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FACULTY OF BIOTECHNOLOGY AND FOOD SCIENCES

SLOVAK UNIVERSITY OF AGRICULTURE IN NITRA

BOOK OF REVIEWED ABSTRACTS

from

the 15th International Scientific Conference

organized by the Faculty of Biotechnology and Food Sciences

of the Slovak University of Agriculture in Nitra

FOOD|BIO|TECH

AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Slovak Republic,
October 5th – 6th 2022

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Keynote talks abstracts

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KEYNOTE TALK 1

Production strategy to obtain insect protein-polyphenol ingredients by spray drying encapsulation

Edilene Silva[1], Karishma Ravichandran[2], Penelope Perkins-Veazie[1], Michael Greenlief[4], Andrew Thomas[5], Kiruba Krishnaswamy[2,3], Mary Ann Lila[1], Roberta Hoskin[1], Marvin Moncada[1]

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Wednesday, 5th October 2022, 9:15

Lecturer: Marvin Moncada

North Carolina State University
Kannapolis, NC, USA

Elderberries have long been used in folk medicine to treat several diseases and flu symptoms. However, different from the European variety, the American elderberry has not been fully explored yet regarding its technological uses and applications. As a contribution to renew the interest in cultivation and increase the commercial expression of the American elderberry, our study investigated the best condition for spray drying encapsulation of elderberry pomace phytochemicals using two different carriers commonly used in the food industry: soy protein isolate (SPI) and tapioca starch (TS) in three temperatures (100C, 120C and 140C). Concentrated elderberry pomace polyphenol extract was prepared and mixed with 8% (w/v) of SPI or TS. Groups showed similar results ($p > 0.05$), but a tendency to a) increased solids recovery, phenolic retention and total phenolic content as SD temperature increased and b) better performance with tapioca as a carrier was observed. The powders have attractive reddish color and the process proved to be efficient as solids recovery were above 50%. We showed that American elderberry pomace polyphenols can be efficiently microencapsulated with both TS and SPI to produce food ingredients with multiple applications. Powders will be further analyzed for their phytochemical content and physicochemical attributes.

Keywords: elderberry, food ingredients, waste revalorization

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KEYNOTE TALK 2

Sensory evaluation of foods with geographical indication

Luis Noguera-Artiaga, Hanán Issa-Issa, Ángel A. Carbonell-Barrachina

Wednesday, 5th October 2022, 9:45

Lecturer: Ángel Carbonell-Barrachina

Miquel Hernandez University of Elche
Orihuela, Alicante, Spain

For Mediterranean people, food is much more than nutritional products, it is a culture; in fact, much of a country/region can be guessed by looking at the food they produced and consumed. The policy of the European Union aims at protecting the names of specific products to promote their unique characteristics, which are linked to their geographical origin, as well as to their traditional knowledge. These products are awarded a quality distinction called "geographical indication, GI", which may be Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) or Traditional Specialty Guaranteed (TSG). These voluntary certification schemes help consumers in getting confidence in the quality of the products they select. The organisms that certify that these products have a differentiated character must establish which are the characteristics that give distinction to the product, such as its location, production technique, use of select varieties and/or its distinguished organoleptic properties. The information related to these processes is collected in a single legal document approved by the European Union, that is public and can be consulted in the eAmbrosia database. It is the function of each IGs to demonstrate that the products meet the established requirements, in addition to validating the method used to do so. In the case of GIs that indicate differentiated organoleptic characteristics, it is necessary to analyze the products using descriptive sensory techniques. But regulatory boards, and more specifically their trained sensory panels, need to be reviewed to make sure they are properly certifying their products. The training and operation of sensory analysis panels is regulated under ISO 17065 (requirements for bodies that certify products, processes and services) and ISO 17025 (requirements for the competence of testing and calibration laboratories) and, in each country, there are control agencies that ensure its compliance and correct operation. At EU level, the European Accreditation Agencies (EAs) operate to ensure that accredited bodies offering testing, examination, calibration, certification, inspection and verification services have the technical competence and impartiality to check the conformity of products and services with the relevant rules and regulations. Details on the operation of these control mechanisms will be presented and case studies of sensory panels evaluating the quality of these products will be shown and discussed. Special attention will be paid to the main problems faced by these panels and the solutions they are taking.

Keywords: designation of origin, geographical protected designation, panel accreditation, traditional foods.

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KEYNOTE TALK 3

Beef production from farm to fork: Environmental and health issues

Giuseppe Maiorano, Siria Tavaniello

Wednesday, 5th October 2022, 11:00

Lecturer: Giuseppe Maiorano

University of Molise
Campobasso, Italy

The global average per capita consumption of meat and the total amount of meat consumed are rising, driven by population growth and rising average individual incomes, as well as by lifestyle changes in some areas of the world. Nevertheless, a growing debate among consumers on red meat is ongoing, as it is commonly considered unhealthy being associated with health risk. Moreover, beef meat is currently under pressure since cattle are considered as the food animals with the greatest environmental impacts as compared to monogastric animals or even more to plant-based proteins. The environmental impacts in terms of carbon, water, soil and biodiversity footprints are highly contextual, and their estimation is often erroneous due to a reductionist use of metrics. The aim of this lecture is to argue the environmental and health issues related to beef production with particular reference to pasture systems. Grassland-based systems can optimize nutrient and energy cycles, while encouraging the use of genotypes that are adapted to low-input environments. Furthermore, well-managed pasture systems can contribute to ecosystem management making use of marginal land with several "ecosystem services", which result in increased ecosystem resilience and economic stability. In fact, in many areas of the world, beef production is economically and socially relevant because it accounts for a significant portion of the agricultural production and represents a vital economic activity in mountain and hill districts of many regions, where few alternatives for other agricultural production exist. As for the nutritional quality of animal products, it is known that meat from ruminants raised on grass has a better n-6 to n-3 fatty acid ratio, a higher concentration of conjugated linoleic acids (CLAs) and higher levels of antioxidants (e.g. Vitamin E) than that of concentrate-fed animals. In particular, Vitamin E is capable of increasing the shelf life of meat and may have a positive effect on preventing the formation of carcinogenic substances during the cooking process (in relationship of cooking method, time and temperature), such as heterocyclic amines and polycyclic aromatic hydrocarbons dangerous for human health. Our recent in vitro studies seem to confirm this hypothesis. Finally, it is right to emphasize that, in spite of the cyclical fears of the mass media about meat, it will always have a prominent role in our diet because we are omnivores. From our point of view, there are several questions to ask whose answers can encourage to identify substantial margin for improvement to reduce the environmental impact of beef production. Among these, one seems very relevant to us: are reduced-meat diets or lab-grown meat better for the environment and human health?

Keywords: beef production, pasture, environmental impact, meat quality, cancerogenic substances

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KEYNOTE TALK 4

Natural food preservatives: A realistic replacement?

Wednesday, 5th October 2022, 11:30

Lecturer: Małgorzata Dżugan

University of Rzeszów
Rzeszów, Poland

The increasing use of chemicals in food products which results in excessive intake of artificial preservatives has caused a negative effect on consumers' health including allergies, asthma, or even cancer. As consumer demand for clean label ingredients continues to trend, natural preservatives are coming into the spotlight. However, their availability can pose a challenge and their application also increases the price of the end product. Still many natural preparations are under testing to check their effects and efficiency as preservatives. Such substances are extracted directly from nature or obtained in biotechnological processes. They can be simple compounds, such as salt, or a blend of many different chemical components, such as rosemary extract. Among them, propolis, a valuable bee product with high antioxidant and antimicrobial properties, has been extensively studied. However, the factor limiting its use in food is its intense resinous smell which reduces the consumer's acceptability of the preserved food. An innovative method of producing a bio-preservative with limited fragrance characteristics based on raw propolis has been developed in Poland (Patent application P.441843). The research showed the effectiveness of both produced propolis preparations: liquid extract and its spray-dried microencapsulates, in extending the shelf life of selected food products without adversely affecting their organoleptic characteristics. The application of the developed bio-preservative in food technology requires further study.

Keywords: food, clean label, natural preservatives, propolis

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KEYNOTE TALK 5

Alternative food sources - new challenges for future

Eva Ivanišová, Judita Lidiková, Adriana Kolesárová

Wednesday, 5th October, 2022, 12:00

Lecturer: Eva Ivanišová

Slovak University of Agriculture in Nitra
Nitra, Slovak Republic

Food and agricultural systems are affected by trends that could jeopardize their future sustainability. Population and income growth drive the demand for food and bring about changes in people's dietary preferences. Edible insects as an alternative protein source for human food and animal feed are interesting in terms of low greenhouse gas emissions, high feed conversion efficiency, low land use, and their ability to transform low value organic side streams into high value protein products. More than 2000 insect species are eaten mainly in tropical regions. Entomophagy has several advantages. First of all insects are a good source of protein, essential fats, and antioxidant peptides. Many insects are rich in microelements such as iron, calcium, and zinc and in vitamins. Secondly, insect breeding is environmentally friendly. Insects emit significantly fewer greenhouse gases and ammonia than most livestock. Moreover, insects require less space, feed, and water for breeding than livestock. Economic factors are also important. Insect rearing can be low-tech or very sophisticated, depending on the level of investment. For these three main reasons, insects have been highlighted as an important food source in response to the growing concerns about the future of world food security.

The consumption of flowers in ancient time is known, on one hand, for being a part of traditional culinary practices, while being also used in the field of alternative medicines. Edible flowers have become a culinary trend. Edible flowers possess nutritional value – being rich in moisture, carbohydrates, and protein, and being low in lipids. They also contain interesting amounts of ash, including dietary minerals such as calcium, iron, potassium, magnesium, phosphorous or zinc. Furthermore, they contain bioactive components, such as phenolic compounds, which contribute to their high antioxidant activity, while also conferring color and aroma.

The increasing human population and nutritional demands has meant that the food industry has tended to look for alternative sources of food production. To this end various studies have been performed in the field of replacement and alternative foods. Replacement foods are based on the substitution of one constituent with another, and these should have the same or similar attributes to ensure optimal organoleptic, microbial, and functional performance.

Keywords: edible insects, edible flowers, perspectives, food industry, innovative foods

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KEYNOTE TALK 6

Three-dimensional cell culture systems

Wednesday, 5th October 2022, 13:30

Lecturer: Petr Sláma

Mendel University in Brno
Brno, Czech Republic

Three-dimensional (3D) cell culture systems are developed for better mimicking of real conditions in animal and human body. In contrary to that, two-dimensional (2D) culture systems create lot of artificial results that are not applicable in *in vitro* conditions. Cells cultured in 2D systems have different shape comparing to normal cells in tissues and these cells are not able to present normal physiological properties. Cells cultured in 3D systems have morphological and physiological conditions similar as in *in vivo* environment. Growing demands on the applicability of *in vitro* experiments force us to develop more perfect 3D systems for cell cultivations. These 3D cultivations include using of nanofiber scaffolds, hydrogels, organoid and organ-on-a-chip. Polycaprolactone nanofiber scaffolds are used for cultivation of immune cells as neutrophils, monocytes, macrophages and dendritic cells. Those nanofiber scaffolds seem to be very good system for studying of phagocytosis and chemotaxis of mentioned cells. In our experiments, we noticed that polycaprolactone nanofiber scaffolds also provide very good conditions for development of dendritic cells from monocytes isolated from peripheral blood.

Keywords: 3D, cell culture, nanofiber, polycaprolactone, dendritic cell

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KEYNOTE TALK 7

Research for the industry – question of expertise, trust and money

Thursday, 6th October, 2022, 9:00

Lecturer: Martin Polovka

National Agricultural and Food Centre
Nitra, Slovak Republic

In the contribution, the concept and unique capacities of the National Agricultural and Food Centre for the cooperation with food industry, but also small food producers on the topics of food safety, innovations and knowledge transfer will be presented.

In the premises in Modra and Lehnice, the outstanding semi-production scale modular technological units are in use, enabling the modelling and test the conditions for innovative food products development, isolation of valuable components from plant residuals, but also food waste processing, energy saving optimisation and many others.

Practical examples of the outputs, many of which found also commercial applications and even awards, will also be demonstrated, together with the current research and knowledge transfer activities. Some of them are connected with recent post-covid situation – they concern the aspects of food security of the population in case of any crisis-induced restrictions and regulations in free food market. Even the approach in covid-syndrome eliminations via novel foods development with specific treatment properties.

The cooperation of research institution like the National Agricultural and Food with the industry is based on couple of pillars. First of all – the reputation, accompanied by the previous experiences, expertise and willingness to find a solution of the problem. Then, by the trust –that the research can find an effective solution of the problem, ideally in real time and with realistic impact and expenses. And – last but not least – it is also the question of money – ideally grands and funds, that allow further development of the suggested ideas or proposed solutions.

Therefore, also the problematic aspects of such cooperation and the potential for its further development will be presented.

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KEYNOTE TALK 8

Spray drying microencapsulation of American elderberry (Sambucus canadensis L.) pomace polyphenols to produce phytochemical-rich ingredients

Edilene Souza da Silva[1], Veronika Valkova[2], Mary Ann Lila[1], Marvin Moncada[1], Roberta Targino Hoskin[1]

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[2]Slovak University of Agriculture in Nitra, Slovak Republic

Thursday, 6th October 2022, 9:30

Lecturer: Roberta Targino Hoskin

North Carolina State University
Kannapolis, NC, USA

Food availability is expected to become critical due to the rapid expansion of human population and less availability of land to grow food. Edible insect protein is an alternative source of high-quality protein with desirable aminoacid profile and health-relevant micro and macronutrients. However, it is still underexplored for human food applications due to technological challenges that jeopardize its practical use. This study investigated the use of insect protein alone or blended with popular proteins in the food industry to encapsulate phenolic compounds from muscadine grape pomace and rosemary leaves. Our goals were to a) establish protocols to complex insect protein and polyphenol extracts and b) determine process efficiency parameters (solids recovery and phenolic retention) to establish the best spray drying conditions to produce encapsulated insect protein-polyphenol ingredients. Our results established a straightforward production protocol for the encapsulation of plant polyphenols with insect protein. Overall, protein blends had higher solids recovery and phenolic retention compared to insect protein alone. The insect protein/pea protein blend showed the best efficiency parameters for both extracts. Our findings contribute to establish efficient production routes using insect protein for food applications. Further analyses regarding functionality and sensory attributes of insect protein-polyphenols particles are in progress.

Keywords: alternative protein, polyphenols, phytochemicals, food ingredients

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Conference abstracts

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THE EFFECT OF BLACKCURRANT (*RIBES NIGRUM*) AND CHOKEBERRY (*ARONIA MELANOCARPA*) ON OVARIAN CELL FUNCTIONS AND SECRETORY ACTIVITY OF HUMAN OVARIAN CELLS HGL5 *IN VITRO*

Simona Baldovska[1], Ladislav Kohut[2], Michal Mihal[2], Oleg Paulen[3], Ales Pavlik[4], Petr Slama[4], Adriana Kolesarova[2]

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Polyphenols and anthocyanins have recently gained increasing research interest due to their therapeutical and pharmacological effects, as well as their possible use for the benefit of human health. According to the structural differences and anthocyanin content of berries such as blackcurrants, chokeberry, and others, there are different biological properties, as well as antioxidant, antimicrobial and stimulatory activities. In this *in vitro* study, the influence of blackcurrant extract (*Ribes nigrum* L.) and chokeberry extract (*Aronia melanocarpa* L.) at the concentrations of 10, 20, 50, and 100 µg/mL (24 h) on the cell viability and the release of progesterone and 17β-estradiol by human ovarian granulosa cell line HGL5 was evaluated. All parameters were investigated spectrophotometrically, the viability was measured by AlamarBlue™ cell viability assay and the release of steroid hormones was assayed by Enzyme-linked immuno sorbent assay (ELISA). Our results showed that the number of viable cells was significantly ($P \leq 0.001$) increased at the concentration of 20 µg/mL of blackcurrant extract and at all used concentrations (10, 20, 50, and 100 µg/mL) of chokeberry extract. Blackcurrant extract failed to affect progesterone release, but slightly ($P \geq 0.05$) stimulated 17β-estradiol release by the cells compared to the control. In addition, a significant ($P \leq 0.05$) stimulatory effect of chokeberry extract on 17β-estradiol release at the concentration of 10 µg/mL was observed, but progesterone release was not significantly ($P \geq 0.05$) affected by chokeberry extract. In conclusion, our study suggests the action of both, blackcurrant and chokeberry extract and their bioactive phytochemicals on human ovarian cell functions including viability and secretory activity, however, understanding the mechanisms of action and effects on ovarian functions, along with their functional interrelationships, requires further elucidation.

