FROM INDUSTRIAL TINCTURE PRODUCTION FROM *PLANTAGO MAJOR* L. LEAVES: INSIGHT INTO CHEMICAL COMPOSITION AND BIOACTIVITY

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Plantago major is a widely cultivated plant, traditionally used as a culinary ingredient and component in various cosmetics products. As a traditional medicinal plant used for centuries, it has been reported to be a remedy for numerous diseases, including those caused by bacteria. Nowadays, there is a rising interest in plant-based products as antimicrobial agents to overcome bacterial resistance issue. Medicinal plants are rich in bioactive secondary metabolites. However, in the process of production and processing of medicinal plants, a large amount of plant residues has usually been discarded as waste and treated as a by-product, so examination of its biological activities would be of great interest. Chemical characterization and bioactivity of ethanol-water extracts obtained from P. major leaves and solid waste of P. major leaves that remains after industrial tincture production have been analyzed and compared. Polyphenolic profiles of extracts were assessed by HPLC analysis. Antibacterial activity was examined by applying MIC assay of the extracts against Staphylococcus aureus and Acinetobacter baumannii. In addition, the MTT assay was performed for the estimation of cytotoxic effects of both extracts against two human cell lines, colon carcinoma (HCT116) and melanoma (Hs 294T). Both extracts are a good sources of polyphenols. Detected phenolic compounds can be classified into phenolic acids (chlorogenic acid, p-hydroxybenzoic acid, caffeic acid and p-coumaric acid) and flavonoids (rutin, quercetin 3-O-glucoside, isorhamnetin 3-O-glucoside, luteolin, quercetin and apigenin). Reduction of cell viability was noted in a dose-dependent manner. HCT116 cells were more susceptible to the agents' treatments. Both agents exhibited antibacterial activity against all tested S. aureus strains at higher tested concentrations (>1.25 mg/mL), but only P. major solid waste showed antibacterial activity on A. baumannii strain at a concentration of 4 mg/mL. Taking into account, extracts show great potential for further investigation, which could provide their potential use as therapeutic agents.

Keywords: Plantago major, plant extract, HPLC, microbial, cytotoxicity

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PHENOLIC PROFILE AND ANTIOXIDANT ACTIVITY OF PEPPERMINT (*Mentha piperita* L.) AGRO-INDUSTRIAL WASTE

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Among medicinal and aromatic plants, peppermint (*Mentha piperita* L.) is one of the most cultivated in the Balkan region, commonly utilized in both food and pharmaceutics industries. The active parts of this plant are the stems, leaves, and flowers. However, some parts of plant, mostly seeds, are excluded at the field as agro-industrial waste.

This study explored the polyphenol profiles and antioxidant activities of ethanol extracts of peppermint's agro-industrial waste from the field. Ethanol-water mixtures in the concentrations of 96 % (v/v) and 70 % (v/v) were used to obtain extracts from dried plant (*M. piperita* L.) waste material. For the separation and quantification of phenolic compounds, ultra-high performance liquid chromatography with a mass spectrometer was used. In addition, total phenol content (TPC) was evaluated in ethanolic extracts by Folin-Ciocalteu method, while antioxidant activity was evaluated by DPPH and FRAP methods.

In total, twelve phenolic compounds were quantified in both samples, including four phenolic acids and eight flavonoids. Chlorogenic acid was the most abundant phenolic compound in both samples prepared with the ethanol-water mixture (M70; 1.77 mg/L) and with ethanol (M96; 0.56 mg/L), followed by caffeic acid with 0.99 and 0.33 mg/L, respectively. Among flavonoids, the most abundant was rutin with amounts 2.93 (M70) and 2.09 (M96) mg/L, while other flavonoids were quantified in small amounts. TPC of M70 and M96 samples were 1587.71 and 786.86 mg/L gallic acid equivalents. The antioxidant activity was 28.70 (M70) and 19.07 (M96) mM Trolox equivalents (TE) according to FRAP method; while 9.05 and 2.69 mM TE according to DPPH method, respectively.

