

**Slovak University of Agriculture in Nitra**

**FACULTY OF AGROBIOLOGY AND FOOD RESOURCES**

**FACULTY OF BIOTECHNOLOGY AND FOOD SCIENCES**

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of FAFR and FBFS with international participation**

-  
**Proceedings of abstracts**



**on occasion of the Science and Technology Week  
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## **Scientific Conference of PhD. Students of FAFR and FBFS SUA in Nitra - Proceedings of abstracts**

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**Studying hot air drying of sweet potatoes with dual frequency ultrasound pretreatments and its influence on the quality and bioactive composition by HPLC-DAD and FTIR analysis**

Muhammad Tayyab RASHID, Haile MA<sup>\*</sup>, Mushtaque Ahmed JATOI, Asif WALI, Bushra Safdar MALIK, Muhammad HASHIM .....67

## Preface

This conference Proceedings collected selected reviewed abstracts and provides an opportunity for students, scientists and other readers to engage with a selection of referred papers that were presented during the most comprehensive academic scientific conference of PhD. students hold on November 06, 2018 in Nitra, Slovak Republic. Submitted full scientific papers were reviewed and the best papers have been published in Journal of Central European Agriculture, Acta Fytotechnica et Zootechnica, Agrochemistry, The Journal of Microbiology, Biotechnology and Food Sciences and Slovak Journal of Food Sciences.

The scientific Conference of PhD. students was organised on the occasion of the Science and Technology Week in the Slovak Republic at the Slovak University of Agriculture in Nitra under the auspices of doc. Ing. Peter Ondrišík, PhD. – dean of the Faculty of Agrobiology and Food Resources (FAFR) and prof. Ing. Ján Tomáš, CSc. – dean of the Faculty of Biotechnology and Food Sciences (FBFS).

The main aim of the Conference was to provide a platform for presentation of research findings, exchange of experiences and knowledge sharing. The abstracts were split equally among the eight conference areas:

- Agrobiotechnologies
- Animal production
- Applied and molecular biology
- Human nutrition
- Multifunctional agriculture, environment and rural development
- Plant production
- Technology, quality and safety of raw materials and foodstuffs of animal origin
- Technology, quality and safety of raw materials and foodstuffs of plant origin

There is no doubt that every our PhD. conference has encouraged the further advancement of interesting sources through fruitful discussions among students and other participants. Committees hope, that participants find valuable their engagement with various ideas in sustaining their own professional development and education; and that the scientific programme have contributed to increasing the knowledge, improving the future work and building new friendships between PhD. students from different countries.

doc. Ing. Peter Ondrišík, PhD.  
Dean of FAFR

prof. Ing. Ján Tomáš, CSc.  
Dean of FBFS



SECTION  
*Agrobiotechnologies*

## **Technogenic degraded soils and their remediation problems**

Bakhrom JOBBOROV, Zafarjon JABBAROV

*Supervisor:* Dr. T. Abdrakhmanov

Nowadays, because of the development of natural deposits, road construction, using land for different purposes causes the impact on soil and of course at the same time it needs of remediation. The problem of remediation of technogenic degraded soils is that they don't have universal method or technology and it can not be. The following obstacles prevent the general approach of technogenic soil remediation:

### **Factors that damage the total remediation of technogenic distributed soils**

To have different chemical properties of pollutants

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To have different types of soils

To have different soil and climatic conditions

Fertility of soil microorganisms

Specificity of flora

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There are also differences in the soil pollution. The strong contamination of most of the elements was detected in Bekabad district, including an increase comparing to ПЭЧУ - AS 4.2 times, Cr - 16 times, Na - 2.5 times. Rb - 3.7 times, Zn - 4.8 times. It has been observed that the man-made elements that have been deposited in the soil and have been affected for many years, and have a negative impact on the biological environment, chemical composition and physical properties of the soil as well. About four km radius of pollutants have been explored and the ecological state of the environment has been significantly damaged.