Keywords: blackcurrant, chokeberry, ovarian cells, viability, steroidogenesis

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IN VITRO EFFECTS OF ELLAGIC ACID ON HUMAN OVARIAN GRANULOSA CELLS

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Ellagitannins are one of the most common plant polyphenols, which are present in common fruits, nuts, seeds, and some mushrooms. Recently, there has been an attracted interest in ellagitannins such as ellagic acid because of their potent antioxidant abilities, which are connected to their anticancer, immunomodulatory, and antiviral activity. However, it is important to note that ellagitannins have low bioavailability. This assumption is based on several facts: ellagitannins have a specific structure (large size), and ellagitannins can bind some salivary proteins which causes the inability to be further metabolized. Also, it could be due to its low water solubility and the ability of ellagitannins to bind irreversibly to cellular DNA and proteins. The aim of the study was to study the *in vitro* effect of ellagic acid at the concentrations of 1, 5, 10, 25, and 50 μM/mL for 24 hours on human ovarian granulosa cell line HGL5 and human ovarian granulosa tumour cells COV434. Cell viability, the secretion of transforming growth factor β2 (TGF-β2), as well as the presence of its receptor TGF-β2R were evaluated. AlamarBlue™ assay was performed to determine cell viability and the secretion of TGF-β2 and TGF-β2R was assayed by the ELISA method. The results from our *in vitro* study showed that ellagic acid had no effect on the viability of healthy HGL5 cells but significantly reduced the viability of tumour cells COV434 ($P \leq 0.0001$) at concentrations of 25 and 50 μM/mL. Similarly, ellagic acid did not affect levels of the TGF-β2 and TGF-β2R in HGL5 cells. But in the case of tumour cells COV434 after ellagic acid treatment, a significant decrease in TGF-β2 secretion at the concentrations of 5 ($P \leq 0.05$), 10 ($P \leq 0.01$), 25 ($P \leq 0.05$), and 50 μM/mL ($P \leq 0.001$) was observed. On the other hand, this ellagitannin did not affect the presence of the receptor TGF-β2R in tumour cells COV434. Our results showed a dose-dependent modulatory effect of ellagic acid on the viability and the mechanism of intracellular communication mediated by the TGF-β signaling pathway in ovarian tumor cells, suggesting their promising antitumor potential without adversely affecting the healthy ovarian cells. However, further research is needed to confirm the hypothesis and ascertain the effect's action.

Keywords: ellagitannins, ovarian cells, viability, cancer

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EPICATECHIN IMPROVES FROZEN SPERM VITALITY BY ITS ANTIOXIDANT AND CRYOPROTECTIVE ACTIONS

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This study served to elucidate the effects of three selected doses of epicatechin (EPI; 25, 50 or 100 µmol/L) on the motion and oxidative profile of bovine spermatozoa subjected to the process of cryopreservation. Furthermore, we focused on the impact of EPI on the expression patterns of heat shock proteins 90 and 70 as well as the BAX and Bcl-2 proteins involved in the process of apoptosis. The experimental samples were compared with a native control as well as specimens cryopreserved in the absence of any supplement. Our dada indicates that particularly 50 and 100 µmol/L EPI assured a higher preservation of the sperm motility ($p<0.001$) in comparison with the cryopreserved control. The best protection of the sperm DNA ($p<0.05$), lipids ($p<0.05$) and proteins ($p<0.01$) were observed in the samples exposed to 100 µmol/L EPI in comparison to untreated frozen samples. The presence of higher EPI concentrations in the cryopreservation medium led to significant stabilization of the heat shock protein 90 ($p<0.01$ in case of 50 µmol/L EPI; $p<0.001$ with respect to 100 µmol/L EPI) as well as a higher maintenance of the BAX:Bcl-2 ratio ($p<0.001$) when compared to the untreated frozen control. In summary, we may assume that EPI exhibits antioxidant properties which enable the molecule from excessive damage to biomolecules essential for the cell survival and protection under low temperatures, all of which may be translated into a higher post-thaw sperm motility and activity.

Keywords: epicatechin, cryopreservation, spermatozoa, oxidative damage, protein expression, heat shock proteins, apoptosis

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OCHRATOXIN A PRODUCTION BY *ASPERGILLUS WESTERDIJKIAE* ISOLATED FROM GRAPE BERRIES OF SLOVAK ORIGIN

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Ochratoxin A (OTA) is one of the most important mycotoxins produced by toxigenic species of *Aspergillus* and *Penicillium* fungi. The aim of the study was to obtain information on OTA production by *Aspergillus westerdijkiae* strains in different cultivation media and cultivation conditions. Two strains of *A. westerdijkiae* (KMi9, KMi12), isolated from grape berries of Slovak origin, were used in this study. High-performance liquid chromatography (HPLC) coupled with a DAD detector was used for confirmation of OTA production by *A. westerdijkiae* strains. As a cultivation media were used MEA (malt extract agar), CYA (Czapek yeast extract agar), PDA (potato dextrose agar), and YES (yeast extract sucrose agar). Production of OTA was tested on 6th, 10th, 14th, 21st and 30th day of cultivation at 18±1°C, room temperature (23±1°C), 25±1°C and 30±1°C. Based on the results from this study, YES medium and 10 days of cultivation were found to be the best for the OTA production. The production maximum achieved by *A. westerdijkiae* strain KMi9 was at 18±1°C (498.74 μg.g^{-1}) and strain KMi12 had the highest OTA production at 25±1°C (574.04 μg.g^{-1}). The OTA production was significantly affected by the used cultivation media and cultivation conditions that were dependent on the requirements of individual strains.

Keywords: ochratoxin A, HPLC, *Aspergillus westerdijkiae*

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EFFECT OF BISPHENOL S ON VIABILITY PARAMETER AND IL-6 PRODUCTION OF HUVEC CELLS

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Bisphenol S (BPS) is endocrine disrupting chemical and represents one of the most prevalent phenols in the environment. Due to its huge production and extensive use, BPS has been continually released and widely dispersed in the natural environment. This ubiquitous chemical compound has been proven to be a safer alternative to bisphenol A in industrial applications. Recently, the global spread of BPS has become an important issue, while research on the toxicity and immunomodulatory mechanisms of BPS remains uncertain. Because we are constantly exposed to these compounds, particularly from birth and in the context of inflammation, the bisphenols' effects on inflammatory processes constitute a formidable task for establishing new standards for their use. The aim of this study was to evaluate effects of bisphenol S on viability parameter and IL-6 production of human umbilical vein endothelial cells (HUVEC). HUVEC cells were cultivated with various concentrations of BPS (0.05–50 μM) for 24 hours and compared to the untreated control group. After 24 hours of incubation, we conducted a metabolic activity assay (MTT), and we also measured IL-6 production by using the ELISA method. Concentrations of implemented BPS treatments were 0.05; 0.1; 1; 10; 25; 50 μM. For the results, MTT assay showed no significant fluctuations of metabolic activity between treated groups. Production of IL-6 displayed significantly raised values in the concentration of 0.1 μM ($P \leq 0.01$), 1 μM ($P \leq 0.001$), 10 μM ($P \leq 0.001$) and 25 μM ($P \leq 0.01$). The submitted study's conclusion established that BPS did not exhibit cytotoxicity at the evaluated concentrations, but on the other hand, we confirmed the production of the pro-inflammatory interleukin IL-6 and thus the pro-inflammatory effect of BPS on endothelial cells. Further analyses are needed for better understanding of interactions between BPS and inflammatory processes.

Keywords: Bisphenol S, Inflammation, Viability, HUVEC

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CRYOCAPACITATION AND ITS ASSOCIATION WITH OXIDATIVE FEATURES IN CRYOPRESERVED BOVINE SPERMATOZOA

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The goal of our research was to evaluate the impact of cryopreservation on the antioxidant activity, lipid peroxidation and protein carbonylation as well as its connection with the progress of cryocapacitation in bovine spermatozoa. As biological material we used semen obtained from 20 sexually mature Holstein bulls. Each ejaculate was divided into three fractions as follows: the first fraction or control (CTRL) was incubated in physiological saline solution, the second was capacitated fraction (CAP) was incubated in a capacitation medium at specific conditions. The third fraction of each sample was cryopreserved (CRYO) and stored in liquid nitrogen at -196°C for further analysis. The motility of spermatozoa was assessed with CASA (computer assisted sperm analysis), while the capacitation status was evaluated by the chlortetracycline (CTC) fluorescent staining. Total antioxidant capacity (TAC) and the level of lipid peroxidation (LPO) was measured with a combined spectro-fluoro-luminometer. The presence of protein oxidation was detected by the traditional DNPH (2,4-dinitrophenylhydrazine) method and evaluated spectrophotometrically. Based on our data, the motility was significantly decreased ($P<0.0001$; $P<0.01$) in the CRYO group against CAP and CTRL. There was a significant increase ($P<0.01$; $P<0.05$) of acrosome-reacted spermatozoa (AR-pattern) in the CRYO group when compared to CTRL and CAP, while TAC was statistically decreased ($P<0.05$) between the CRYO and CAP experimental group. In the case of protein oxidation and LPO, both parameters were significantly higher ($P<0.0001$) in the CRYO group when compared to CAP or CTRL. In summary, cryopreservation induces capacitation-like changes and promotes oxidative damage in frozen-thawed bovine spermatozoa.

Keywords: cryocapacitation, bull, spermatozoa, antioxidant activity, lipid peroxidation, protein oxidation

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DEVELOPMENT OF LONG-LIFE PASTRY FOR SPECIFIC PURPOSES

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Currently, a great variety of bakery products are marketed, from consumer bread types made from traditional raw materials to specific bakery products containing various protective components, which increase their nutritional value and are a source of biologically active substances. This is a prospective way into the future, with the aim of satisfying the demanding needs of modern consumers. However, the production of products for consumers with specific nutritional requirements is a serious problem. Celiac disease is chronic inflammatory autoimmune disease of gastrointestinal tract caused by intolerance to certain prolamin protein fractions, mostly affecting mucosa of proximal parts of small intestine, which belongs to diseases requiring consumption of food with specific composition. The only effective treatment of celiac disease is "gluten-free" diet (diet free of celiac active protein components of certain cereal crops). Other kinds of intolerance are often associated with celiac disease, such as lactose intolerance, alternatively other metabolic disorders, mainly diabetes mellitus. In lactose intolerance, it is necessary to exclude milk and dairy products containing lactose from diet; in diabetes mellitus it is necessary to follow a diet based on controlled or decreased intake of saccharides. Products that would be suitable for such "combined" consumers are not common on the market, and due to the specific composition, their technological and sensory quality is a problem. The output of the research that took place in the past years (2013-2020) was the filing of patent application no. 125-2017, and the decision of the Slovak Industrial Property Office granted patent no. 288733. Subsequently, European patent application no. 18209743.6 and the European patent EP3491925 "Long life pastry for specific nutritional purposes and method of its production" was granted. As part of the patent, optimal combinations of naturally gluten-free raw materials are designed, which ensure the required energy value of the food, but also the supply of biologically significant components, e.g. fibre, protein and mineral substances, or substances with antioxidant potential. The goal was to provide long-life pastry goods primarily intended for those consumers who suffer from several disorders at the same time, and is therefore suitable for celiacs, or for celiacs with lactose intolerance, or also with diabetes. Another advantage is that the addition of eggs, which are also among the substances causing allergies or intolerance, was excluded from the recipes. From a technological point of view, the manufacturing method is described in the patent, starting with a variable recipe and ending with individual steps, while the sensory acceptability of the final products was taken into account. The philosophy of the products (it is not a single recipe) is based on the creativity of possibilities and settings of different sensory and nutritional profiles. Research on the development of designed foods with the aim of making them suitable for consumers with other diagnoses, e.g. HIT (histamine intolerance) or IBS (irritable bowel syndrome) continues, as does the work on nutritional reformulation of cereal durable products. Other naturally gluten-free raw materials, especially marginal, forgotten, or non-traditional ones, were verified, and the possibilities of their further food use and technological and sensory properties were described. It can be concluded that by applying such raw materials (pseudocereals, minor cereals, modified berries with a high anthocyanin content, medicinal and spice plants) to foods, it is possible to significantly increase their nutritional parameters and suitability for consumers.

Keywords: specific nutritional requirements, gluten-free, marginal, forgotten, non-traditional raw materials, consumers with specific nutritional requirements

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PHYSIOLOGICAL ASPECTS AND ECONOMICAL APPROACH OF CONTAMINATION BY MYCOTOXINS IN FOOD

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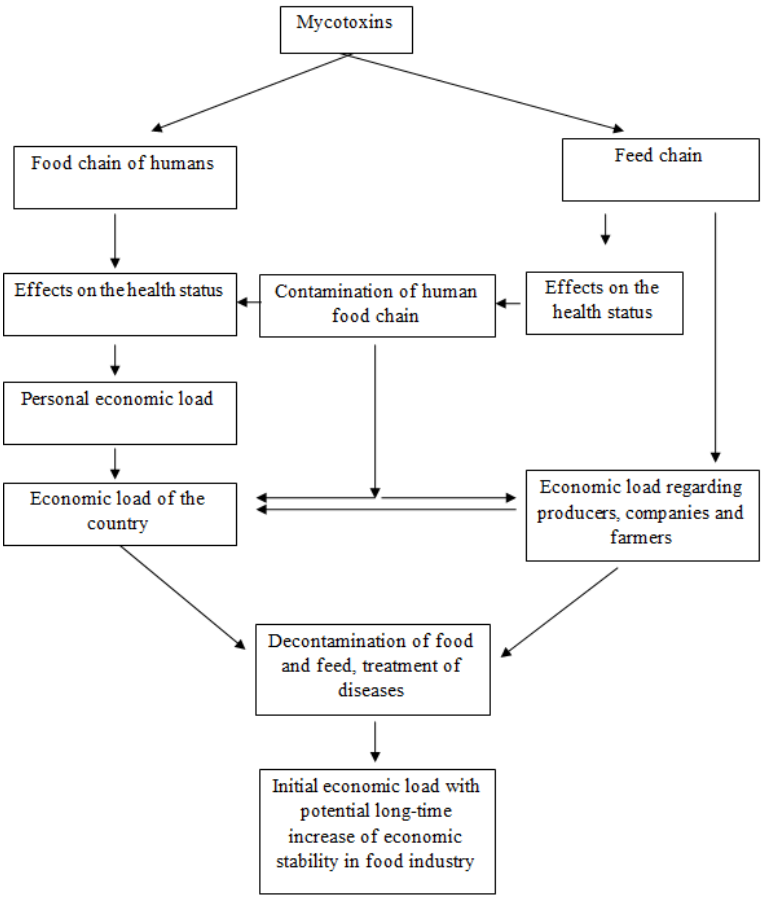
The contamination of food and feed by toxic metabolites of fungi is not risk only for consumers resulting in various embarrassment regarding health status and well-being, but also for producers, companies and export market on the ground of economic losses and ruined stability of economic trade. What is more, the detection of mycotoxins is not easy and many of them can remain hidden. Chronical intake of low amount of mycotoxins can break out into illness that has not to be inevitable attributed to specific mycotoxins. Manifestation of various symptoms is not considered as consequence of mycotoxin contaminations but as another similar disease or disorders. Thus, therapy is ineffective and the hidden intake of mycotoxins hold over what sometimes leads to insoluble situations. Ultimately, the existence of mycotoxicosis may be more common that suspected. The attention and controlling, monitoring and methods of their occurrence in foodstuffs authorised by state surveillance agencies have to be systematic. The cycles of mycotoxin occurrence in food chain and consequent economic impacts is given:



Keywords: Mycotoxins, food, contamination, economical approach

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MERCURY CONTENT AND RISK ASSESSMENT OF THE WILD EDIBLE *LECCINUM* BOLETES COLLECTED IN DIFFERENT LOCALITIES IN SLOVAKIA

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Boletes are consumed as a delicacy, mainly for their unique texture and aroma. However, they are known to accumulate mercury and other heavy metals, which can represent a significant risk to the health of consumers. Mercury is considered the most toxic heavy metal in the environment. It has the potential to damage almost all organs of the human body. Considering the position of boletes in the human food chain, it is necessary to monitor the content of heavy metals in order to protect and preserve human health. The samples of 4 boletes from the genus *Leccinum* ((*Leccinum scabrum* (Bull.) Gray, *Leccinum pseudoscabrum* (Kallenb.) Šutara, *Leccinum albostipitatum* den Bakker & Noordel and *Leccinum duriusculum* (Schulzer ex Kalchbr.) Singer), collected from 6 forested areas of Slovakia in 2020 (Hliník and Hronom, Kurima – Taraš, Mníšek and Popradom, Snina – Štefekovo, Čačín – Jelšovec, Žákylské pleso) were analyzed in this study. Total mercury content was determined by AMA 254 cold-vapor AAS analyzer. Mercury content in analyzed soil samples ranged from 0.07 to 0.18 mg.kg^{-1} DM. The limit value for Hg in soil (0.50 mg.kg^{-1}) was not exceeded in any sample. The contamination factor (C_f) ranged from 0.23 to 0.60, the index of geoaccumulation (I_{geo}) ranged from -2.68 to -1.32, and the potential ecological risk factor (E_r) ranged from 9.33 to 24.00. Based on the environmental risk assessment, all soils could be classified as low contaminated and low risk regarding the Hg content. Mercury content in analyzed *Leccinum* cap samples ranged from 0.41 to 7.52 mg.kg^{-1} DM. Mercury content in analyzed *Leccinum* stipe samples ranged from 0.40 to 2.91 mg.kg^{-1} DM. The EU limit value in edible mushrooms for Hg (0.75 mg kg^{-1} FW) was exceeded in caps from Mníšek nad Popradom. Bioaccumulation factor (BAF) ranged from 3.00 to 45.04 for caps and from 2.12 to 19.77 for stipes. Based on the BAF, analyzed samples are indicators of Hg. The % of PTWI ranged from 2.64 % to 48.33 % for caps and from 2.28 % to 18.71 % for stipes. The toxic hazard quotient (THQ) ranged from 0.050 to 0.920 for caps and from 0.043 to 0.356 for stipes. This indicates that the moderate consumption of *Leccinum* boletes from studied localities should not pose a serious possibility of adverse health effects, but consumption of other sources of mercury must be taken into the account.