The ethanolic extracts of peppermint's agro-industrial waste exhibited noteworthy antioxidant activity. Considering the presented results, this high-value and low-cost material should be more extensively studied and encouraged for further exploitation in the food industry sector, thus contributing to waste reduction.

Keywords: agro-industrial waste, phenolic compounds, antioxidant activity.

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AVAILABLE APPROACHES FOR OVERCOMING LOW BIOAVAILABILITY OF RESVERATROL

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The lack of appropriate delivery systems of resveratrol (RSV), mostly due to its poor bioavailability, may be limiting factor for its broader clinical use and cause the discrepancy between the results obtained from experimental and clinical studies. However, over the years researchers have been trying different methods aiming to overcome its low water solubility: co-administration with inhibitors of glucuronidation, use of RSVs methylated analogs and designing the new delivery systems - using cyclodextrins (found in lemon juice) or endogenous proteins with high binding capacity to RSV: serum albumin, haemoglobin, fibrinogen or LDL cholesterol. Besides, many proteins used in the food industry may serve as RSV carriers: β-lactoglobulin (a major whey protein), whole buttermilk, α- casein and gliadin. Nowadays, however, nanotechnology-based RSV delivery systems which use various biocompatible materials, represent the most sophisticated method. Weather nutraceutical (biopolymer-based nanoparticles, emulsions) or parenteral (microbubbles, liposome) formulations, those delivery systems provide compelling alternatives for increasing RSV application potential and have the tenacity to evolve into widely applicable RSV carriers. Peanut oil, for example, is promising food-compatible way of RSV delivery but it seems that different liposomes: zwitterionic (made from saturated phosphatidyl-choline1, 2-dipalmitoyl-sn-glycero-3-phosphocholine and cholesterol) or cationic (made from soya lecithin, cholesterol and stearylamine) show precedence among other vehicles. RSV in cationic liposomes even have improved biological activity comparing to free RSV. In one study, liposomal RSV improved biochemical and histopathological alterations in doxorubicin-induced cardiomyopathy via upregulation of sarco/endoplasmic reticulum calcium ATPase2a expression. Ultra-flexible liposomes called transferosomes offer interesting delivery method that is suitable for transdermal application. Encapsulation of RSV using grape seed or orange oil, additionally, protects RSV against UV-light isomerization and degradation. It is very important to carefully monitor the dose of RSV since higher dose may cause adverse effects and lower dose may fail to exert any beneficial effect.

Keywords: bioavailability, resveratrol, delivery systems, liposomes, carriers.

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APPROACHES FOR POTENTIATING THE EFFECTS OF PIPERINE

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Piperine is an alkaloid found in several species of the Piperaceae family that are traditionally used as spices all around the world. Its nutraceutical properties have been investigated in recent years, and this compound has attracted scientific attention, as many researchers have shown its antioxidant, anti-inflammatory, anticancer, immunomodulatory, antibacterial, antifungal, antispasmodic, analgesic, with the number of confirmed actions constantly rising.

The absorption and metabolism of piperine have been studied. Compared to other nutraceuticals with low water solubility, studies report piperine reaching bioavailability as high as 97% in animal models. However, its low water solubility and strong pungency can present a limitation to reaching its full potential. Until recently, piperine has mostly been regarded as a bioavailability enhancer itself in combination with other bioactive, such as curcumin. But current research also deals with designing innovative delivery systems, as nanoparticles or other platforms and approaches in the formulation that may be valuable in potentiating the beneficial effects of piperine. Most approaches use nano- and micro-emulsification, polymer (protein- or polysaccharide-) based carriers, or complexation and conjugation with certain molecules as techniques generally employed for improvement of bioavailability of low-soluble bioactive. This work sums up the most employed approaches for potentiation of the effects of piperine. According to our research, piperine has been formulated and investigated after complexation with cyclodextrin, loaded into nanocapsules and liposomes, in solid dispersion, solid lipid nanoparticles, and microemulsion with self-emulsifying drug delivery system and even bile-salt based nanovesicles.

Although cyclodextrin complexation is the most used technique for improving the bioavailability of piperine to date, it is expected that forthcoming methods will be direct to various nanotechnology tools. In addition, it is expected that methods will be modeled according to the need of a specific target/site of action, thus enabling targeted delivery of this active substance.