Their negative impact on the living population in the surrounding area, their livestock and gardens can be understood from their severe objections. The aim of our research is to determine the levels of man-caused effects, identifying the mechanism of impact on soil properties and developing appropriate recultivation technology which based on individual approaches. Currently, technical re-cultivation of the soil cover is being carried out and technical arrangements are underway in field conditions. Based on the results, concrete recommendations on recultivation will be given.

**Key words:** *soil, pollution, remediation, productivity, method, heavy metals*

**Acknowledgement:** The study was implemented within the project funding by the grants "OT-A-QX-2018-399" we thank to all of the project coordinators for their kind help and support.

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### **Monitoring techniques for western corn rootworm in Croatia**

Martina MRGANIĆ, Renata BAŽOK, Katarina M. MIKAC, Darija LEMIC

*Supervisor:* Renata Bažok PhD, Katarina M. Mikac PhD, Darija Lemic PhD

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, (WCR) is economically the most important pest of maize in Croatia. First occurrence of WCR in Europe was in 1992 near Surčin, Serbia. Till nowadays different monitoring techniques have been conducted in Croatia to define and predict WCR population abundance and variations through all invasion phases. Traditional population metric surveys were conducted in first years of WCR invasion in Croatia with the aim to define population abundance and dispersal. Later a modern monitoring technique such as population genetics (using microsatellite markers) was used to provide information on population variations within and among WCR populations in Croatia and its neighbouring countries. To better understand WCR invasion process and to define the impact that this invasive species have on global agriculture production and food resources during the next few years we will use a single nucleotide polymorphisms (SNPs) method. SNPs are single base substitutions found at a single genomic locus. For use in population genetics, SNPs have surpassed microsatellites as the marker of choice. Compared with microsatellite loci SNPs have lower allele diversity and provide less statistical power to discriminate unique genotypes but have a denser and uniform distribution within genomes which make them useful for population and mapping studies. Furthermore, SNPs has become an affordable and readily accessible means of generating important data on a species. During my PhD thesis investigation we will use SNPs on total WCR populations from invaded area in Croatia as well as populations from most infected regions in U.S. with the main purpose to establish detail genomic description of this invasive species. WCR in last decade in its native region in U.S. developed resistance on all control measures. Considering ongoing multiple introductions from U.S. to Europe, which was proved in many surveys, the resistance could become serious problem in Croatia and Europe as well. Detail genomic description could give us an answer about resistance which is probably genetically conditioned. Considering this our aim will be to find genetic marker which could be used in detection of resistance pattern in WCR.

**Key words:** *Diabrotica virgifera virgifera, monitoring techniques, Croatia, maize, population genetics, SNPs*

**Acknowledgement:** This work was supported by Croatian Science Foundation through project: Monitoring of Insect Pest Resistance: Novel Approach for Detection, and Effective Resistance Management Strategies (MONPERES).

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**Antioxidant activity of *Corylus avellana* L. cultivars – Hallská obrovská,  
Lombardská biela and Webbova**

Nataliia NIKOLAIEVA

*Supervisor:* doc. Ing. Janka Nôžková, PhD.