Keywords: Leccinum, boletes, mercury, health risk assessment, environmental risk assessment

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MICRO AND MACRO MORPHOLOGICAL EVALUATION OF *BORAGO OFFICINALIS*

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Morphological, anatomical, and physiological plasticity may play different functions in plant adaptation to environmental changes. The influence of the environment on plant morphological expression is very significant and can induce changes at the morphological and physiological levels. The morphological characters of borage - plant, flower and trichomes were measured in the laboratory of medicinal plants of NPPC - VÚRV in Piešťany. Some of the morphological traits measured: plant height, leaf length and width, flower diameter or trichome length, each character was measured from 10 plants of all genotypes. A CI-203 laser analyser was used for leaf morphological analyses, and a Carl Zeiss Discovery V20 microscope was used for trichome measurements. The result of research: 1. the average plant height varied from 701 to 765 mm (locality Piešťany). 2. petiole lengths 20-80 mm are mostly elliptic, entire edged, with a distinct reticulate veining. 3. leaf characteristics were found: leaf length 115.6-166.0 mm, leaf width 69.9-99.4 mm, leaf circumference 311.4-450.5 mm and leaf area 5403-11426 mm2. 4. flowers of borage - predominantly large, long-stalked, 15-30 mm in diameter, mostly blue in colour, except for genotype 7/17 which had white flowers. 5. seed morphological characters, its average length 5,698 mm and width were found 2,714 mm. Chwyl and Borowy (2018), based on research on stem and leaf micromorphology, determined three types of cover trichomes according to the measured length and classified them as: short (326 - 451 μm), medium (650 - 865 μm) and long (1043 - 2196 μm). In addition to these parameters, the number of basal cells of the cover trichomes was also monitored. Short trichomes have 2-4 typical basal cells, medium trichomes have 5-8 large, linearly arranged basal cells, and long trichomes are located on massive, multicellular, multi-row basal cells. According to our results, the length of leaf cover trichomes at the flowering stage of the plant varied from 1,94 mm to 2,39 mm. The description of morphological characters is helpful to understand the ecological strategies of the plants. For example, plant height represents the competitive ability of plants for light, as taller plants have better access to light and higher photosynthetic activity; another trait, plant foliage and leaf area size, are important for aldehyde production, as it is in the leaves that their high content was found.

Keywords: borage, morphology, variety, evaluation, character

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EFFECT OF HEAT TREATMENT IN TECHNOLOGICAL PACKAGING ON PHTHALIC ACID ESTERS CONTENT IN PORK MEAT

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The aim of experiment was analyzed chemical composition and content of DBP (di-n-butyl phthalate) and DEHP (di-2-ethylhexyl phthalate) in pork shoulder before and after heat treatment was analyzed. The meat was analyzed raw and after heat treatment at 50 °C and 60 °C. The heat treatment time in the sous vide water bath was 4 hours, 4 hours + 1 hour after 24 hours, 8 hours and 8 hours + 1 hour after 24 hours. Of the additives added in the production of plastics, plasticizers - plasticizers - pose the greatest risk.
Co-extruded EVA/PVD/EVA three-layer films are most often used for vacuum packaging in the form of a cover into which the product is placed, and air is sucked out in a chamber packaging machine and the bag is hermetically sealed. Another possibility is to use shrinkable films (PE, PP, PC, PVDC), in which the effect of heat tightly wraps around the product around the product and thus reduces the dimensions of the packaged raw material. In some cases, they make up 40% of the total packaging material and are highly lipophilic, making them easy to extract with the fatty components of the food. The most common plasticizers used in packaging are phthalic acid esters, especially di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP). Preparation of meat samples and heat treatment pork shoulder were cut into slices 18-20 mm thick and immediately vacuum-packed in cooking bags with a thickness of 60 μm at room temperature 20 °C. The meat slices were packed individually. The fat content in meat treated at 50 °C increased. The content of fatty acids during the sous vide treatment did not significantly change with exception of vaccenic acid. The vaccenic acid statistically significantly reduced at temperatures of 50 °C and also at 60 °C. The DBP content in raw shoulder at 50 °C and 60 °C during sous vide heat treatment increased. The DEHP content during the heat treatment 50 °C increased but at 60°C decreased. The content of DBP and DEHP in the packaging gradually decreased with the length of the heat treatment. This study also confirms that the migration of phthalates is affected by the heat treatment of the meat sample.

Keywords: heat treatment, pork shoulder, di-n-butyl phthalate, di-2-ethylhexyl phthalate

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THE EFFECT OF ZINC NANOPARTICLES ON THE EQUINE SPERMATOZOA *IN VITRO*

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Zinc plays an important role in many biological processes. This element is essential for the synthesis of DNA and proteins, proper cell division, and participating in the metabolic activity of numerous enzymes. In the male reproductive system, zinc is involved in ensuring hormonal balance, spermatogenesis and regulation of capacitation and acrosome reaction. Zinc deficiency can cause imbalances in testosterone levels or abnormal spermatozoa morphology. Even though zinc is considered as biogenic trace element in the body, nanoparticles of this element can have deleterious effect on the physiological functions of reproductive cells and organs. The aim of this study was to evaluate the effects of zinc oxide nanoparticles (ZnO NPs) on the motility and viability of stallion spermatozoa *in vitro*. Ejaculate samples were obtained from randomly selected breeding stallions (n=8) aged 4-19 years from breeding stables in western Slovakia. The effect of different concentrations of ZnO NPs (0; 0.75; 1.5; 3; 6; 12 and 24 mg/ml) was evaluated after 30, 60 and 90 minutes after collection. Spermatozoa motility and progressive motility were assessed by CASA analysis. Spermatozoa motility significantly decreased ($P<0.001$) after the addition of 12 and 24 mg/ml ZnO NPs 90 minutes after collection. In the case of progressive motility, there was a significant increase ($P<0.001$) after 30 minutes of exposure to 3 mg/ml ZnO NPs and after 90 minutes ($P<0.01$) at concentration of 3 and 6 mg/ml ZnO NPs, followed by a nonsignificant decrease at concentrations of 12 and 24 mg/ml. The metabolic activity of spermatozoa was evaluated by the mitochondrial toxicity test. A notable decrease ($P<0.001$) was recorded in samples with a concentration of 6, 12 and 24 mg/ml ZnO NPs. The results of this study suggest that low concentrations of ZnO NPs have a positive effect on spermatozoa quality parameters, but in the case of higher concentrations, their effects are negative. Due to their increasingly frequent use in the pharmaceutical, food industry or cosmetics and lack of results in this area of research, it is necessary to examine their effects on living organisms closely.

Keywords: zinc, nanoparticles, spermatozoa, motility, CASA, MTT, stallion

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ANTIOXIDANT ACTIVITY OF YOUNG GREEN BARLEY (*Hordeum vulgare* L.)

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Barley grass is the young leaf of the barley plant (*Hordeum vulgare* L.). In the present it is called as superfood, which is a common ingredient at health stores. Young barley is available on the market in several forms such as tablets or powdered form dissolved in drinks. Barley grass is used as a supplement to boost weight loss, enhance immune function, and support overall health. It has been associated with several potential health benefits considering high antioxidant activity. Barley grass is widely accepted as a source of antioxidants and various compounds with antioxidant activity have been isolated from young barley. The aim of our study was to verify claims whether the green matter of barley plants shows antioxidant properties. Six malting varieties (Kangoo, Marthe, Odysey, Tangoo, LG Tosca and Overture) were used to estimated antioxidant activity of young spring barley plants (*Hordeum vulgare* L), each variety in six replicates. For analysis of antioxidant activity, young green leaves, and stem of vegetative growth stage from seedling at 7 days after sprouting, were used. Antioxidant activity was determined using ABTS method. Trolox was used as an antioxidant standard. The highest antioxidant activity was determined in variety Overture (891,6 μmol TE.g^{-1} FW) and the lowest antioxidant activity was determined in variety Marthe (319,6 μmol TE.g^{-1} FW). Based on results, there were evident differences in antioxidant activity between tested varieties. Samples of young barley show high antioxidant activity and can be potentially recommended as food supplements in human nutrition.

Keywords: barley grass, young barley, antioxidants

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ACTIN CYTOSKELETON, MITOCHONDRIAL AND LYSOSOMAL STATUS OF BOVINE VITRIFIED OOCYTES IN RELATION TO EMBRYO DEVELOPMENT

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Vitrification is used for several decades for long-term preservation of animal gametes. During vitrification oocytes are exposed to unnatural conditions that cause several damages to them, including dysfunction of intracellular organelles. The aim of this study was to assess how vitrification affects the status of mitochondria, lysosomes, actin cytoskeleton in bovine oocytes and further embryo development. Oocytes, aspirated from cow ovaries obtained from a local slaughterhouse, were *in vitro* matured (TCM199, 10% FCS, 0.25mmol.l^{-1} sodium pyruvate, 50ug/ml gentamicin, 1/1 I.U FSH/LH (Pluset)) for 21 hours (38.5°C and 5% CO_2) and vitrified in minimum volume on the nickel electron microscopy grids by ultra-rapid cooling technique. Fresh oocytes served as a control group. Oocytes post-warming were stained for mitochondrial (MitoTracker Green), lysosomal (LysoTracker Deep Red) activity and actin cytoskeleton (phalloidin-TRITC). Part of vitrified/warmed (n=504) and control (n=731) oocytes were *in vitro* fertilized using frozen bull semen and cultured until the blastocyst stage. Mitochondrial and lysosomal activities were measured on the basis of relative fluorescence intensity by ImageJ software (control group set by the arbitrary unit at 1). Actin cytoskeleton was evaluated by classifying to 3 quality grades (excellent, good and poor). Embryo development was evaluated by counting cleavage (day 2) and blastocyst (day 7-8) rates. Mitochondria and lysosomes were occurred in the ooplasm mostly in diffused or aggregated form, and their distribution patterns were not affected by vitrification. However, vitrification caused significant decrease in mitochondrial (0.66 AU; $p<0.05$) and especially lysosomal activity (0.23 AU; $p<0.05$) compared to control group (1 AU). Proportions of oocytes with excellent actin (grade 1; 71.43%) and good actin (grade 2; (18.37) quality in control group was higher compared to vitrified oocytes of grade 1 actin (59.70) or grade 2 actin (28.36%) quality. Nevertheless, the percentages of poor quality actin cytoskeleton (grade 3) were almost similar in both groups (vitrified=10.20%; control=11.94%). Therefore, the overall actin status showed no significant difference between vitrified and control oocytes. Impaired organelle status in vitrified oocytes was coincided with declined cleavage (55.75%) and blastocyst (15.08%) rates compared to control group (66.21 % and 30.37 %, resp.; $p<0.05$). In conclusion, mitochondrial and lysosomal status of vitrified oocytes reflects their developmental potential rather than the actin cytoskeleton, which was not significantly affected by vitrification.

Keywords: bovine, oocytes, vitrification, organelle

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THE EFFECT OF CORNELIAN CHERRY ON SELECTED SERUM BIOCHEMICAL PARAMETERS OF SPONTANEOUS DIABETIC RATS

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Diabetes type 2 is a global disease with an increasing prevalence. One of the diabetic complications is kidney damage, which can lead to diabetic nephropathy. The aim of our study was to monitor biochemical parameters such as total proteins, albumins, globulins, urea, uric acid and creatinine in blood of Zucker Diabetic Fatty (ZDF) rats. ZDF rats (n=24) were divided to the three groups: C (diabetic non-treated group), E1 (cornelian cherry´s stone 250 $mg.kg^{-1}$ body weight) and E2 (cornelian cherry´s pulp 1500 $mg.kg^{-1}$ body weight). The animals were regularly given crushed fruit through a gastric tube. After the end of the experiment, the animals were humanely sacrificed, and the blood was collected. After centrifugation the blood serum was used to determine selected parameters. All parameters were analysed by a fully automated analyser Rx Monaco. The results of the analyse showed that there were no significant differences among the groups in urea, uric acid, total proteins, albumins, and globulins after the addition of cornelian cherry. When determining creatinine, there was a significant increase ($P < 0.05$) after the application of cornelian cherry´s pulp 1500 $mg.kg^{-1}$ body weight. In general cornelian cherry had no significant effect on selected biochemical parameters in ZDF rats, but creatinine values increased after the addition of cornelian cherry´s pulp, which could mean impaired kidney function. For this reason, further studies dealing with the effect of cornelian cherry in diabetic rats are needed.

Keywords: diabetes, cornelian cherry, ZDF rat, biochemistry

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NARINGENIN, CURCUMIN AND ISOQUERCITRIN MITIGATE DAMAGES IN SPERMATOZOA CAUSED BY *STAPHYLOCOCCUS AUREUS* DURING BACTERIOSPERMIA *IN VITRO*

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The presence of bacterial contamination in bovine insemination doses may lead to unexpected economic loss. Currently used antimicrobial supplements are often ineffective due to antibiotic resistance of the common bacterial contaminants. *Staphylococcus aureus* belongs to the frequent inhabitant of bovine semen with a deleterious effect on spermatozoa quality. Bioactive substances including naringenin, curcumin and isoquercitrin were previously studied with various beneficial effects on spermatozoa. Therefore, the study aimed to simulate bacteriospermia *in vitro* using *S. aureus*, which was previously isolated from the bovine ejaculate, and to evaluate if the bioactive substances mentioned above would bring some protective properties against the bacterial damage. Density gradient centrifugation was used to avoid contamination by naturally present bacteria. *S. aureus* was inoculated in BGM-3 medium, and co-incubated with pre-washed spermatozoa and bioactive substances. At times of 0, 2, and 4 h, progressive motility, the membrane potential of mitochondria, reactive oxygen species (ROS) formation, and sperm DNA damage were evaluated. The results showed that *S. aureus* significantly increased ROS production, sperm cells with damaged mitochondrial membrane potential, and sperm DNA integrity which led to a decrease in the progressive movement of spermatozoa. On the other hand, all three bioactive substances significantly scavenged elevated ROS. Especially, the groups treated with naringenin and isoquercitrin showed preserved mitochondrial membrane, DNA integrity inside the sperm head, and improved sperm progressive movement under bacteriospermia *in vitro*.