Keywords: piperine, nutraceutical, nanoparticles, bioavailability

GREEN TECHNOLOGIES FOR THE EXTRACTION OF BIOACTIVE COMPOUNDS FROM *SATUREJA HORTENSIS*

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Satureja hortensis is a medicinal herb with a wide application in traditional medicine due to its numerous benefits such as antioxidant, antifungal, antibacterial, antinociceptive, and antiproliferative effects. In order to achieve maximal exploitation of the material and obtain high quality extracts, supercritical carbon dioxide ($ScCO_2$) was applied in this study to extract lipophilic components. Next, the exploited raw material was subjected to ultrasound-assisted extraction (UAE) to separate more polar components.

$ScCO_2$ under different pressures 100, 200, and 300 bar (40°C and 4h) was applied. For comparison, UAE (50% ethanol as solvent) was conducted on both the exploited and unexploited raw material and the impact of extraction time (20 and 40 min) and temperature (40 and 50°C) on the efficiency of the extraction of bioactives was investigated. The content of total phenols in the obtained extracts was investigated as well.

The yield of lipophilic components of the $ScCO_2$ extraction was in the range from 1.05 to 1.61% and the highest one was achieved at 200 bar pressure. Next, it was determined that the extracts obtained with UAE after $ScCO_2$ extraction were richer in phenols which is most likely the consequence of weakening of the cells' wall and easier release of phenols due to the exposure to CO_2 under high pressure. At constant time of 20 min, the higher temperature of 50°C was more adequate for phenols extraction, while at constant temperature of 40°C, longer exposure to the ultrasound (40 min) resulted in a higher yield of phenols as well. Additionally, the extract obtained from the material which was exposed to the lowest pressure was the richest in content of phenols with 1.059 mg GAE/mL of extract. It can be concluded that apart from isolating lipophilic components, the application of $ScCO_2$ significantly improves the separation of more polar components coupled with UAE which provides high-quality extracts and better exploitation of the *S. hortensis* material.

Keywords: Satureja hortensis,Ultrasound, Supercritical carbon dioxide

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IMMOBILISATION OF LACTOBACILLUS RHAMNOSUS ON INDUSTRIAL CARRIERS IN COMBINATION WITH CALCIUM ALGINATE FOR L-(+)-LACTIC ACID FERMENTATION

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Lactic acid (LA) is the most important hydrocarboxylic acid widely distributed in nature, that has numerous applications in variety of industries such as food, pharmaceutical, textile, leather, and chemical industries. LA bacteria represent one of the most promising microorganisms for application in extensive bioconversion of various substrates into value added products. Although LA production is majorly accomplished by batch fermentation using free cells, application of immobilized cells (biocatalyst) offers various advantages, such as biocatalyst (BC) reusability, higher fermentation rate, easier separation of microorganism from the fermented media, protective effect against substrate and product inhibition, and cheaper inoculum preparation. Sodium alginate (Na-alginate) is a natural biopolymer, composed of polysaccharide backbone with two repeating monosaccharide units (guluronic acid, and manuronic acid), with hydroxyl and carboxyl groups in its structure that enable further structure modifications.

In this study immobilisation of *Lactobacillus rhamnosus* ATCC 7469 on industrial carriers (ICs) without and with aid of calcium alginate (Ca-alginate) for application in lactic acid (LA) fermentation was investigated. Three types of IC were investigated, and possibility of biofilm formation was observed. Originally ICs were made for waste water treatment (at Faculty of Technology and Metallurgy) from polyethylene (PE) in the shape of ribbed sphere, round leaf (with cavities) and hollow gear. To further enhance cell attachment the IC were coated with Ca-alginate. Obtained IC and IC/Ca-alginate biocatalysts were applied in batch L-(+)-LA fermentations on MRS broth. The formation of biofilm on all ICs was observed and the highest viability of *L. rhamnosus* cells was 9.8 log CFU/g of the carrier. *L. rhamnosus* cells immobilised in ICs coated with Ca-alginate had even higher viability reaching values of 11.7 log CFU/g of the carrier. In LA fermentation, the highest LA yield and volumetric productivity of 97.4 % and 2.1 g/l h^{-1}, respectively, was achieved with ICs coated with Ca-alginate. Obtained results suggest the possibility of highly efficient LA fermentation by application of ICs without (preferred biofilm forming microorganisms) and with Ca-alginate coating.