The European hazelnut, *Corylus avellana* L., is the species of commerce and is genetically diverse. This species has economic importance as edible nuts and good quality timber. Hazelnuts are an excellent source of antioxidants. Hazelnut antioxidants were studied in hazelnut oil, kernels, skin, leaves, seed husk. There was not enough attention given to catkins and pollen antioxidant activity of *C. avellana*. In our work cultivars of Hallská obrovská Lombardská biela and Webbova were studied. Samples were prepared from Botanical garden in the SUA Nitra. Antioxidant activity of hazelnut catkins and pollen were analyzed. Antioxidant activity determined by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method by Brand-Williams et al. (1995). Extracts were prepared from 1 g of dry substance and 25 ml of methanol (99.5%) and distilled water, mixing on the shaker during 12 hours. For basic solution the 0.025 g of DPPH and 100 ml of methanol were used. The measurements were carried out by a spectrophotometer GENESYS 20 (USA) at the wave length 515 nm. The percentage of inhibition DPPH determined according to the formula:  $((A_0 - A_{10})/A_0) * 100$ , where  $A_0$  – is a value of sample with 3.9 ml of working DPPH solution and  $A_{10}$  – is value of sample with working DPPH solution and pollen extract. The antioxidant activity of hazelnut pollen in methanolic extracts was in the range from  $60.80 \pm 1.10\%$  to  $63.33 \pm 1.08\%$ , and in aqueous extracts – from  $31.56 \pm 1.58\%$  to  $34.26 \pm 3.06\%$ . In methanolic extracts the antioxidant activity of hazelnut catkins was in the range from  $78.03 \pm 0.36\%$  to  $79.58 \pm 0.24\%$ , and in aqueous extracts the antioxidant activity of hazelnut catkins was in the range from  $51.03 \pm 0.73\%$  to  $76.53 \pm 1.16\%$ . In methanolic extracts cultivars Lombardská biela, among hazelnut catkins, and Hallská obrovská, among hazelnut pollen, had the strongest antioxidant power. In aqueous extracts cultivars Webbova, among hazelnut catkins, and Lombardská biela, among hazelnut pollen, had the highest antioxidant activity. In general, antioxidants activity of dry hazelnut catkins in methanolic and aqueous extracts is higher than hazelnut pollen.

**Key words:** *antioxidant activity, Corylus avellana* L., *cultivar*

**Acknowledgement:** This publication is partly carried out within the International project “FarmersEduca – Neglected and Underutilized Species in Socio-Economic Rural Development” under the serial number 2164044.

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### **Soil changes around the reservoir and their ecologic state**

Adiba TURSUNQULOVA, Charos DJALILOVA, Zafarjon JABBAROV

*Supervisor:* Prof. Sadulla Abdullaev

It is known that the natural change of soil is a long-lasting evolutionary process, but its rapid change takes place as a result of various human influences, especially in the process of soil erosion, which is an urgent issue for the history of soil as a result of complex change of soil coating, transition to other types of physical, chemical and biological properties of soil, hydrothermal, nutrition and air modes. This situation is aggravated by reservoirs. The research is based on the study of the natural landscape of the Kattakurgan reservoir, (39°50'00-66°15'00) which was built during 1940-1951 years studying soil cover and its changes, the study of hydro geological and hydro ground waters, modern hydromorphism process and the work on elaborating scientifically-based recommendations, on the determination of the impact of the reservoir on the process of its expiration the effective use of land and water on the basis of the obtained results, conservation, restoration and enhancement of soil fertility. Relying on the obtained results there has been some changes focused on keeping soil fertility and its natural properties. This includes soil cover, mineralization level of underground waters, the types of moisture, the moisture content of the soil, and their properties in the following sequence were determined (table).

#### **The sequence of changes occurring in soils that are spread around the reservoir**

Physiologic al and quantitative variation of microbio- logical universe	Changes in physical properties, and the deterioration of the mechanical composition	The accumulati on of organic substances and the erosion process	Changes in chemical composition of spill, increase or decrease of pH environment	Degradation of the hydrothermal process, overheating of the air regime	Reduction of quantity of nutritional elements reduction of productivity indicators
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The change of soil cover around water reservoirs is mainly caused by the following two 1). Additional moisture increase in soil layers (extraction, gulling, wetting ): 2) Continuous changes in the flora are due to the change in factors such areas: The remaining changes are influenced by the two factors, including Heterotrophic microorganisms, actinomycetes, fungi, nitrifiers, and moisture content the moisture content of the soil drips to the humidity and the amount of the vegetation is reduced. The accumulation of organic matter is accompanied by fast water, annual biomass accumulates in moisture conditions and organic matter is optimally concentrated, moisture is high, and the air volume decreases, resulting in a reduction in the amount of humus over the years.