Keywords: *Staphylococcus aureus*, spermatozoa, ROS, progressive motility, sperm DNA, mitochondrial membrane potential

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MYCOTOXIN PRODUCING *ASPERGILLUS* SPP. AND *PENICILLIUM* SPP. AND OTHER FUNGI ISOLATED FROM GRAPES FOR WINE PRODUCTION IN THE CENTRAL SLOVAK WINE REGION

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From the Central Slovak wine region were collected 7 samples of red wine grapes (Alibernet 2x, Cabernet Sauvignon 2x, Blaufränkisch, Pinot Gris, and Pinot Noir) and 4 of white wine grapes (Chardonnay, Devin, Irshay Oliver, Welschriesling) during harvesting 2021. These 11 samples were collected from the 4 wine producing wineries in Slovakia: Selvino (n = 3), Movino (n = 3), Historic Wine Cellar (n = 3) and Chateau Selešťany (n = 2). The aim of this work was the identification of surface and endogenous mycobiota colonisation of grapes, primarily focused to the current spectrum of the *Aspergillus* and *Penicillium* species due to their relevance for mycotoxin production. Direct plating of grapes on DRBC agar plates was used for analysis of surface mycobiota of grapes while surface sterilised grapes were used for endogenous mycobiota analysis. The identification of fungi was performed using the morphological and microscopical characteristics. Overall, from exogenous mycobiota we isolated 574 strains belonging to 12 genera of filamentous microscopic fungi and unidentified genus *Mycelia sterilia*. Almost all samples were colonized by genus *Alternaria* (91% IF), followed by *Aspergillus* (73%), *Cladosporium, Penicillium*, and *Rhizopus* (64%, each). The most abundant genus was *Alternaria* (53% RD) of all the fungi found, *Rhizopus* was the second one (9%). From exogenous mycobiota 23 strains of 6 *Penicillium* species, namely P*. brevicompactum, P. crustosum, P. expansum, P. glabrum, P. oxalicum,* and *P. thomii* were isolated. Seven samples were colonised by *Penicillium* species. Fungi of genus *Aspergillus* (*A. clavatus, A, flavus* and *A.* section *Nigri*) were found in 8 grape varieties. *Aspergillus* section *Nigri* were the most frequent and abundant isolates (58% IF, 77% RD). From endogenous mycobiota we isolated 512 strains belonging to 8 genera and *Mycelia sterilia*. All samples were colonized by genus *Alternaria* (100%), followed by *Rhizopus* (82%), *Penicillium* (73%), *Aspergillus* (64%), and *Cladosporium* (45%). The most abundant genus was *Alternaria* (61% RD) of all the fungi found, *Cladosporium* was the second one (13% RD). Two *Aspergillus* species were found: *A. clavatus*, and *A.* section *Nigri*. *Aspergillus* section *Nigri* were the most frequent and abundant isolates (54% IF, 98% RD) from all aspergillus isolates. Fifteen strains of 3 *Penicillium* species, namely *P. brevicompactum*, *P. expansum*, and *P. glabrum* were isolated. Potentially toxigenic *Aspergillus* and *Penicillium* species were tested for their toxigenic ability by thin layer chromatography method. All tested strains were able to produce at least one mycotoxin, except potential producer of OTA *Aspergillus* section *Nigri* and *Aspergillus flavus* which were negative for aflatoxin B_1, G_1, and cyclopiazonic acid.

Key words: wine grapes, mycobiota, mycotoxins, TLC method

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MODERN ANALYTICAL APPROACHES USED AT THE EVALUATION OF QUALITY AND HEALTH SAFETY IN WINES OF DIFFERENT ORIGIN IMPORTANT FOR CONSUMERS

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Wine is one of the oldest alcoholic beverages consumed and its price is related to the origin and quality. The quality and safety of the wine depends on several factors, these include grape variety, geographic origin, vintage conditions, wine production etc. Analytical approaches in combination with chemometrics can help to prove wine origin by identifying specific chemical markers important for quality, health safety and consumers as well. In this work, main chemical parameters of wines such as sugar content, total acids, malic and tartaric acids, glycerol and alcohol content, total phenolic content and the antioxidant activity of 9 white wines with PDO at varieties Pinot Blanc, Riesling, Sauvignon blanc from different countries (Slovakia, Hungary, Austria) were studied in order to identify possible differences/similarities affected by their origin. The wine samples originated from the 2020 vintage and were examined by the FTIR and UV-VIS spectrophotometry methods. Sensory evaluation (by OIV) of wines was performed as well. For the statistical evaluation, ANOVA and PCA methods were applied. High sugar content, phenolic content and antioxidant activity were parameters characteristic especially for the Austrian varieties, such as Pinot blanc and Sauvignon blanc. Both originated from the Steiermark area located in the southeast of the country. Sauvignon blanc and Pinot blanc from Hungary were characterized by higher content of alcohol and glycerol, as they are produced by the sugar fermentation. Statistical evaluation was helpful to show several differences, especially in the case of PCA application. Just Riesling variety from all countries was so similar at selected parameters that it was not possible to be strictly distinguished using PCA. The results of sensory evaluation show that as the best individual sample was classified Pinot blanc from Slovakia (classified as "very good"). Slovak wines were the best sensorically evaluated samples significantly exceeding the samples of wines from other countries. Using multivariate statistical methods (PCA) was useful tool to follow several differences among varieties/localities. We can recommend to use the PCA to find several markers for locality and quality of wines combined with other statistical and/or chemometrical methods as well.

Keywords: grape, wine, quality, PDO, origin, traceability

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THE KETOTIC CONDITIONS THAT MIMIC FOOD DEPRIVATION, AFFECT MACROPHAGE INNATE IMMUNE RESPONSE *IN VITRO*

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Ketogenic diet and various dietary regimens that aim to mimic the metabolic state achieved during fasting have gained much interest due to their beneficial effects in patients with various noncommunicable diseases. Ketogenic diet or fasting induce the state of ketosis, which is manifested by normoglycemic glucose concentration with elevated plasma ketone bodies (5-8 mM of mainly beta-hydroxybutyrate, bHB). However, little is known about the impact of ketosis on immune system, particularly on macrophages, which coordinate innate and adaptive responses. Our study was planned to assess how the ketosis in cell culture model (5.5 mM glucose, 5 mM bHB; as compared to standard 25 mM of glucose, no bHB addition) affects the main innate effector functions of macrophages. The obtained data demonstrated that ketotic conditions elevated secretion of pro-inflammatory IL-1β and anti-inflammatory IL-10 in response to bacterial lipopolysaccharide (LPS), but decreased the synthesis of ROS and nitric oxide, as compared to the standard cell culture. The average phagocytic index was lower in the macrophages cultured in the ketotic conditions, but the heterogeneity of the phagocytic capacity was significantly greater than in the standard conditions, which suggests higher plasticity. In conclusion, our results indicate that ketosis modulates the macrophage innate immune functions.

Keywords: ketogenesis, macrophages, inflammation, phagocytosis

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ANTIOXIDANT CAPACITY OF EXTRACTS FROM HOP PELLETS OF DIFFERENT VARIETIES OF COMMON HOPS

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Currently, hops and its products are gaining attention due to their unique biological properties. Among other things, it is the biosynthesis of secondary metabolites with high antioxidant potential. In this work, we analyzed methanolic and ethanolic extracts of hops of the Polaris, Saaz Late and Premiant varieties, whereby the samples were prepared by mechanical homogenization or pulverization. The total antioxidant capacity (TAC) of the obtained extracts was determined by two TEAC methods. In the first one, using the DPPH radical, the highest TAC was shown by the ethanol extract of the Premiant variety, prepared by mechanical homogenization at 100 °C (1.13 mmol.dm^{-3} TE). In the second method using the radical ABTS, the pulverized sample of the Premiant variety, extracted with ethanol at a temperature of 100 °C (1.05 mmol.dm^{-3} TE), had the highest TAC. The total content of polyphenols was determined by the Folin-Ciocalteu method, while the highest concentration of polyphenols was obtained from the Polaris variety after extraction with methanol at an extraction temperature of 50 °C (2.28 g GAE.dm^{-3}). The results show that hop extracts have significant antioxidant activity, and their addition can increase the content of biologically valuable substances in beer, without unwanted bitter flavors.

Keywords: *Humulus lupulus*, extraction, antioxidant capacity, polyphenols

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EFFECT OF *LACTOBACILLUS* SPECIES TO *ASPERGILLUS OCHRACEUS* GROWTH AND MYCOTOXIN PRODUCTION

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Aspergillus ochraceus is an important mycotoxin producer which produce ochratoxin A (OTA) with a several toxicological effects on human. There is a lot of studies about OTA molecule which can have toxicological effect such as immunotoxic, nephrotoxic, hepatotoxic, teratogenic or neurotoxic. Therefore, the study of elimination or decreasing of mentioned mycotoxin is very desirable in food and feed production. Many authors describes elimination of mycotoxins by different ways such as physical, chemical or biological. Each of the methods has its positive and negative aspects. Not all methods can be used in the same conditions and not all methods are 100% effective in eliminating mycotoxins. In this study was used some selected *Lactobacillus* species such as *L. plantarum, L. paraplantarum, L. paracasei, L. fructivorans* against growth and ochratoxin A production of *Aspergillus ochraceus*. All lactobacilli strains were isolated from the fermented dairy products and identified by MALDI-TOF Mass Spectrometry in cooperation with Maldi Biotyper software. *Aspergillus ochraceus* was isolated from bread samples and identified by MALDI-TOF MS and genetically based on ITS region. Lactobacilli were incubated on MRS agar at 37 °C during 72 hours. After incubation and visible growth SDA agar was overlaid and inoculum of *Aspergillus ochraceus* was transferred onto the agar. Co-cultivation took place at 37°C for 8 days. After co-cultivation a diameter of fungal mycelium were measured and growth was evaluated. Thin Layer Chromatorgraphy was used as qualitative method for ochratoxin A detection. Obtained results showed that *Lactobacillus plantarum* and *L. paraplantarum* inhibited growth of *A. ochraceus* absolutely. *Lactobacillus paracasei* inhibited growth on 83 % and *L. fructivorans* about 10 % only. On the other hand *Lactobacillus fructivorans* inhibited production of ochratoxin A in the case of all tested samples. The same results was detected in *Lactobacillus paracasei*. Other lactobacilli strains were could not be a tested because *Aspergillus ochraceus* was inhibited absolutely. The results indicate that some lactobacilli strains inhibite growth of *Aspergillus ochraceus* and other species have potential inhibit mycotoxin production. There is a heavy potential in food and feed protection or biopresevartion as well as biological control agents by biotechnological processes.

Keywords: *Lactobacillus, Aspergillus ochraceus*, inhibition, OTA, growth

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SCoT MARKERS AS A SUITABLE TOOL FOR DETECTION OF COMMON BEAN GENETIC DIVERSITY

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Common bean represents a worldwide cultivated and consumed legume crop. Beside supplying the main daily intake of calories for poor communities living in developing countries, it also represents an important source of protein, starch, dietary fibre, micronutrients, and bioactive compounds, associated with many health benefits. In the recent years, many breeding activities have focused on the improvement of various key traits of economically important crops using the process of marker assisted selection. Many types of DNA markers are currently applied in genetic diversity evaluation, quantitative trait loci analysis and genetic mapping. The aim of the work was to detect genetic variability among 10 bean genotypes using 6 Start Codon Targeted (SCoT) markers (SCoT14, SCoT15, SCoT29, SCoT33, SCoT62, SCoT63). Used primers amplified altogether 55 evaluable DNA fragments, of which 9 were monomorphic and 46 fragments were polymorphic. The percentage of polymorphism ranged between 55,56 % (SCoT62) and 100 % (SCoT15, SCoT29), with relatively high average of 81,62 %. The size of the amplified fragments varied from 180 to 2800 bp. Altogether three coefficients were calculated to evaluate the level of polymorphism in the set of bean varieties. Values of polymorphic information content (PIC) varied from 0,655 (SCoT14) to 0,766 (SCoT63), with an average of 0,725. A mean value of diversity index (DI) was of 0,736, with values varying from 0,664 (SCoT14) to 0,777 (SCoT63). The probability of identity (PI) values ranged from 0,017 (SCoT63) to 0,070 (SCoT14), and the mean value was of 0,032. The dendrogram representing genetic relationships among varieties was constructed based on hierarchical cluster analysis using UPGMA algorithm and Jaccard´s similarity coefficient. The hierarchical cluster analysis divided 10 bean varieties into two main clusters. The first cluster separated variety Enso (Sweden), and the second cluster included remaining varieties further separated into subclusters. Based on the dendrogram, as well as the highest value of Jaccard´s similarity coefficient (0.857), variety Cabernet (Netherland) and variety Amulet (France) are considered to be genetically the closest. On the other hand, genetically the furthest is variety Enso (Sweden) from variety Marika (Czech Republic), with the value of Jaccard´s similarity coefficient 0.048. The results obtained in the work present high level of polymorphism and confirm the usability of SCoT markers for the study of bean genetic variability.

Keywords: common bean, SCoT markers, dendrogram, genetic diversity

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NUTRITIONAL AND TECHNOLOGICAL QUALITY OF EMMER WHEAT AND SPELT WHEAT GENOTYPES

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Cereals are the most widespread agricultural crops from all plant production. They are very important source of energy and contain biologically valuable substances for nutrition. Wheat proteins have not only important nutritional, but also technological function. Wheat technological quality depends on presence of protein glutenin and prolamins fractions because formed gluten, which is essential in the production of bakery products. Very productive wheat species are used in intensive agriculture. Although productive wheat species provide high yields, they are very sensitive to environmental conditions. Whereupon old wheat species are used in breeding program. We analysed 7 genotypes of the emmer wheat (*Triticum dicoccum* (Schrank) Schuebl) and 3 genotypes of spelt wheat (*Triticum spelta* L.) in our work, which serve as a donor of new features in breeding program. Aim of the study was to identify individual protein subunits of the high molecular weight glutenin subunit (HMW-GS), because they are involved in the gluten properties, which significantly influence of the bakery quality. In our work, we also focused on the percentage determination of individual protein fractions of individual genotypes with regards to bakery utilization as well as their use in a gluten-free diet. HMW-GS was determined by electrophoretic method SDS PAGE. However, the composite structure of HMW-GS could only by identified in the genotypes of spelt wheat. In the analysis of spelt wheat genotypes, we have identified only one electrophoretic profile with the composite structure of HMW-GS 2*, 7+8, 2+12, which are implicated as a good baker wheat. Based on the protein profiles obtained by SDS-PAGE electrophoresis we managed to construct the dendrogram of relatedness between the individual genotypes of spelled and emmer wheat, which allows us to keep track of individual differences among genotypes of wheat.

Keywords: cereals, nutritional and technological quality, protein identification, wheat

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A RAPID METHOD VALIDATION AND DETERMINATION OF AFLATOXIN B1 BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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A high-performance liquid chromatography method coupled with fluorescence detection has been optimized for the determination of AFB_1 produced by *Aspergillus* section *Flavi* in isolates from the food commodities. The study was aimed at evaluation of HPLC-FLD method and characterisation of the method by several validation characteristics. A rapid validation was based on intraday and interday precision and repeatability of retention time and concentration, linearity of the method and calculation of limit of detection (LOD) and limit of quantification (LOQ) for Aflatoxin B1. The validation characteristics tested showed high sensitivity, allowing determination of the AFB1 in isolates at the concentration levels of sub-$\mu g.mL^{-1}$. The detection limit for AFB1 was 0.032 $\mu g.mL^{-1}$ and limit of quantification was 0.102 $\mu g.mL^{-1}$. The linearity of the method (R^2 = 0.99999) was confirmed within the whole calibration range from of 0.01 to 10.0 $\mu g.mL^{-1}$.
Under the optimized conditions, three *Aspergillus* section *Flavi* isolates were analyzed by the method. The isolates were cultivated by submerse cultivation in yeast extract sucrose (YES) liquid medium at 25±1°C. The samples were simply extracted in ethyl acetate and measured directly in water/methanol mobile phase by HPLC-FLD in isocratic mode. The results showed that the concentration of the AFB1 was in the range from <LOD (0.03 $\mu g.mL^{-1}$) to 49.38 $\mu g.mL^{-1}$. The results showed that the method is sensitive, reproducible, and accurate and is suitable for determination of AFB1 in isolates or similar matrices.

Keywords: aflatoxin B1, HPLC-FLD, validation method, *Aspergillus* section *Flavi*

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INVESTIGATION OF THE CYTOTOXIC POTENTIAL OF *TRIBULUS TERRESTRIS* IN A TIME-DEPENDENCY

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Tribulus terrestris is well known for its use in the traditional medicine of many countries for the treatment of cardiac diseases, eye trouble, and skin itch as well. Several studies have also reported that the main active compound of the plant was steroidal saponins, which act as natural testosterone and highly active surface substances. At the same time, the biologically active compounds of *Tribulus* find application in contemporary medicine as a component of drugs effective in impotence treatment. It is also included in many dietary supplements claimed to have a biostimulating activity and could positively affect human health. The aim of our in vitro study was to evaluate the potential effect of *Tribulus terrestris* on the mitochondrial activity of mice Leydig cells. TM3 cells were cultured in the presence of different doses of *Tribulus* (37.5 – 600 µg/mL) during 24-h and 48-h exposure. Determination of the presented parameters was evaluated using the cytotoxic (MTT) assay. The obtained results confirmed time- and dose-dependent effect of experimental extract. A significant ($p>0.0001$) cytotoxic effect has been observed at 250 µg/mL; 300 µg/mL, and 600 µg/mL after 24-h exposure. In case of prolonged time of cultivation, significant changes ($p>0.05$) have been started at 150 µg/mL until the highest applied dose ($p>0.0001$). Currently, there is necessary to extend this pilot data and identified other cellular parameters in detail.