Keywords: lactic acid fermentation; immobilisation; sodium alginate; industrial carriers; biofilm formation

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THE INFLUENCE OF TEMPERATURE AND AIR FLOW RATE ON DRYING KINETICS OF APPLE CHIPS

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A fruit snack is an important part of the daily diet, which can help consumers increase the demand for healthier food. Apple is an ideal fruit for snacks according to its high nutritional content. Dried apple slices have a good market potential as a raw material in many processed foods and can be an alternative to deep fat fried chips for health-conscious consumers. The purpose of this research was to study the effect of the temperature and air flow rate on convective drying of 1 mm apple slices. A laboratory-scale drying system was used as a conventional drying method which consisted of a drying chamber, air duct system with associated control elements and air heaters. Greeny Smith apple slices were dried in an experimental dryer at different air flow rates (3.5; 4 and 4.5 m/s) and different temperatures (60, 65 and 70°C). Prepared apple slices were spread in a single layer on the perforated stainless-steel tray. The samples were withdrawn for moisture content estimation at an interval of 5 min. When the mass of the samples no longer changed, drying was stopped. The results showed that the optimal parameters are a temperature of 65°C and air flow velocity of 4 m/s, at which the change in mass of the sample is most uniform and constant over time. The drying process itself is shorter compared to other examined parameters of the convective drying process. At the optimal temperature of 65°C the stationary drying time is established in 70 minutes. Stationary drying time for a temperature of 60°C is 75 minutes and for a temperature of 70°C, the time is 80 minutes. The "stationary drying time" is the time after which the changes in the mass of the sample are very small and the drying process is almost complete. At the optimal temperature of 65°C and air flow rate of 4 m/s, the stationary drying time is established in 80 minutes, for a flow rate of 3.5 m/s that time is 90 minutes, and for a flow rate of 4.5 m/s, the steady-state is established after 100 minutes of the drying process. Considering organoleptic quality the determined optimal parameters are suggested parameters for drying of apple slices. The results of experiments also showed that high-quality dried apple chips are provided due to less browning and a brighter appearance.

Keywords: Apple chips, conventional drying, temperature, air flow rate

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FOOD PRODUCTION, PROCESSING, SUSTAINABILITY, ADDED-VALUE FOOD

EFFECT OF USING NATURAL COLORANTS EXTRACTED FROM *ROSA RUBIGINOSA*AND *OPUNTIA STRICTA*FRUITS AND*HIBISCUS SABDARIFFA* FLOWERS ON THE QUALITY OF SALAMI

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The present study aims to evaluate the effect of the substitution of synthetic food dye by natural pigments extracted from *Rosa rubiginosa* and *Opuntiastricta* fruits and *Hibiscus sabdariffa* flowers on the salami quality during storage at 4°C.

The phenolic compounds content of the aqueous plant extracts was evaluated by Folin Ciocalteu, $AlCl_3$ and Vanillin methods for total polyphenols, flavonoids and tannins, respectively. The pigment contents were quantified by spectrophotometer and the antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and ferric reducing antioxidant power (FRAP) tests.The plant extracts were supplemented at two concentrations (0.5 and 2.5%) during salami production and a monitoring of physicochemical quality (pH, dry matter, color parameters, water holding capacity and lipid oxidation) were conducted during storage. The results revealed that the fruits of *R. rubiginosa* contained the highest amount of total polyphenols recording a value of 86.61 ± 9.08 mg GAE/g of extract. The carotenoids value in the *R.rubginosa* was 62.52 ± 2.79 mg/g DM, for betalains the value was 0.87 ±0.02 mg/g DM in *O. stricta* and the anthocyanins content was 10.95 ± 1.57 mg C_3GE/g DM in the *H. sabdariffa* flowers. For antioxidant activity, *R. rubiginosa* extract had registered the best capacities for both tests. The results of salami quality showed that the plant extracts improved its physicochemical quality in comparison with control. The impact of plant extracts addition on the salami color, showed that the red index is higher in salami with 2.5% supplementation compared to 0.5% (p<0.05). The addition of *O. stricta* extract showed better effect than the other two extracts, before cooking, giving an appreciable color. However the hibiscus-based treatment showed the highest intensity of red index after cooking. The plant extracts succeeded in maintaining an antioxidant activity in salami samples even for the 0.5% concentration. The use of natural colorants can be successfully applied during the production of safety foods.