**Key words:** *water reservoir, soil, plant, humus, ecological condition, microbiological properties, biomass*

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SECTION  
*Animal Production*

## **The differences in fatty acid composition of sheep milk and suckling lamb meat**

Martin JANÍČEK

*Supervisor:* Assoc. Prof. Milan Margetín

The fatty acid (FA) composition of ewes' milk were compared to the FA composition of intramuscular fat (IMF) of *Musculus longissimus lumborum et thoracis* (MLLT) of light lambs from traditional rearing system. The milk of experimental flock of three hundred and twenty-eight ewes belonging to three breeds (148 Tsigai, 124 Improved Valachian, and 56 Lacaune) grazed on natural pasture was taken and sampled during the morning milking. The total eighty light slaughtered lambs of four breeds (20 Tsigai; 20 Improved Valachian; 20 Slovak dairy sheep and 20 Merino) were used for comparison. *Musculus longissimus lumborum et thoracis* were taken between 9th and 13rd thoracic vertebra of each lamb. Milk and intramuscular lipids were extracted and transesterified to fatty acid methyl esters (FAME) for determination of milk and meat FA composition by using capillary gas chromatography. Differences between milk and meat FA composition were analysed by ANOVA using General Linear Model procedure in statistical software SAS 9.2. Estimated least squares means were compared using Scheffe's tests. Intramuscular fat of MLLT detected lower proportion ( $P < 0.001$ ) of C12:0 lauric acid (0.69 vs. 3.68 g/100g FAME), C14:0 myristic acid (5.28 vs. 9.58 g/100g FAME), C16:0 palmitic acid (22.23 vs. 23.25 g/100g FAME), *c*9-C16:1 palmitoleic acid (0.46 vs. 0.82 g/100g FAME), C18:1 elaidic acid (0.23 vs. 0.50 g/100g FAME), *c*9,*r*11-C18:2 rumenic acid (0.67 vs. 1.54 g/100g FAME), the total medium-chain FAs (1.02 vs. 14.94 g/100g FAME), the total saturated FAs (SFA) (45.12 vs. 66.45 g/100g FAME) and the sum of conjugated linoleic acid's isomers (CLA) (0.67 vs. 1.54 g/100g FAME). On the contrary intramuscular fat of MLLT had higher proportion ( $P < 0.001$ ) of C18:0 stearic acid (12.68 vs. 10.88 g/100g FAME), *c*9-C18:1 oleic acid (31.21 vs. 18.07 g/100g FAME), C18:2 *n*-6 linoleic acid (LA) (6.90 vs. 2.31 g/100g FAME), C20:4 *n*-6 arachidonic acid (3.14 vs 0.20 g/100g FAME), C20:5 *n*-3 eicosapentaenoic acid (EPA) (0.68 vs. 0.06 g/100g FAME), C22:5 *n*-3 docosapentaenoic acid (DPA) (0.91 vs. 0.15 g/100g FAME), C22:6 *n*-3 docosahexaenoic acid (DHA) (0.39 vs. 0.05 g/100g FAME), the total monounsaturated FAs (38.83 vs. 26.89 g/100g FAME), the total polyunsaturated FAs (PUFA) (16.04 vs. 6.47 g/100g FAME), the total long-chain (LC) FAs (98.05 vs. 86.24 g/100g FAME), the total *n*-6 PUFA (10.37 vs. 2.63 g/100g FAME) and the total *n*-3 PUFA (3.20 vs. 1.26 g/100g FAME). Ratios of important FA groups were lower ( $P < 0.001$ ) in ewes' milk fat: PUFA : SFA (0.10 vs. 0.37), total *n*-6 PUFA : total *n*-3 PUFA (2.15 vs. 3.29), LC *n*-6 PUFA : LC *n*-3 PUFA (1.22 vs. 1.57) and C18:2 *n*-6 : C18:3 *n*-3 (2.43 vs. 6.98). Atherogenic index and the index DFA (desirable FAs) were more favorable in IMF of lambs' meat than in the milk of ewes.