Keywords: *Tribulus terrestris*, Leydig cells, mitochondrial activity

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EFFECT OF BEE BREAD SUPPLEMENT ON MEAT QUALITY CHARACTERISTICS IN MALE OF JAPANESE QUAILS *(COTURNIX JAPONICA)*

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Bee products present an important alternative as they influence a number of processes in the animal body. The goal of the experiment was to determine the effect of bee bread on selected parameters of meat quality (content of water, proteins, fat) of male Japanese quails after adding the bee bread into feed. Animals were divided into four groups (C, control; E1, E2 and E3 experimental groups). Experimental quails received bee bread in feed mixture in various doses as follows: E1-2000 $mg.kg^{-1}$, E2-4000 $mg.kg^{-1}$, E3-6000 $mg.kg^{-1}$. Feeding period lasted 180 days. After 180 days of feeding animals were slaughtered and meat samples (n=5 in each group) were collected. Meat quality was analysed using a sample of *Musculus biceps femoris* for parameters characterizing the content of nutrients (content of water, proteins, fat). The content of water, proteins, fat was analysed by method FT IR (Fourier Transform Infrared Spectroscopy) using Nicolet 6700 (Pragolab s.r.o.). The following parameters (content of water, proteins, fat) were not affected ($P>0.05$) by bee bread treatment. Detailed physiological mechanism should be further investigated and explained.

Key words: bee bread, Japanese quails, quality meat

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THE PRODUCTION OF TRANSGENIC TOBACCO PLANTS OVEREXPRESSING OAK DEHYDRIN GENE

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Dehydrins (PF00257) are thermostable and highly hydrophilic proteins that are associated mainly with later stages of plant embryogenesis. Besides that, many studied indicate a role of dehydrins in plant stress tolerance. They are accumulated under low temperature, water deficiency, osmotic or heavy metal stress. The work is focused on the production of transgenic tobacco plants overexpressing oak dehydrin gene AY607707.1. The oak dehydrin gene is structurally characterized as a K_3 type and is associated with response to altered water and osmotic conditions. Transgenic tobacco plants were generated via *Agrobacterium*-mediated transformation. The T-DNA of the plant transformation vector pMK contained the dehydrin gene fused to the constitutive double dCaMV35S promoter and the selectable neomycin phosphotransferase gene. The sequence of the dehydrin gene was isolated from *Quercus robur* by PCR approach and cloned. The constructed binary vector pMK was introduced into *Agrobacterium tumefaciens* LBA4404 and used in the transformation experiments. Transgenic plants were generated with an efficiency of 32.7%. Transgenic nature of regenerated T_0 plants was confirmed by PCR analyses. The expression of the oak dehydrin gene was proved by RT-PCR and quantified by qPCR analyses. Selected T_0 transgenic plants with the strongest expression of Dhn3 gene were transferred to greenhouse conditions and the seeds were obtained after self-pollination.

Keywords: *Agrobacterium tumefaciens*, dehydrins, *Nicotiana tabacum L., Quercus robur*, transgenic plants

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EFFECTS OF DIETARY LIPIDS TYPE ON THE MICROSTRUCTURE, DROPLET SIZE AND PHASE SEPARATION IN OIL-IN-WATER EMULSIONS STABILISED BY WHEY PROTEIN CONCENTRATE

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There are well-known differences between the physical, structural, or thermal properties of saturated versus unsaturated lipids in their native forms, however the properties of new processed lipid rich matrices remain scarce. In this study, oil-in-water emulsions were fabricated with 20% (w/w) of rapeseed oil as a naturally low in saturated fatty acids and high in unsaturated fatty acids, and with 20% of porcine lard or milk fat as a naturally high in saturated and a low in unsaturated fatty acids. In addition, a combination of porcine lard or milk fat was mixed with rapeseed oil in a ratio of 1:1 (10% + 10%). A remaining phase of 80% (w/w) contained of 4% (w/w) whey protein in a water continuous phase. All constituents were homogenised by a high shear mixing at 30 000 rpm for 150 seconds. The produced emulsions were subjected to a particle sizing, microstructure, and phase separation measurement. The phase separation resulted in a lowest for a milk fat rich emulsion, followed by emulsions with mixed milk fat and rapeseed oil and a rapeseed oil emulsion alone. Porcine lard also reduced the phase separation but without a clear difference between the emulsion with mixed rapeseed oil or lard alone. Inspected microstructures of samples validated the droplet size distribution. The droplet size of emulsions was similar across all the samples (peak at 7.1-7.8 μm), irrespectively to content and type of added lipids, with the exception of lard rich emulsions indicating a greater content of droplets above 50 μm. This finding provides a hint to further examine their functionality with fixed physical droplet's structure.

Keywords: emulsions, rapeseed oil, porcine lard, droplet size

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ANTIOXIDANT EFFECT OF HONEY ENRICHED WITH VARIOUS TRADITIONAL AND NON-TRADITIONAL FRUIT ADDITIVES AS A FUNCTIONAL FOOD WITH ENHANCED HEALTH BENEFITS

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Nowadays, innovation in the area of functional food products in the food industry is very intense and desirable. The aim of the study was to investigate the effect of elderflowers (*Sambucus nigra*), blue honeysuckle fruits (*Lonicera caerulea* var. *kamtschatica*), sea buckthorn berries (*Hippophae rhamnoides*), and goji berries (*Lycium chinensis*) (6 and 12% concentrations) on the antioxidant activities of rape honey. Honey (control) and honey enriched with lyophilized plant material powders were analyzed regarding total polyphenol content (TPC) and total phenolic acid content (TPAC), as well as antioxidant activity measured by the DPPH method. All parameters were investigated spectrophotometrically. Our results demonstrated that TPC varied in case of 6% concentration of additives from 1.10 mg/g gallic acid equivalent (GAE) DW for sea buckthorn, 1.13 mg GAE/g for goji, 2.18 mg GAE/g for blue honeysuckle to 3.04 mg GAE/g for elderflowers. In case of 12% concentration, TPC varied from 1.80 mg GAE/g for goji, 2.04 mg GAE/g for sea buckthorn, 3.82 mg GAE/g for blue honeysuckle to 4.11 mg GAE/g for elderflowers. TPAC ranged in case of 6% concentration of additives from 0.16 mg/g caffeic acid equivalent (CAE) DW for goji, 0.17 mg CAE/g for sea buckthorn, 1.46 mg CAE/g for blue honeysuckle to 2.00 mg CAE/g for elderflowers. In case of 12% concentration, TPAC ranged from 0.25 mg CAE/g for goji, 0.34 mg CAE/g for sea buckthorn, 2.80 mg CAE/g for blue honeysuckle to 3.67 mg CAE/g for elderflowers. The addition of all used plant additives significantly increased the antioxidant potential of honey. Sea buckthorn was the most abundant in antioxidants with antioxidant capacity ranged from 0.74 to 0.78 mg/g Trolox equivalent antioxidant capacity (TEAC) DW, following by blue honeysuckle (0.62–0.76 mg TEAC/g), elderflowers (0.52–0.61 mg TEAC/g), and goji (0.10–0.25 mg TEAC/g). To summarize, better properties were found in samples with 12% concentrations of plant additives compared to control and/or 6% concentration. From investigated plant sources, honey enriched with sea buckthorn berries and elderflowers are the most valuable sources of antioxidants and polyphenols and could be recommended as a novel functional food used in pharmacological studies and the food industry.

Keywords: honey, fruit, antioxidant, polyphenol, food innovation

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THE ANTIOXIDANT PROPERTIES OF RUTIN-RICH ELDERBERRY EXTRACT ON HUMAN OVARIAN CELLS HGL5 *IN VITRO*

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Black elder (*Sambucus nigra* L.) has been recognized for a wide range of beneficial effects on the human body. Elderberry fruits contain high levels of flavonoids and anthocyanins, which exert anti-inflammatory and antioxidant properties. The aim of our study was to determine the content of bioactive substances in the elderberry extract from berries and its effect on the human ovarian granulosa cells HGL5 *in vitro* after 24-hour treatment with extract at different concentrations (12.5; 25; 50; 100; 200 µg/mL). Cell viability was determined by AlamarBlue™ test, the secretion of epidermal growth factor (EGF) and insulin-like growth factor I (IGF-I), as well as their receptors, were assayed by ELISA. Reactive oxygen species (ROS) production was quantified by chemiluminescence. Chemical analysis was performed by qualitative and quantitative screening using HPLC-DAD analysis, total polyphenol content (TPC) was examined by Folin-Ciocalteau reagent, and total antioxidant activity was determined by the DPPH method. Our results showed that elderberry extract presents a rich source of polyphenols with the most abundant bioactive compound rutin. Total polyphenol content was 78.91 Gallic acid equivalent (GAE) mg/g DW elderberries and total antioxidant capacity was 87.52 mg Trolox equivalent antioxidant capacity (TEAC) mg/g DW elderberries. The number of viable cells HGL5 was not significantly affected by elderberry extract treatment. On the other hand, elderberry extract significantly ($P \leq 0.05$) increased the EGF level at the concentration of 200 µg/mL, but it did not cause any changes in the presence of EGF receptor. Similarly, we observed a significant ($P \leq 0.01$) increase in IGF-I secretion at a concentration of 200 µg/mL and a slight increase in the level of IGF1 receptor at a concentration of 12.5 µg/mL ($P \geq 0.05$). Moreover, elderberry extract treatment at the concentration of 100 µg/mL ($P \leq 0.01$) and 200 µg/mL ($P \leq 0.001$) led to significantly lower levels of ROS. In conclusion, rutin-rich elderberry fruit extract presents a strong antioxidant agent, which may play an important role in the antioxidant signaling pathway by the reduction in ROS production in ovarian granulosa cells and may have modulatory effects by regulating growth factors.

Keywords: elderberry, ovarian cells, viability, growth factors, ROS, oxidative stress

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TECHNOLOGICAL AND SENZORY EVALUATION OF BISCUITS ENRICHED WITH NON – TRADITIONAL FRUITS OF SASKATOON BERRY (*AMELANCHIER ALNIFOLIA* NUTT.)

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Biscuits can be included among the cereal products that we can use as a supplement to improve nutrition. Nowadays, it is very popular to enrich biscuits with less typical ingredients and thus increase their nutritional value. The aim of the work was to evaluate the technological quality (moisture, ash, falling number, crude protein, gluten, acidity) of mixed flour prepared from wheat flour and lyophilized Saskatoon berry (*Amelanchier alnifolia* Nutt.) in the amount of 5 %, 10 % and 15 % and to compare the chemical (a_w, dry matter, crude protein, ash) and sensory parameters (appearance, aroma, taste, consistency, overall perception) of biscuits prepared from these flours. Due to the relative humidity of the lyophilized Saskatoon berries (8.64 %) and the humidity of the control sample (12.52 %), the moisture of the flour mixture was reduced by the addition of berries. Compared to the control sample, the ash content increased from 0.59 % (control) to 0.87 % (15 %) with the addition of fruits, as did the acidity of the flour ($p < 0.05$). On the contrary, with the increasing proportion of the addition of the powder of this fruit in the mixture with wheat flour, some technological parameters decreased ($p < 0.05$) (gluten content and its quality, falling numbers). The dry matter content in the biscuits ranged from 95.31 % (control) to 95.67 % (15 %). The ash content of the biscuits increased significantly ($p < 0.05$) from 0.88 (control) to 1.14 % (15 %), but the crude protein content decreased with increasing Saskatoon berry addition (8.34 % - control, 7.92 % – 15 %). In biscuits with the addition of this fruit, the value of water activity (a_w) was significantly reduced from 0.57 (control) to 0.31 (15 %) ($p < 0.05$). Based on organoleptic evaluation, the replacing of up to 10 % and 15 % of wheat flour with Saskatoon berry powder improved the sensory properties (taste and overall perception) of the prepared cookies compared to control wheat cookies ($p < 0.05$). Sensory characteristics such as appearance, aroma, and intensity of aroma, presence of foreign smell and consistency of biscuits did not show any significant differences ($p > 0.05$) in the sensory evaluation. Nevertheless, the higher the addition of Saskatoon berry in the mixed flour, the more positive the individual characteristics were evaluated, especially the colour of the product. We can recommend this non-traditional fruit for application in the production of biscuits (adding 10 and 15 %). Even in conjunction with the colour of the dough, this fruit can increase the attractiveness of products and can be interesting when designing new products with increased potential for health.

Keywords: Saskatoon berry (*Amelanchier alnifolia* Nutt.), wheat flour, biscuits, technological quality, sensory quality

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CAN HUMIC ACIDS AFFECT THE HEALTH STATUS OF THE BROWN HARE?

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In recent times, the trend has been to use various organic compounds in animal feed that would otherwise be unused. Humic acids (HAs) are natural organic compounds resulting from the chemical and biological decomposition of organic matter and the synthetic activity of microorganisms. In our study, we used 24 brown hares. The animals were divided into three groups (control and experimental groups – EG1, EG2). Experimental groups received 1% of humic acids, and group EG2 also received the addition of green feed for 90 days. As part of the assessment of the health status of the animals, we evaluated selected haematological and biochemical parameters from the blood and blood serum. We subjected the obtained results to statistical analysis, first we subjected the set of results to the normality test. Subsequently, we performed an ANOVA analysis followed by Tukey's multiple comparison test. As part of the assessment of haematological parameters, we noted a statistically significant difference at the lowest level of evidence only for the total content of red blood cells between the control group and experimental group 2 (decrease compared to control). Other monitored haematological parameters showed some variations, but without statistical evidence. Evaluation of biochemical parameters showed a similar tendency. Different variations were shown, for example, in the concentrations of the mineral profile and the hepatic profile, but without statistical significance. We noted a statistically significant difference between the experimental groups for the cholesterol content (increased cholesterol concentration in experimental group 2). Humic acids are relatively well researched as a nutritional supplement in poultry, where production parameters (weight gain, proportion of muscle, etc.) were mainly monitored. There are relatively few studies on mammals, so it is first necessary to determine possible effects on the individual's health. The presented results and findings indicate a relatively high safety of using humic acids in the nutrition of hares in the tested concentrations. In the future, we recommend increasing the number of animals in the experiment, possibly choosing several economically important species, as well as testing higher concentrations of humic acids in the feed.

Keywords: humic acids, brown hares, serum chemistry, diet, health status

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VARIABILITY OF PROMOTER REGIONS OF *MUTE* HOMEOLOGOUS GENES IN BREAD WHEAT

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Stomatal biogenesis is an important issue for consideration of possible ways of crop improvement in condition of reduced water regime without a loss of grain quality and yield. Among the key genes in stomatal development is *MUTE*, an essential stomatal regulator, particularly, of guard mother cell division.
Our preliminary study of *MUTE* genes in six Ukrainian winter wheat cultivars, demonstrated specific and reproducible expression patterns in leaf seedlings with certain diversity among the cultivars. In current study we investigated promoter regions of the *MUTE* genes in the Ukrainian cultivar Natalka. Based on the available sequence of Chinese Spring genome, sub-genome specific primers were developed, and *MUTE* promoters were isolated and sequenced from cv Natalka. Sequences upstream of the predicted start codons were amplified by PCR and sequenced: (i) approximately 3600-bp of *MUTE-A1*, (ii) 1600-bp of *MUTE-B1*, and (iii) 2600-bp of *MUTE-D1*. The study revealed significant heterogeneity of promoter sequences between three *MUTE* homeologous genes (*MUTE-A1*, *MUTE-B1*, *MUTE-D1* from wheat sub-genomes A, B and D respectively), the reference sequences of the cultivar Chinese Spring (IWGSC RefSeq v2.1, https://wheat-urgi.versailles.inra.fr, Alaux et al., 2018) and cultivar Natalka. We observed much higher sub-genome diversity in the promoter region upstream of the transcription start compared to the protein-coding downstream sequences. The sequencing showed numerous single nucleotide polymorphisms, and large deletions in every Natalka sequence compared with Chinese Spring: 276-bp deletion in *MUTE-A1*, 205-bp in *MUTE-B1*, 1291-bp in *MUTE-D1.* These changes led to elimination of a range of *cis*-regulatory elements (light-and stress-responsive elements, as well as tissue-specific elements) within the investigated DNA sequences. This comprehensive study will assist in understanding the nature of cultivar-specific gene expression patterns and wheat improvement for saving yield and quality of grain under restricted water conditions.

Keywords: *in silico* analysis, stomatal biogenesis gene, promoter, *cis*-regulatory element, sequence homology

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EFFECT OF LONG-TERM STORAGE ON WINTER WHEAT GRAIN QUALITY

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The effect of storage period on grain quality parameters of winter wheat (*Triticum aestivum* L.) was observed in this work. The assessment was made from the grain after two years of cultivation, in the regular three-month intervals of storage. Storage of wheat was performed during 14 months. Wheat grain was stored in a warehouse with active ventilation in the region of Nitra. Results showed statistical evidence in qualitative changes at standard stored raw material. Changes in individual components of dry matter were different. The primary significant changes during storage of wheat grain occurred after six months of storage. Gradually the grain of wheat decreased at wet gluten content, but its ability to swell was maintained. The activity of alpha-amylase gradually increased. Significant effect of length of storage on changes in individual components was confirmed. Results found at the changes of components in stored products indicate the need for regular replacement of the stocks of cereals and oilseeds, with a maximum storage period of 1 year. The results show that the duration of storage affects individual component of the protein and carbohydrate complex with increasing length of storage. Influence of the storage length was highly statistically demonstrated at the main parameters of the protein and carbohydrate complex: starch, falling number, gluten content and Zeleny sedimentation index. The effect of storage on a dry matter content was statistically significant. Similarly, the year showed a highly statistically significant effect on starch content, falling number, gluten content and Zeleny sedimentation index.