Keywords: food additives, natural colorants, antioxidant activity, salami quality.

EXPRESSION OF AMYLASES IN ADULT HONEY BEES FED WITH DIFFERENT PATTIES

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Fed of honey bee (*Apis mellifera*) is challenging for beekeepers and formulation of supplemental food is improved continuously. When natural food sources are scarce or not available, supplemental foods are widely used to support and build up honey bee colonies. Influence of commercial patty and patty enriched with 12.5% pollen on amylase expression in honey bee adults is presented. This is part of a wider study aimed to compare the activity of digestive enzymes when using different patties. We assume that data collected in this way can be used for the development of better food supplements for honey bees.

Honey bees were kept in an incubator for 21 days, at a temperature of 35°C and at 80% humidity. In each cage, there were one hundred bees and a piece of honeycomb. Midgut and hindgut samples were taken after 7, 14 and 21 days and midgut without hindgut was taken after 21 days. Samples were homogenized and used for amylase zymogram, IEF and enzyme assays.

There was no mortality during the experiment. A high protein concentration was detected in the midgut in both groups of bees. Amylase activity was significantly higher in bees fed pollen enriched patties, which is shown by enzyme assay and by zymograms. There are different amylase isoforms present in bees fed by pollen enriched patties in comparison to bees fed by commercial patties, but the major isoforms were the same.

The observed decrease in the amylase activity over time is probably due to dilution caused by the accumulation of water and undigested substances in the hindgut. Reduced amylase activity in the intestines of bees fed by commercial patties is due to a lack of starch or some other inducers present in pollen.

Because pollen is honey bee natural food, we conclude that food supplements that induce similar enzyme expression as pollen can be superior in comparison to supplements that induce very different enzyme expression.

Keywords: amylase, honey bee, nutrition.

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INFLUENCE OF EXTRACTION PARAMETERS ON SOME QUALITY CHARACTERISTICS OF TANNIN EXTRACTS FROM ACORN KERNEL

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Acorn (*Quercus ithaburensis* subsp. *macrolepis*) is the fruit of the oak tree and natural spreading area of acorn oak is Eastern Mediterranean countries. Acorn is rich in tannin which is one of the most important forest by-products exported by Turkey. After removal of the tannin by extraction, the acorns are discarded or used as animal feed. However, acorn flour can be used as a substitute for wheat flour in a wide variety of applications in gluten-free food production of cakes, cookies, pasta, and thickener. In the present study, the removal of tannin from shredded acorn kernel to a certain concentration (0.03 mg tannin/mg of dry matter) by aqueous extraction with different acorn/water ratios (1:2 and 1:20 w/v) at different temperatures (20-40-60°C) was investigated. The effects of extraction process parameters on pH, brix, turbidity, and tannin content were determined. It was determined that acorn/water ratio did not have significant effect on pH value ($p > 0.05$) however, higher brix and turbidity values were obtained by 1:2 w/v ratio ($p < 0.05$). Also, it was revealed that the acorn/water ratio did not have a significant effect on the tannin removal rate from acorns ($p > 0.05$). The highest turbidity was obtained for the extraction temperature at 60 °C ($p < 0.05$). While all extraction temperatures had similar effects on pH, the lowest brix value was obtained for 20 °C ($p < 0.05$). In addition, it was determined that the target concentration value was reached in 8.0±0.4 h at 60 °C, 10.5±0.5 h at 40 °C and 16.0±0.8 h at 20 °C. The results of the present study will give valuable information for the efficient removal of tannin from acorn being used in novel gluten-free products.

Keywords: Acorn, extraction, tannin

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