**Key words:** *sheep milk, lamb meat, fatty acids, conjugated linoleic acid*

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### **Fatty acid profile of different plant oils**

Renata KOLLÁTHOVÁ, Branislav VARGA, Eva IVANIŠOVÁ, Branislav GÁLIK

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The aim of this research was to determine the fatty acid profile of four plant oils (sunflower, soybean, flaxseed and rapeseed). Analyzed crops were grown in University Experimental Farm in Kolíňany. Seeds were processed in the Laboratory of Fats and Oils (AgroBio Tech Research Centre of the Slovak University of Agriculture in Nitra). Pressing unit FARMER 10 (Farmet, Czech Republic) was used. Laboratory samples were analysed in the Laboratory of Quality and Nutritive Value of Feeds at the Department of Animal Nutrition at the Slovak Agricultural University by standard laboratory methods and procedures. Fatty acid profile analysis was performed using the Agilent 6890 A GC machine by Agilent Technologies, USA. The analyzed oils mainly composed of polyunsaturated fatty acids (PUFA), with the exception of rapeseed oil which primarily contained monounsaturated fatty acids (MUFA). Flaxseed oil has significantly ( $P < 0.05$ ) proven to be the richest in PUFA content (76.46%), but on the other hand it contained the least amount of MUFA (13.47%). The saturated fatty acid (SFA) content, except for soybean oil, was below 10%. The most optimal ratio between n-6 and n-3 unsaturated fatty acids (USFA) was found in flaxseed oil (2.22:1). Linoleic acid,  $\alpha$ -linoleic acid (except sunflower seed oil), oleic acid, palmitic acid and stearic acid were the most common fatty acids detected in analyzed samples. On the contrary, arachidonic acid, eicosenoic acid, behenic acid and lignoceric acid were present in the analyzed oils the least, mostly below 1%. Significant ( $P < 0.05$ ) differences in the content of all the mentioned acids between the analyzed oils were found. Only in rapeseed oil were trace amounts of palmitoleic acid (0.22%), heptadecenoic acid (0.10%) and docosahexaenoic acid (0.13%) detected. Soybean oil as the only one contained margaric acid (0.11%). Eicosadienoic acid was not in the analyzed samples found.

**Key words:** *oils, fatty acids, MUFA, PUFA, SFA*

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## **Genetic diversity of Barbary lion based on genealogic analysis**

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The aim of this study was to evaluate the state of genetic diversity in population of Barbary lion based on the genealogical analysis. To evaluate the state of genetic diversity in the population of Barbary lion the indicators derived from the common ancestor were used. One of the main factors that affect the reliability of genetic diversity indices is the completeness of pedigree. Currently, this lion subspecies doesn't occur in the wild, and its population is considered to be critically endangered. The pedigree file consisted of 545 animals, while the reference population included 445 individuals. Alongside pedigree completeness, the parameters derived from common ancestor were used to analyse the state of genetic diversity in target population. The completeness of pedigree data had significantly decreasing tendency with increasing generations. The pedigree completeness index was the highest in the first generation (68%). The average value of the inbreeding coefficient was very similar in the reference population and the pedigree file ( $F = 0.05$ ). Across generations, the trend of inbreeding increase was positive mainly due to the long-term use of specific lines and families for mating. The relative high average relatedness among individuals ( $AR = 0.06$ ) only reflected the individual increase in inbreeding (3.18%). As expected the higher level of individual increase in inbreeding was found in the pedigree file (3.41%). The effective population size at level 26.66 confirmed that the Barbary lion is critically endangered by the loss of diversity. Because of this, the future continuous monitoring of genetic diversity of this subspecies is necessary, especially for long-term conservation purposes. In the population, there is an increase in inbreeding, and the population becomes less demographically stable. Size of the population and inbreeding are related and lead to the loss of genetic diversity. When comparing our results, it must be pointed out that the loss of the population is actually the result of the interaction between inbreeding, the impact of human activity, demographic instability and the loss of genetic diversity. These are the most frequent factors affecting the loss of population, except for other factors; in particular reducing adaptability, reproduction and population survival, etc. It would be appropriate to introduce a system of regular (annual) monitoring of the genetic diversity of lions kept in zoo gardens in the Czech Republic and Slovakia as a joint project in relation to the international database. The results of this work can be taken as an initial assessment.