Keywords: quality, winter wheat, starch, storage

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OVARIAN FOLLICULAR GROWTH QUANTIFICATION IN RABBITS

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Quantification of the ovarian follicular growth in rabbits is reported. An evaluation of relative volume of developmental stages of follicles revealed that the primary follicles form over 84%, growing follicles without antrum formation 10%, antral follicles almost 4% and Graafian follicles almost 2% of the relative volume of the ovary. In primary follicles, the oocyte forms over 70% and granulosa cells less than 30%. In follicles with less than 2 layers of granulosa cells the relative volume of granulosa cells is almost 48% and that of oocyte 52%. In follicles with more than 2 layers of granulosa cells and without antrum formation the granulosa cells form more than 70% of the relative volume. In follicles with antral formation, the oocyte forms less than 11%, antrum 25% and granulosa cells over 64%. In antral follicles the highest relative volume of antrum and lower of granulosa cells and oocyte was observed. The diameter of an oocyte is over 20 μ in primary follicles, higher in follicles with antrum, but in the follicles with antrum formation and Graafian follicles the diameter of oocyte is almost the same. On the other hand, our morphometric data demonstrated a linear increase in the follicular diameter.

Keywords: ovary, follicle, development, morphology, rabbit

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THE EFFECT OF DIFFERENT METHODS OF DRYING SMALL FRUITS ON THE CONTENT OF TOTAL POLYPHENOLS AND ANTHOCYANIN DYES

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The aim of our work was to dry selected types of small fruits and to monitor changes in the content of anthocyanin dyes and total polyphenols after drying. In this work we used small fruits that are characterized by the presence of anthocyanin dyes. These were the following species - chokeberry (*Aronia melanocarpa* Michx.) variety Nero, elderberry (*Sambucus nigra* L.) variety Haschberg, sour cherry (*Prunus cerasus* L.) variety Fanal, red currant (*Ribes rubrum* L.) variety Lake, black currant (*Ribes nigrum* L.) variety Triton, cherry elaeagnus (*Elaeagnus multiflora* Thunb), raspberry (*Rubus idaeus* L.) variety Heritage and blueberry (*Vaccinium corymbosum* L.*)* variety Bluecrop*.* Drying was performed in a Concept S1060 hot air dryer at 60 °C and a Yden CI IR D5 infrared dryer. The analysis of the content of anthocyanin dyes was performed by the pH differential method, and the determination of the total content of polyphenols was performed by the Folin - Ciocalteu method. By analyzing fresh fruits, we found the highest content of anthocyanin dyes in the fruits of black chokeberry (3 036,97 mg.100 g^{-1} dry matter - DM) and the lowest content in the fruits of cherry elaeagnus (83,86 mg.100 g^{-1} DM). The total content of polyphenols in our monitored species ranged from 726,07 mg GAE.100 g^{-1} DM to 7 895,61 mg GAE.100 g^{-1} DM. Similarly to the polyphenols, the highest content was found in the fruits of black chokeberry and the lowest in the fruits of cherry elaeagnus. After drying in a hot air dryer, the content of anthocyanin dyes dropped to values 23,04-979,13 mg.100 g^{-1} DM. In percentage terms, the decrease in the content of anthocyanin dyes was from 62,2 % (elderberry) to 72,52 % (cherry elaeagnus). The total polyphenol content after drying in a hot air dryer ranged from 339,53 mg GAE.100 g^{-1} DM to 4 329,56 mg GAE.100 g^{-1} DM. The percentage decrease in the content of total polyphenols after drying ranged from 45,16 % (chokeberry) to 53,34 % (red currant). After drying in an infrared dryer, we found the content of total anthocyanin dyes from 24,13 mg.100 g^{-1} DM to 1 363,72 mg.100 g^{-1} DM, which represented a decrease in content by 50,41 % (elderberry) to 71,22 % cherry elaeagnus). The total content of polyphenols after drying in the infrared dryer ranged from 387,81 mg GAE.100 g^{-1} DM to 4 654,49 mg GAE.100 g^{-1} DM, which represented a percentage decrease of 41,05 % (black chokeberry) to 47,89 % (sour cherry).
The statistical evaluation confirmed that infrared drying has the most favourable effect on black chokeberry, black elderberry, and black currant fruits in terms of retention of total polyphenols and anthocyanin dyes content.

Key words: small fruits, quality, drying, chemical composition, anthocyanins, polyphenols

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PROLAMIN AND GLUTEN PROTEINS CONTENT IN BEERS PRODUCED IN POLAND

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Gliadin-related proteins, prolamins, are cereal proteins which are a source of polypeptides toxic to people showing intolerance or allergy to gluten. Safety of beer consumption by persons with gluten intolerance depends on the content of immunoreactive amino acid sequences present in prolamin molecules and the peptides derived from it during technological processes. The aim of the work was to determine the level of prolamins and calculate gluten content in samples of beers produced in Poland. The prolamins content of seventy-two commercially available beers were determined using a competitive ELISA method. Prolamin level ranged from 5 to 3326 mg/l. 31.9% of samples contained prolamins less than 10 mg/l, 43,1% of samples contained prolamins within the range 10-50 mg/l and 25% contained prolamins more than 50 mg/l, what corresponds to less than 20, 20-100 and more than 100 mg/l of gluten respectively. The highest level of prolamin content was in wheat beers.

Keywords: beer, prolamin, gluten, ELISA, immunoblot

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ISORHAMNETIN AND QUERCETIN: THE EFFECTS ON HUMAN OVARIAN GRANULOSA AND CANCER CELLS *IN VITRO*

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Isorhamnetin and quercetin are the most important active ingredients in the fruits of sea buckthorn (*Hippophae rhamnoides* L.), which possess extensive pharmacological activities. At present, there have been numerous investigations on isorhamnetin an also quercetin, which exert effects on cardiovascular and cerebrovascular protection and can act as anti-inflammatory, anti-apoptotic, antioxidant, and anticancer agents. The aim of this *in vitro* study was to determine the impact of isorhamnetin at concentrations of 5, 10, 20, 40, and 80 µmol/mL (24 h) and quercetin at concentrations of 5, 10, 20, 50, and 100 µmol/mL (24 h) on the viability of human ovarian granulosa cells HGL5 and human ovarian carcinoma cells OVCAR-3, as well as the secretion of steroid hormones (17ß-estradiol and progesterone) by granulosa cells. Cell viability was evaluated by AlamarBlue™ cell viability assay, and ELISA methods assayed the release of steroid hormones. Our results confirmed the effect of isorhamnetin and quercetin on cell viability. Isorhamnetin treatment significantly ($P \leq 0.001$) reduced the viability of cancer cells OVCAR-3 at the concentrations of 10, 20, 40, and 80 µg/mL, but at the same time significantly ($P \leq 0.001$) reduced the viability of granulosa cells HGL5 at the highest concentration (80 µg/mL). Quercetin significantly decreased ($P \leq 0.001$) the viability of cancer cells OVCAR-3 at the concentration of 100 µmol/mL. We did not observe any changes in the number of viable granulosa cells HGL5 at any concentration of quercetin treatment. Isorhamnetin did not affect steroid hormone release. On the other hand, a significant ($P \leq 0.05$) increase in 17ß-estradiol level at the concentration of 50 µmol/mL quercetin was observed, but with no effect of quercetin on progesterone release at any used concentrations. To summarize, our data showed the potential of phytochemicals from sea buckthorn to inhibit ovarian cancer cell viability *in vitro* and the possible involvement of quercetin in the regulation of steroidogenesis. Understanding the mechanisms of action of isorhamnetin and quercetin is essential due to their pro-apoptotic effect on ovarian cancer cells. Further studies are needed to fully understand the impact.

Keywords: isorhamnetin, quercetin, sea buckthorn, ovarian cells, cancer, apoptosis

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DETERMINATION OF GENETIC DIVERSITY OF MAIZE GENOTYPES USING SCOT MARKERS

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Maize (*Zea mays* L.) is an important cultivated crop from the family *Poaceae*, originated from Mexico and Central America. Worldwide production reaches about 1100 million tons per year, making corn one of the most cultivated cereals. Thanks to its versatile use, it is of great importance in human and animal nutrition and at the same time it is a very important raw material for industry. The aim of the study was to analyze the genetic diversity of maize using the SCoT amplification technique. Seven SCoT markers were used to analyze ten maize genotypes. A total of 92 DNA fragments were detected by the SCoT markers used, of which 71 were polymorphic. The average number of polymorphic fragments was 10.14. Most polymorphic fragments were identified using the marker SCoT26 (13). The marker SCoT6 reached the highest percentage of polymorphism (84.6%). The highest DI value was detected by the marker SCoT26 (0.890), similarly the PIC value. PIC values ranged from 0.823 (SCoT28) to 0.889 (SCoT26). PI values were low and the mean value was 0.005. Using hierarchical cluster analysis using the UPGMA algorithm, maize genotypes were divided into two main clusters (I and II) in the created dendrogram. In cluster I, one genotype (CORD_334) originating from Colombia was separated. Cluster II could be divided into two subclusters (IIa and IIb), in which the other 9 genotypes were grouped. A total of 5 genotypes were grouped in subcluster IIb, of which all four analyzed Argentine genotypes were grouped together. From subcluster IIb, two genotypes, ARZM_18_047 and ARZM_06_99, originating in Argentina were genetically the closest. The genotypes studied were also partially grouped by country of origin. Since the DI and PIC values were higher than 0.8, we can conclude that the SCoT markers used are highly polymorphic and are suitable for analysing the variability of maize genotypes and determining the genetic relationships between them, which is used mainly in the breeding process.

Keywords: *Zea mays* L., SCoT technics, polymorphism, dendrogram

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MILK PROTEINS - STRUCTURE, FUNCTIONS AND TYPES - REVIEW

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The main purpose of this review is to analyse the milk protein, by examining different protein variations, protein benefits and functional properties. Every section is analysed on a case-by-case basis, to form conclusions on variations and advantages of protein within different foods. The review compares milk protein of different species, drawing on conclusions of interspecies differences, which will affect growth rate of neonates of the species. The review provides a clear definition of protein and evaluates protein variations in milk, such as casein and whey protein. A clear difference is highlighted by highlighting different types of milk protein, affecting how protein is distributed within different foods. Circumstances of protein nutritional benefits are examined, and examples are given on therapeutic, anticarcinogenic, immunomodulatory, and antiviral effects of milk protein. Dairy-derived bioactive peptides are attracting much interest because of their health-promoting properties. Based on information available, milk protein allergy is analysed to discuss the two primary categories, IgE-mediated, and non-IgE-mediated. Symptoms of both are explained and compared to milk intolerance, clearly highlighting the differences. Lastly, the review focuses on milk protein's functional properties, concluding protein usage and advantages. The review compiles evidence that Milk proteins contribute enough positive health and therapeutic benefits, such as promoting muscle growth, fat breakdown, and bone growth in adults and children. Due to their diverse properties, they can be digested in several different forms.

Keywords: milk proteins, casein, whey protein

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THE ASSESSMENT OF MILK SELECTED PROPERTIES DURING THE LACTATION PERIOD OF LIPIZZANER MARES

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Not only for their nutritional composition and their possible use in various aspects of human nutrition, but there is also a growing interest in different types of alternative milks. One such milk is mare's milk. Because of its nutritional characteristics, mare's milk is used as a low-allergenic substitute for bovine milk, as a substitute for human and bovine milk for premature new-borns and as a healing agent for several diseases and disorders; it beneficially against skin diseases, supports general physical health, immune system, and stomach function, keeps vitality of muscles, joints and bones, and aids with metabolism problems, liver diseases, cardiovascular diseases, and cancer. The aim of the present study was to analyse the selected physicochemical properties of Lipizzaner breed mare milk. Mares (n=6) were kept under the same conditions and fed the same ration. The mares were 5-6 years old. Milk samples were tested for 6 months, and the following physicochemical parameters were evaluated: density, electrical conductivity, pH, titratable acidity, dry matter, and content of calcium, fat, and lactose. On the base of our results, we can state that the average value of titratable acidity was 2.07 °SH, electrical conductivity 1.61 $mS.cm^{-1}$, density 1028.64 $kg.m^{-3}$, pH 7.25, content of dry matter 9.55%, lactose 6.71%, fat 0.92%, and calcium 105.03 mg. L^{-1}. However, to obtain more information about the Lipizzaner mare's milk and to define its quality aspects, it is necessary to carry out further studies.

Keywords: milk, Lipizzaner breed, mare milk, physicochemical properties

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CHANGES IN TEXTURAL PROPERTIES AND COLOR DUE TO THE PROCESSING METHOD OF GREEN *Coffea Arabica*

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Choosing a suitable method of green coffee beans processing is one of the first steps that can significantly affect the final product. Thus, the objective of this study was to evaluate how the processing method affects the selected observed parameters (dry matter, aw, pH, hardness, color). On-farm postharvest coffee processing is essential for ensuring high quality of coffee cup and constitutes a chain of interlinked phases mainly aimed at removing the cherries' mucilage and drying the beans to a low moisture content of 10 to 12% (mass/mass). The coffee samples were of American origin *Coffea arabica*. A total of 12 samples from multiple regions were used. Samples of Coffea arabica were collected into three groups of processing. The highest dry matter value was indicated in dry processing, and the highest value of water activity was in wet processing. The average value of pH was 5.74. Natural processing showed the most significant differences between individualgroups concerning color processing. On the other hand, wet and dry processing showed very similar colors. Values of hardness were in range 86549.05 - 100674.90 g. The finalquality of the green coffee beans is thus dependent on the different agricultural, and farmpractices applied.

Keywords: green coffee, post-harvest processing, textural parameters, natural method, semi washed method, wet method, *Coffea arabica*

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OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*) ETHANOLIC EXTRACT PROTECTS YEAST CELLS FROM GLUCOSE STRESS

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The objective of the present study was to investigate the putative positive effect of the oyster mushroom *Pleurotus ostreatus* ethanolic extract on the growth, oxidative stress, antioxidant capacity and apoptosis of *Schizosaccharomyces pombe* yeast cells undergoing glucose stress. Ethanolic extract of fruiting bodies of *P. ostreatus* was tested for their anti-hyper- and hypo-glycemic stress activity. Yeast cells were grown under standard conditions in YE+5S media containing 3% glucose as control, hypoglycemic conditions were achieved by cell incubation in media containing either 0 or 0.1% glucose, while hyperglycemic conditions were achieved by cell incubation in media containing 6, 10, or 20% glucose. Compared were cells un- and treated with *P. ostreatus* 1 or 2% ethanolic extract. Growth intensity, generation time (gt), metabolic activity, ROS generation, MDA content, and cell death and apoptosis were determined in *S. pombe* cells treated with indicated amount of glucose in the growth medium for 3 hours. Growth retardation and delayed cell doubling time (gt) resulted from glucose deprivation or over-supplementation was significantly restored by oyster mushroom extract, predominantly by the 2% concentration. Similarly, ROS overproduction and enhanced MDA formation as a consequence of glycemic stress was reduced in cells treated with oyster mushroom extract. Strikingly, 2% extract was capable to increase metabolic activity of cells over-supplied with glucose that resulted in cell protection from death. Results of our study suggest that ethanolic extract of *P. ostreatus* has the ability to protect *S. pombe* cells from glucose stress through reduction of oxidative stress and the increase of metabolic activity.

Keywords: *Pleurotus ostreatus*, *Schizosaccharomyces pombe*, glucose stress, ROS, MDA, metabolic activity

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THIOREDOXIN FUSION TAG IMPROVES PROTEIN YIELD OF RECOMBINANT *DROSERA BINATA* GLUCANASE

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The production of soluble recombinant proteins in *Escherichia coli* remains one of the major bottlenecks of prokaryotic expression systems. The addition of fusion tags to enhance the solubility of the produced protein is one of the best solutions for expressing enzymatically active eukaryotic proteins. In our work, we over-expressed the basic β-1,3-glucanase from the carnivorous plant *Drosera binata* in *Escherichia coli* cells. Two forms of enzymatically active protein were produced, which differ in the presence (DbGluc+Trx, ~50 kDa) or absence (DbGluc-Trx, ~30 kDa) of the thioredoxin fusion tag. Both forms of β-1,3-glucanase also contain the 6xHis-tag sequence for purification by affinity chromatography. Successful production of both forms of recombinant β-1,3-glucanase was detected by SDS-PAGE and the presence of both forms of β-1,3-glucanase was confirmed by on-gel detection of Ni-NTA conjugated fluorescence dye signal. Although both forms of β-1,3-glucanase showed similar specific activity (68.12 $U.mg^{-1}$ DbGluc+Trx and 64.61 $U.mg^{-1}$ DbGluc-Trx), thioredoxin fusion tag increased protein yield of β-1,3-glucanase two-times during purification on Ni-NTA agarose. We estimated that 69% of total enzymatic activity was preserved after purification of DbGluc+Trx, in comparison with DbGluc-Trx, where β-1,3-glucanase retained only 31% of its activity. Thioredoxin fusion tag significantly increased the yield of recombinant β-1,3-glucanase and its presence during purification steps is crucial for sufficient transgene production.