**Key words:** *Barbary lion, diversity, endangered species, pedigree analysis.*

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## **The analysis of factors affecting the calving difficulty in Slovak Spotted cattle**

Barbora OLŠANSKÁ, Juraj CANDRÁK

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The aim of this study was to analyse several genetic and non-genetic factors that can affect the calving difficulty of Slovak Spotted cattle and to find out their statistical significance. A total of 417030 calving difficulty records from 174795 dams were collected during the calving parity from 2001 to 2017. For evaluation of the calving difficulty was used 4 – point scale: 1 – spontaneous calving (no assistance required); 2 – easy calving (assistance of 1 – 2 person); 3 – difficult calving (assistance of 3 or more person or a veterinarian); 4- caesarean section. The impact of factors affecting the calving difficulty was analysed by using procGLM implemented in SAS 9.3 on the basis of multifactor analysis of variance. Within the evaluation of calving difficulty, the highest occurrence for the spontaneous calving during months March and April (97.60%) was observed. The lowest occurrence was found for calving requiring the caesarean section during month May and June (0.06%). The analysis proved that the most calving difficulty was occurred in the heifers. In the second lactation was calving difficulty compared to the first lactation noticeably reduced. The value of determinant coefficient for the evaluated effect was  $R^2 = 1.04\%$ . In the dataset of Slovak spotted cattle were observed 1803 sires but for the evaluation were used only 14 most important sires. In the population of Slovak spotted cattle were the most used sire with the state register code HAT001 (Name: Xirno, line: Haxist) and the highest occurrence of difficult calving was observed in sire DIK005 (Name: GS Dionis, line: Dirteck). The effects of the herd, year of calving, sex of calf, breed type, month of calving, the parity and the sire were tested. The sex of born calf was the most statistically significant factor ( $R^2 = 25.5\%$ ). The determinant coefficient (R-square) of 0.254796 indicated that 25.5% variability of calving difficulty is explained by the examined effects, and the remaining 74.5% variability of calving difficulty can be explained by other reasons than the linearity between the variables. The calving difficulty was significantly affected also by the herd, year of calving, month of calving, parity and sire. Each of these effects showed high level of significance ( $P < 0.001$ ). The lowest level of statistical significance for effect of breed type was found. Based on obtained results is recommend to use the continuing recording of calving difficulty for the purposes of evaluation of effect of sire on inheritance.

**Key words:** *cattle, calving difficulty, non-genetic factors, reproduction*

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### **Intensity of inbreeding and diversity in Slovak spotted cattle in selected farm**

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The aim of the study was to analyze the pedigree of the Slovak spotted cattle in the selected farm, to estimate trends in inbreeding intensity, genetic diversity and loss of genetic diversity. The pedigree population consisted of 2688 individuals (334 bulls and 2354 cows). In reference population were 1119 individuals. The reference population consisted of living individuals. In the reference population there were 3 bulls used in AI and 1116 live cows and heifers. Bulls used in the insemination on the farm belonged to the TOP in the year 2016. Among the cows were pure-bred animals, but also crossbred with a larger or smaller proportion of other breeds in genotype. Wright (1965) defined the inbreeding coefficient as the probability that an individual has two identical alleles with another individual. The average coefficient of inbreeding intensity in whole population was 0.3540% and in the reference population was 0.7435%. When compared to a purebred population, we can see a good selection of bulls, but an increase in inbreeding coefficient in cows above 1%. There are 125 cows in the given population, which aren't inbred. Genetic diversity can be defined as the variety of alleles and genotypes in the population, whose express in morphological, physiological and behavioural differences between individuals in populations (FRANKHAM et al., 2002). The genetic diversity was at 96.15%. The total genetic loss in the observed population was 3.85%. This loss was caused by a bottleneck effect, which was 0.62% and the genetic drift was 3.23%. The problem of existing decrease in diversity is that the excessive use of a single line or family results in increase of inbreeding. The secondary effect of mating of close relatives is increase of inbreeding depression, which negatively affects the reproduction and fitness of animals (BEZDÍČEK, J. et al., 2007). Diversity can be maintained by a proper mating plan where we focus on genetic gain in production traits, but also on eliminating the increase of inbreeding and improving offspring fitness. In terms of indicators derived from the probability of origin of genes, the effective number of ancestors in the reference population was 17. All results can be determined by the high completeness of pedigrees, which was in the reference population 90% in the fourth generation. Pedigree analysis is important for the assessing the completeness of data which are base for further calculations and the validation of the calculated data (KADLEČÍK, KASARDA, 2007). This study can serve as a starting point for preparation of mating plans.