Keywords: *Drosera binata* β-1,3-glucanase, Ni-NTA purification, thioredoxin fusion tag

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ALTERNATIVES TO ANTIBIOTICS FOR MEAT PRODUCTION IN JAPANESE QUIALS (*COTURNIX JAPONICA*)

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The goal of the experiment was to analyse the effect of probiotic preparation on the base of *Bacillus subtilis* and *Lactobacillus paracasei* and humic acid given to the feed mixture on selected parameters of meat quality (breast and thigh muscle) of Japanese quails. Total of 30 males were used. The animals were given standards basal feed mixture and were divided into 3 groups: males with probiotic preparation (PM, n=10) and males with humic acids (HM, n=10). The probiotic preparation was applied in dose of 1g/1kg of feed mixture and humic acids 3g/1kg. The group of animals without any addition served as the control (CM, n=10). The parameters were not affected by consummation of tested substances. The effect of probiotic preparation and humic acids is probably dependent on the dose. Further studies with higher doses are needed.

Key words: Japanese quails, probiotics, humic acids, meat quality

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NATURAL BENZALDEHYDE FROM *PRUNUS* SPECIES

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Benzaldehyde belongs to the group of highly desirable aromatic compounds. Based on consumption, it is the second most important molecule in the flavour industry after vanillin. Benzaldehyde has a hint of almonds and cherries, with a characteristic sweet aroma. It is widely used as a food and flavouring ingredient. Benzaldehyde occurs in a number of plants, especially in the family *Rosaceae* and in particular in the genus *Prunus*. In nature, there are more than 100 genera and 3,000 species in the *Rosaceae* family. In this research, leaf essential oils of peach (*Prunus persica* L.) and cherry laurel (*Prunus laurocerasus* L.) extracted by hydrodistillation in different seasons was investigated as a new potential source of natural benzaldehyde. The chemical constituents were determined by GC-FID, GC-MS and chromatographic profiles were compared with each other. The oils were characterized by elevated level of benzaldehyde (95.5%–99.7%). Laboratory and pilot experiments confirmed high yield of benzaldehyde (0.33g - 0.45 g/100 g fresh leaves), i.e. by processing of 200 - 300 kg of green leaves of various species of the genus *Prunus*, especially peach and cherry laurel, 1 kg of benzaldehyde can be obtained. The results confirmed that essential oil extracted from leaves of peach and laurel appear to be a promising source of natural benzaldehyde.

Keywords: amygdalin, benzaldehyde, biotechnology, essential oil, hydrodistillation

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EFFECT OF DIFFERENT ADDITIVES IN FEEDING MIXTURES ON GROWTH AND INTERNAL ENVIRONMENT INDICATORS OF BROILER CHICKEN

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The aim of the study was to evaluate the effect of addition of various additives in feeding mixtures on growth rate and selected internal environment indicators of broiler chickens. At the beginning of experiment, 45 one day old broiler chickens (ROSS 308) were used. The animals were divided to three groups. Control group (C) included chickens fed only with BR feeding mixtures. To group O a defined amount of crushed milk thistle (*Silybum marianum*) seeds was added to the feeding mixtures, and to group P a defined amount of the commercially supplied product Presan® - FY was added to the feeding mixtures simultaneously with the application of Selko® - pH into drinking water, both from Trouw Nutrition Biofaktory (Czech Republic). Chickens were fattened for 60 days with BR1 (20 days), BR2 (25 days) and BR3 (15 days). It can be seen from the results that during the control weighing on day 4 and 11, there was no difference in growth intensity among the groups. However, the weight of broilers at control weighing on day 33 and the live weight at the end of fattening were significantly higher in the P group compared to the control. From the point of view of the internal environment indicators, a significantly lower activity of AST, ALT, GGT, as well as the total bilirubin concentration was defined in group O, compared to the control. The addition of milk thistle increased LDH activity and decreased plasma lactate concentration. The highest concentrations of TAG and glucose were recorded in group P. Higher total antioxidant capacity as well as catalytic activity of GSH-Px and SOD were determined in groups O and P, compared to control.

Keywords: chicken broilers, feeding additives, production, blood biochemistry

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MALTING POTENTIAL EVALUATION OF DIFFRENT SPRING BARLEY VARIETIES

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Currently there are demands on malting industries to produce high quality barley malt in order to satisfy the needs of the customers e.g. brewery. The aim of this work was to analyze the barley grain technological quality and malting potential of five samples of four different spring malting barley varieties intended for malting process. The micro-malting trials of the varieties Overture (SK), Odyssey (SK), Malz (SK), Malz (HU), LG Tosca (SK) were carried out. Varieties proved to be of satisfactory malting quality in terms of analysed technological parameters. First-class grain percentage in the analysed samples ranged from 80.4 to 94.6 %. Hectolitre weight reached values from 634 to 737 $g.dm^{-3}$. Barley samples also contained a relatively high starch content of 63 to 68 %. Starch is one of the most important indicators of the technological quality of barley. Nitrogenous substances ranged from 9.9 to 11.3 % and all tested varieties met the requirement for nitrogen content. The proteins present in barley grain are essential for the production of quality malt and beer. From biological parameters the germination capacity was measured. The average value of germination capacity in tested varieties was 98 % without statistical differences among samples. These samples were micro-malted and resulting malt was analysed. All tested malt samples achieved high and satisfactory extract content of 82.4 - 83.4 %. Malt extract is another crucial trait while choosing promising malt variety. Statistically significant ($p<0.05$) the highest extract content was confirmed in variety LG Tosca (84.3 %) which was found to be relatively the best technological quality also for its kernel size and optimal starch and protein content. The content of the relative extract at 45 °C in tested samples ranged from 41.8 to 48.01 %. The average value of friability was 98 %. The intensity of proteolytic modification among varieties was satisfactory (Kolbach index 39.18 – 47.17 %). The viscosity of the wort reached values from 1.4 to 1.5 $mPa.s^{-1}$, varieties provided clear wort in all cases. Mashing process consists of gelatinization, liquefication, and saccharification. Saccharification time was less than 10 min which indicates good activity of malt amylolytic enzymes. Based on the results of grain and malt quality parameters it can be concluded that all investigated spring barley varieties are suitable for malting process.

Keywords: barley, micro-malting, malt, malting quality

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FERMENTATION OF SPONTANEOUSLY FERMENTED BEER

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Spontaneously fermentation is the old traditional process of beer preparing. Inoculation is not using in this process, beer is fermented only with microorganisms from enviroment and cask contamination. Fermentation is slow without the massive inoculation of dominant yeast culture. Every specie must grow from a few members of wild inoculum, speed of growing is connecting with substrate usability and other condition like pH or temperature. This parameters discriminate microorganims to part based on part of fermentation with top of the fermentation of specie. Fermentation is not divided from same phases like inoculated fermentation, phases are still there, but they are unique for every from more than 100 species of yeast and more than 50 species of bacteria. Fermentation is divided in 4 phases: phase of Enterobacteriaceae, main fermentation, phase of acidification, phase of maturation. Phase of Enterobacteriaceae is the first phase and start the fermentation. Phase start 3-7 days after cooling wort and fermented with mixed-acid fermentation. A lot of monosacharides are in a young wort so species with fast grow and bad fermentation capacity of higher saccharides are predominat here. After fermentation of monosaccharides and fall of pH level phase end. During first phase Saccharomoces species are growing, but place for full fermentation they have after this phase. Main fermentation runs during degradation of disaccharides. After depletion of fermentable sources the phase ends. Third and fourth phases run with same species: wild Brettanomyces species of yeast and Pediococcus species. This species have the same sign, they can ferment higher saccharides – dextrines. During these phases the biggest part of taste and aroma are making. The difference between these two phases is that acidification run in cask and maturation run in bottles. All processes have been running for three years and fin the end is a unique and expensive product with terroir taste and aroma from indigenous population of microorganisms. When we understand this process, we can use parts of it to improve our fermentation technologies and or construct new one to prepare different new kind of beverages.

Keywords: spontaneously fermentation, beer, sour beer

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BEE BREAD SUPPLEMENTATION AND IMPACT ON MEAT QUALITY CHARACTERISTICS OF FEMALE JAPANESE QUAILS *(COTURNIX JAPONICA)*

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Bee bread is product of beehive with high nutritional value and beneficial properties. Since legislation does not allow the addition of growth promoters into animal feed, bee bread might have potential as possible supplement into basal diet. The aim of the study was to investigate the effect of bee bread incorporated into feeding mixture in 0.2 %, 0.6 % and 0.8 % for 8 weeks on selected parameters of meat quality (content of water, proteins, fat) of female Japanese quails. The total number of animals was 40, divided into four groups as follows: E1 (n = 10) 0.2 % bee bread, E2 (n = 10) 0.4 %, P3 (n = 10) 0.6 %, and the control without additives (C). The food and water were given *ad libitum*. After slaughter, meat samples were collected. Meat quality was determined on sample of *Musculus biceps femoris* for content of water, proteins, fat by method FT IR (Fourier Transform Infrared Spectroscopy) using Nicolet 6700 (Pragolab s.r.o.). Addition of bee bread into basal diet did not affect (P>0.05) following parameters: content of water, proteins, and fat. Our results can be used for further research.

Key words: meat quality, bee bread, Japanese quails

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EFFECT OF SELECTED ESSENTIAL OILS IN THE VAPOUR PHASE ON THE GROWTH *OF RHIZOPUS STOLONIFER* STRAINS

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Species of the genus *Rhizopus* are involved in the spoilage of many food products (breads, fruits, vegetables, etc.). The research was aimed on testing the effect of essential oils in the gas phase on the growth of *Rhizopus stolonifer* strains (2). Essential oils obtained from the following plants were used: *Oscimum basilicum* L., *Thymus vulgaris* L., *Satureja hortensis* L., *Salvia officinalis* L., *Origanum majorana* L., *Thymus serpyllum* L., *Origanum vulgare* L., *Pimpinella anisum* L., and *Carum carvi* L. Antifungal activity was tested by the micro-atmospheric method, during a seven-day cultivation (25 °C). Essential oils that completely inhibited the strains growth at a primary (selection) concentration of 625 µl EO.l^{-1} air were used to determine the minimum inhibitory doses. EOs dissolved in dimethylsulfoxid (DMSO) were prepared at different concentrations (from 500 to 15.63 µl EO.l^{-1} air). Obtained data were then used to calculate minimal inhibition doses (MIDs$_{90}$) using probit analysis. The strains reacted differently to the presence of essential oils. But *Thymus vulgaris*, *Satureja hortensis*, *Origanum majorana*, *Thymus serpyllum*, *Origanum vulgare*, and *Carum carvi* EOs completely inhibited the growth of all strains. *Oscimum basilicum*, *Salvia oficinalis*, and *Pimpinella anisum* EOs inhibited the strains growth only partially, and their effect gradually decreased. MIDs90 of *Thymus vulgaris* EO were 156.11 µl EO.l^{-1} air for the first *Rhizopus stolonifer* strain, and 204.40 µl EO.l^{-1} air for the second strain. MIDs$_{90}$ of *Origanum vulgare* EO were 160.63 and 151.51 µl EO.l^{-1} air. These EOs were evaluated as the most effective. MIDs$_{90}$ of *Satureja hortensis* EO were 167.60 µl EO.l^{-1} air for the first strain, and 311.26 µl EO.l^{-1} air for the second strain. MIDs$_{90}$ of *Thymus serpyllum* EO were approximately the same, 256.76 and 261.89 µl EO.l^{-1} air for the first and the second strain, respectively. *Pimpinella anisum* and *Carum carvi* EOs were rated as the least effective with the highest MIDs$_{90}$ (>500 µl EO.l^{-1} air).

Keywords: Essential oils, antifungal activity, vapour phase, *Rhizopus stolonifer*

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PHENOLICS CONTENT AND ANTIOXIDANT ACTIVITY IN SEEDS OF CHOSEN LEGUMES – WHITE BEAN, CHICKPEA, LENTIL

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The main goal was to determine the content of total polyphenols and the antioxidant activity in 9 varieties of white bean, 6 varieties of chickpea and 6 varieties of lentils which were cultivated in the soil with the same conditions. We priority evaluated a soil hygiene and risky toxic metals in observed legumes. Of the risk elements, the total content of cadmium and mobile forms of lead in the soil of the monitored location exceeded the limit value set by Law No. 220/2004 valid in Slovakia by 40%, 180 %, respectively, but met the threshold value by the European Commission (2006). In all lentil varieties, the toxic elements lead, and cadmium were below the detection limit. In the case of chickpea and bean, limit values of lead content were measured in the varieties Kráľová z Krajová, Maškovský Bagovec, Bušinský (chickpeas) and Albena, Luna, Sina (white beans). In the chickpea varieties, the cadmium content was below the detection limit with exception variety Slovák – reached the limit value. In white beans, the cadmium content exceeded the limit values only in the Petra variety. The highest content of total polyphenols (1333-1973 mg.kg^{-1}) and the highest percentage of antioxidant activity (17,4-33,5%) was determined in lentil varieties, therefore we can conclude that lentils belong to legume foods with excellent antioxidant properties. We measured the lowest amount of total polyphenols (501-836 mg.kg^{-1}) in white bean varieties. On the other hand, the antioxidant activity in the same bean varieties (2,05-11,06 %) was higher than the percentage of antioxidant activity in chickpea varieties (0,6-5,0 %). There are statistically significant differences in the content of total polyphenols and antioxidant activity between the individual varieties of the chosen legumes. Internal variability was confirmed in the selected legume varieties, the variety has an effect on the content of total polyphenols and antioxidant activity. A statistically significant difference was also found between legume species. The dependence between the antioxidant activity and the total polyphenols was confirmed in lentils. In chickpeas, the content of total polyphenols has no effect on the value of antioxidant activity. A partial effect of the total polyphenols content on the antioxidant activity was confirmed in beans.

Keywords: legume, polyphenols, variability

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THE EFFECT OF AMYGDALIN ADDTION ON SOD AND GPX ACTIVITY

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The Rosaceae family is characterized by cyanogenic glycosides. Amygdalin is non-toxic but together acid or appropriate hydrolytic enzymes compound produce toxic hydrocyanic acid (HCN). In our study we investigated influence of amygdalin on activity of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) in the kidney. The amygdalin was short applicated. Male California meat rabbits of the P91 rabbit line (n = 20) was investigated in the experiment. The first control group was fed by standard commerce feed mixture. The experimental groups E1 and E2 was fed by standard commerce feed mixture. Solution of amygdalin was applied by intramuscular injection to musculus in concentration 0,6 and 3,0 mg/kg b. w. daily per 14 days. Animals from experimental group E3 and E4 were fed by crushed apricot seeds, at dose 60 and 300 mg/kg b. w. which were mixed into the feed mixture. We found out the significant differences ($p < 0.05$) between SOD activity in the control group (mean 143,48±5,12 U of SOD/mg of protein) and experimental groups E2 (mean 258,56±46,26 U of SOD/mg of protein) and between the control group and the E4 group (mean 256,46±60,83 U of SOD/mg of protein). We observed differences between GPx activity in the control group (mean 30,08±2,6 U of GPx/mg of protein) and experimental groups E1 (mean 72,26±15,01 U of GPx/mg of protein) and E2 (mean 123,6±16,55 U of GPx/mg of protein). The information about effects of amygdalin is contradictory and these is necessary to research more.

Keywords: amygdalin, Glutathione Peroxidase, Superoxide Dismutase, rabbit, kidney

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DETECTION OF LUNASIN GENE IN DIFFERENT PLANTS USING ddPCR

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In the last years the health-promoting effects of whole grains have gained attention as they can protect against many diseases. Lunasin is a bioactive peptide whose anticancer properties are widely reported in cereals. It was originally isolated from soybean seeds, but lunasin peptide was also found in other cereal (wheat, oat, amaranthus, barley etc.). However, there are still doubts about its origin because lunasin gene was detected in seed storage proteins of dicotyledonous plants, but not in cereal seeds, even though the peptide itself was present in wheat which was attributed to possible pathogen contamination. Due to its anticancer properties, it is very requested and new transgenic varieties of soy were prepared to increase the amount of this peptide, which can be isolated and purified for medical purposes. In our work we tried to identify lunasin gene in different plants to confirm its presence using droplet digital PCR because of very high sensitivity of this method. For detection of lunasin gene we tried different combination of primers previously used by other authors but using standard PCR procedure and different plants, commonly produced in Slovakia (peas, chickpeas, wheat, barley, oats, amaranth, sorghum, maize, sunflower). First, we analyzed soybeans to obtain information about best primer combination for the identification of the lunasin gene in our collection. Lunasin gene was identified in all studied plants, but with different occurrence not only between crops but also between different varieties of each crop. Based on our analysis beside soybeans also chickpeas seeds can be used as a source of lunasin peptide in food industry to prepare healthy food.

Keywords: Lunasin, peptide, droplet digital PCR

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THE EFFECTS OF VARIOUS FERTILIZATION AND TILLAGE ON UPTAKE CHROMIUM AND NICKEL IN FLOUR OBTAINED FROM SELECTED VARIETIES OF BARLEY

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In our study, the samples of barley flour were analysed, where chromium and nickel contents were determined depending on different agrotechnical methods of growing cereal plants. The field trials were based on a plot of land in Dolná Malanta near the city of Nitra. In the experiment, three different dosages of macroelements into the soil were applied, and two methods of soil treatment by ploughing were also performed. Barley flours were obtained by grinding of grains of four varieties of spring barley: *KM2084, Lédi, Xanadu and Marthe*. Flour samples were mineralized by wet way using of microwave digestion, and after precise dilution, the final chromium and nickel contents were determined by AAS method. The highest value of chromium in ground barley flour (0.77 mg.kg-1) was determined in the second variant by Xanadu variety and in the third variant by Lédi variety grown on the area where minimization ploughing was applied, conversely, the lowest value of Cr in flour (0.17 $mg.kg^{-1}$) was determined by Marthe variety in the control (unfertilized) variant. According to the Food Codex of the Slovak Republic, the maximum permissible amount of chromium in cereals is 0.5 $mg.kg^{-1}$ in grain - in our examined cereals of the Lédi variety (in all variants where minimization ploughing was applied), the maximum permissible amount of this contaminant in flour was exceeded. The amounts of nickel in barley flour ranged from 0.05 to 0.76 $mg.kg^{-1}$. Both of these extremes were evaluated by the variety KM2084, which was grown on an area with conventional ploughing. The highest permissible content of this heavy metal is 1 $mg.kg^{-1}$ in grain according to the Food Code of the Slovak Republic and it was not exceeded in our analyses. Fertilization in the dose of $N_{70}+P_{4.4}+K_{16.6}$ (2^{nd} variant) compared to the control resulted in an increase in chromium contents in the flour obtained from all varieties of barley plants that grew on the plots where the minimization ploughing (statistically significant by three varieties) was applied and, on the contrary, its statistically significant decrease by Xanadu and Marthe varieties growing on areas with conventional ploughing. In the remaining two varieties (KM2084 and Lédi), this ratio of nutrients did not affect the Cr contents in their barley flour. In the case of conventional tillage, statistically non-significant increased Cr contents were recorded in the barley flour from plants that grew on limed and fertilized soil compared to cereals growing on the same fertilized soil. Increased contents of this element in the grain were also recorded in the comparison of the fourth variant with the control. On the contrary, in the last variant (conventional ploughing), a decrease in the amount of nickel in barley flour was observed compared to the penultimate variant in all varieties of spring barley, except for the variety Marthe. Ploughing itself with the absence of NPK macroelements in the soil also had the effect on the change in the contents of both monitored heavy metals in the barley flour obtained from the grain of our cereal varieties. The application of conventional ploughing compared to minimization ploughing caused an increase in nickel contents in grain in all varieties of spring barley and at the same time a decrease in chromium contents, except for the Marthe variety.

Keywords: fertilization, tillage, flour, heavy metals

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RAPID DETECTION OF PATHOGEN MICROORGANISMS FROM SEEDS OF VEGETABLES WITHOUT DNA ISOLATION

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Many pathogenic microorganisms are able to contaminate the seeds of important types ofvegetables, such as tomatoes, peppers, garlic, onions and subsequently infect their plantsin the ripening process, causing significant economic damage. Despite the chemical treatments of the seeds, some pathogenic microorganisms have a certain probability to survive and therefore it is necessary to have quick specific proof of their presence before their distribution.
An effective and rapid procedure was developed for the detection of pathogenic microorganisms *Xanthomonas euvesicatoria, Xanthomonas gardneri Xanthomonas perforans, Xanthomonas vesicatoria, Xanthomonas campestris, Clavibacter michiganesis, Pseudomonas syringae* contaminating the above vegetable seeds without the necessary of DNA isolation before PCR application.

Keywords: pathogen microorganism, DNA isolation, vegetable seed

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CFU ASSAY OF RABBIT BONE MARROW CD45 DEPLETED CELLS

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Recently, we reported the possibility to use the specific MethoCult™ medium for hematopoietic colony-forming unit (CFU) assay of rabbit bone marrow mononuclear cells (BMMCs). These promising results indicate the usefulness of this CFU assay to measure proliferation and differentiation of individual progenitor cell within a rabbit sample enriched for hematopoietic progenitors such as BMMCs depleted for mature hematopoietic $CD45^+$ cells. Briefly, rabbit BMMCs were isolated from bone marrow using density gradient centrifugation (Biocoll, Biochrom). The cells were firstly incubated with antibody against rabbit CD45 (L12/201, mouse IgG1, Bio-Rad), washed and incubated with anti-mouse IgG1 MicroBeads (Miltenyi Biotec) and washed again. Afterwards, $CD45^+$ cells were magnetically depleted from the sample using AutoMACS Pro Separator (program Depletes, Miltenyi Biotec). Enriched cell sample was expanded in a serum-free StemSpan™ SFEMII medium containing 10% of $CD34^+$ Expansion Supplement (Stemcell Technologies) and 0.5% of penicillin/streptomycin mixture (Life Technologies) for 2 days. The expanded cells were diluted in Iscove's Modified Dulbecco's Medium with 2% Fetal Bovine Serum (FBS; Stemcell Technologies) to prepare three different 10x plating concentrations. Then, each cell suspension was mixed with complete MethoCult™ medium (H4435 Enriched; Stemcell Technologies) at the ratio of 0.1:1 ml to get final plating concentration (500, 1000, and 2000 cells per well) and carefully dispensed in a volume of 1.1 ml to 6-well SmartDish™ culturewares as reported previously. CFU assays were performed in duplicates for each cell concentration. Samples were cultured for 14 days in order to proliferate into CFU-E (Colony-forming unit-erythroid), BFU-E (Burst-forming unit-erythroid), CFU-GM (Colony-forming unit-granulocyte, macrophage) and/or CFU-GEMM (Colony-forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte) colonies. Afterwards, plates were observed under Zeiss Axio Observer.Z1/7 microscope and CFU colonies were identified and counted using STEMgrid™-6 (Stemcell Technologies). Expression of typical CFU markers (CD14 and CD115 – monocyte/macrophage, CD15 – granulocyte, CD71 and CD235a – erythroid) were also analyzed in CFU samples using RT-PCR. The experiment was performed three times. The depleted and expanded bone marrow samples produced mainly CFU-GM colonies and rarely also CFU-GEMM colonies as observed by light microscopy. RT-PCR analysis confirmed expression of all CFU markers (CD14, CD15 and CD115 typical for CFU-GM, and CD71 and CD235a typical for erythroid cells – most probably CFU-GEMM). Moreover, we determined the optimal plating concentrations for CFU assay of CD45 depleted and expanded BMMCs to 2000 cells per well. In conclusion, depletion of $CD45^+$ cells might enrich the rabbit BMMCs for hematopoietic progenitors.

Keywords: rabbit, bone marrow, MACS, hematopoietic progenitors, CFU assay, RT-PCR

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GENETIC POLYMORPHISM OF MAIZE (*ZEA MAYS* L.) DETECTED BY PROTEIN MARKERS

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Maize (*Zea mays* L.) is an annual, cross-pollinated by wind and the only monoecious among cereal crops to have male and female inflorescences on separate branches of the same plant. It belongs to grass family *Poaceae* (*Gramineae*) which is leading in importance in the order Poales. This family contributes to the world economy, food and industry through valuable crops i.e. wheat, rice and maize. The aim of the work was a general analysis of the biochemical composition the grain and subsequent molecular analysis of the 30 genotypes and 34 lines of maize grain (*Zea mays* L.) proteome with the main emphasis on the detection of its polymorphism and diversity. Maize genotypes (30) were obtained from the Gene Bank VURV Praha-Ruzine (Czech Republic) and 34 line of maize were obtained from the breeding station Zeainvent Trnava Ltd. (Slovakia). The proteome of maize grains was analyzed by SDS-PAGE and A-PAGE. The number of evaluable protein bands detected by SDS-PAGE ranged from 18 to 22 and by A-PAGE from 14 to 19. Dendrograms based on SDS-PAGE and A-PAGE were constructed with UPGMA and Jaccard coefficient and the genotypes were divided into 2 main clusters. Based on the obtained results, we can state that the SDS-PAGE technique is more efficient and better for the study of maize polymorphism than the A-PAGE technique. This is because using SDS-PAGE we could not distinguish only 4 genotypes and using A-PAGE we could not distinguish up to 16 maize genotypes and lines. It seems to be effective tool for the maintenance, evaluation and utilization of maize germplasm which is the only source to be utilized for the development of new varieties during breeding programs.

Keywords: maize, dendrogram, protein markers, diversity

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CURRENT OVERVIEW IN THE FIELD OF APPLICATION OF EDIBLE COATINGS/FILMS TO MEAT AND MEAT PRODUCTS

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Due to its high content of nutrients, pH, water activity and content of autolytic enzymes, meat is characterized as a food subject to rapid spoilage, as it is a suitable environment for the growth of a wide range of microorganisms, the action of contained autolytic enzymes or the natural oxidative changes of fats and proteins. Currently, several innovative meat preservation techniques and packaging technologies are being introduced with the aim of eliminating the effects of negative factors during storage. Consumers are currently increasingly asking for the minimization of the addition of chemical preservatives and the application of so-called green technologies and packaging, which are environmentally friendly and at the same time effective in extending shelf life. Edible coatings/films, as part of active and intelligent packaging, can meet these requirements. Edible coatings and films are produced from biopolymers of plant and animal origin, which are characterized by their biocompatibility and biological degradability and, in some cases, natural antimicrobial and antioxidant properties. Because of its biocompatibility, it is possible to incorporate biologically active compounds with antimicrobial, antioxidant, and health-promoting effects into the structure of edible coatings/films, thereby ensuring added value. From the overview of published studies dealing with this issue, it can be concluded that attention is mainly focused on phytochemicals, probiotics, protein hydrolysates, bioactive peptides, or the application of nanotechnology. A particularly interesting area is the possible application of bioactive peptides, which are being investigated for their broad bioactive effects from the point of view of pharmacy or medicine. Bioactive peptides are characterized by their antimicrobial, anticoagulation, immunomodulatory, antiproliferative, and antioxidant properties, which makes them an attractive compound that can be incorporated into the matrix of edible coatings/films, which is also confirmed by the partial results of our ongoing research on their application *in vivo*. The advantage of bioactive peptides is their possible synthesis in laboratory conditions with a previously required structure of amino acids and also their non-influence on the natural sensory attributes of meat, as is more often the case with the application of phytochemicals in the form of separate polyphenolic compounds or in the form of essential oils and extracts. Currently, as the only bioactive peptide is registered Nisin under the EU legislation as a food additive with the code mark E 234. Overall, the subject area of research breaks down the boundaries between food, food packaging and preservation and takes it to the next level. The current legislation lacks specifics that would deal with this issue, despite the existence of a wide range of legislative regulations regarding packaging materials, additives, as well as active and packaging materials and objects in contact with food.

Keywords: Active Edible coatings, active packaging, bioactive compounds, bioactive peptides.

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STORAGE OF FRESH AND MARINATED MEAT INTENDED FOR GRILLING

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The aim of the work was to evaluate selected aspects of health safety and quality of fresh and marinated poultry meat intended for grilling. We focused on microbiological and physico-chemical analysis of fresh, marinated and grilled meat. Marinated meat was kept under various conditions until it was heat-treated. During the experiment period, we monitored total viable count of microorganisms (TVC), coliform bacteria, filamentous microscopic fungi and yeast, but also water activity, pH, salt content and fat content of fresh, marinated and grilled meat. The highest value of TVC (4.91 log CFU/g) was in marinated meat after 12 hours of storage at 0 – 4 °C (B). TVC were not detected in the samples of grilled marinated meat (BG, CG, DG, EG). Microscopic fungi did not form the predominant microflora of the analyzed samples. The incidence of coliform bacteria was differentiated in meat samples. The highest value (3,02 log CFU/g) was detected in marinated meat (D). In the meat samples, coliform bacteria achieved zero values after heat treatment. The highest aw (0.99) was measured in samples AG and D. In contrast, the lowest aw (0.92) was measured in the sample EG. The highest pH (6.30) was measured in the sample EG. The range of mean pH values ranged from 5.82 – 6.15. The salt content was very different in each sample. The highest salt content of salt content (1.00 g/100 g) was in the sample CG. The lowest value of salt content (0,10 g/100 g) was measured in the sample AG. No sample of heat-treated meat exceeded the maximum permissible salt content (max 1.3 g/100 g). The fat content of the raw meat samples was higher compared to the heat treated meat samples. The difference between the lowest and the highest fat content was very pronounced. The lowest value of fat content 3.40 g/100 g was obtained in the sample AG. The highest value of fat content (10.24 g/100 g) was detected in the sample D. All the obtained results were statistically evaluated. The data were subjected to the analysis of variance (ANOVA) in the general linear models (GLM), Scheffe's test and Pearson correlation coefficients (r_{xy}). The level of significance associated to the statistical test was 0.05. Under the Decree of Ministry of Health of the Slovak Republic No. 125/2017 on requirements for catering facilities, the meat intended for grilling must be stored for a maximum of 48 hours at a temperature of 0 to 4 °C. Performed microbiological analyses of meat have shown that the temperature range from 0 to 4 °C is truly the most suitable one and it also respects the requirements for storage of fresh poultry meat, but in terms of storage we have achieved better microbiological quality results at 12 hours. However, from a culinary point of view, this period is not sufficient for the penetration of substances from the marinade into the meat. It is therefore necessary to find in practice such a combination of temperature and time conditions, so that both hygienic and culinary criteria are met, respecting the legislative regulations.

Keywords: poultry meat, marinating, grilling, microorganisms, food safety

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THE CYTOTOXICITY ASSESSMENT OF *TRIBULUS TERRESTRIS* L. *IN VITRO*

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Tribulus terrestris L. is an annual herbaceous plant frequently used in the traditional medicine of India, Turkey, and the southern part of Europe. Due to its unique mixture of biologically active compounds could be used to treat urological infections, cardiovascular difficulties, and prostatic hypertrophy as well. *Tribulus* is extremely rich in saponins, flavonoids, alkaloids, and phytosteroids, which makes it useful in herbal mixtures and drugs to treat sexual dysfunction and enhance sexual performance. The main objective of *in vitro* study was to determine the potential consequences of *Tribulus terrestris* on cell membrane integrity and lysosomal activity in TM3 cells. Mice Leydig cells were exposed to different experimental doses starting from 37.5 µg/mL to 600 µg/mL of ethanolic extract for 24-h. The analyses of cell membrane integrity were performed by CFDA-AM assay, followed by determination of the lysosomal activity (Neutral Red uptake). The gained results revealed significant ($p>0.0001$; $p>0.01$) damage of cell membrane integrity at 300 µg/mL and 600 µg/mL. A similar tendency was recorded in the case of lysosomal activity, where the same experimental concentrations inhibit the presented parameter significantly ($p>0.001$; $p>0.05$). Considerably more detailed and systematic research is definitely required for a better understanding of consequences associated with the effect of *Tribulus terrestris* on animal and human reproduction as well.

Keywords: *Tribulus terrestris*, Leydig cells, membrane integrity, lysosomal activity

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TEXTURAL PROPERTIES OF VACUUM-FRIED CHIPS, PREPARED FROM DIFFERENT VARIETIES OF POTATOES

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A potato chip is a thin slice of potato that has been fried until crunchy. Given the popularity of chips, the good news is there are now several technological processing that results in a lot lower fat and acrylamide. One of them is the preparation of potato chips using a vacuum fryer. We compared seven potato chip varieties using TA from a textural poinft of view.XT plus analyzer. A rupture test was performed on the vacuum-fried potato chips obtained from seven potato varieties. To describe the texture of the samples, we focused on the hardness, defined as the force at maximum compression. We analyzed seven varieties of potato chips prepared using a vacuum fryer under common conditions: Chateau, Agáta, Melody, Taisiya, Anne Bell, Manitu, and Colomba. The highest hardness, 668,09g, had variety Taisiya, and the lowest hardness, 551,09g, had variety, Chateau. The analysis was supplemented by a sensory assessment performed by a sensory evaluation panel.

Keywords: potato, texture, frying, varieties, hardness

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