**Key words:** *inbreeding, genetic diversity, Slovak spotted cattle, pedigree completeness*

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### **The influence of probiotics on the quality of pork**

Patrycja RESZKA, Joanna BOGUĆKA, Wojciech KAPELAŃSKI

*Supervisor:* prof. Wojciech Kapelański

Meat quality is affected by mostly environmental factors, i.e. management conditions, pre-slaughter handling, slaughter and post-slaughter procedures- 70% and genetic factors- 30%. The main indicators of meat quality, which determine its eating attributes, are: acidity, color, water-holding capacity, drip loss, water-binding capacity, emulsifying properties, appearance (color and marbling), texture (tenderness and juiciness) and palatability (Karpiesiuk and Falkowski, 2009). The quality of meat has become an important consideration in recent years. One of the ways to improve the quality of meat may be the use of probiotic in animal nutrition. Probiotics (greek pro bios - for life) are living microbial cultures which are intended to act advantageously animal organism (Augustyniak and Nawrotek, 2014). For the production of probiotics uses lactic acid bacteria, and in particular the following strains: *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus lactis*, jak również *Bifidobacterium bifidum*, *Pediococcus acidilacti*, *Enterococcus faecium*, *Bacillus subtilis* and *Bacillus toyot*. They are also used some yeast species: *Saccharomyces cerevisiae*, *Saccharomyces boulardi*, or mold mycelium *Aspergillus oryzae* and *Aspergillus Niger*. Probiotic preparations can additionally be enriched with elements (Fe, Cu, Co), vitamins (A, E, D3, B12) and immunoglobulins colostrum (IgG). Bacteria used for production probiotic preparations should be isolated from representatives of this species, at which they are to be used, because probably a part of health benefits effects is species-specific (Janik et al., 2006). Research Liu et al. (2013) shows that diet supplemented with probiotics reduced the drip loss and cooking loss of pork ( $P<0.05$ ), significantly decreased the content of triglycerides and low-density lipoprotein ( $P<0.05$ ). Suo et al. (2012) also showed that addition of probiotic preparations (*L. plantarum*) improving pig growth and pork quality. These results indicate that the use of probiotic preparations positively affects the quality of pork. Although work on this subject has been created a lot over the past years, the vast majority of them concerned poultry. Therefore, there is a need to explore this issue based on pigs.

**Key words:** *probiotics, meat quality, quality of pork*

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**The effect of different feeding system on fatty acids composition of cow's milk**

Patrícia VAŠEKOVÁ, Renata KOLLÁTHOVÁ, Norbert ANDRUŠKA

*Supervisor:* doc. Ing. Miroslav Juráček, PhD.

The aim of the study was determined the effect of different feeding system on fatty acids profile of cow's milk. Tank's samples of milk were collected from two farms which are breeding Slovak Spotted breed. Feeding system on the base pasture + supplementary feeding (without silage) on the farm A and silage feeding system on the farm B was realized. The crude fat content and fatty acid profile in the milk samples were analysed (Agilent 6890A GC, Agilent technologies, USA). Feeding system affects fatty acid profile of cow's milk. Significantly higher proportion of C4:0, C17:0, C18:1 cis n9, C18:3 n3, C18:2 cis n9 and C20:0 in milk samples from farm with pasture + supplementary feeding system was detected. Milk samples only from this feeding system contained C20:5 n3. The results confirmed in the milk from the farm with silage feeding system significantly higher content of 18:2 cis n6 and presence of C13:0, C20:3 n6 and C20:4 n6 only in these samples were determined. Significantly lower proportion of saturated fatty acids was typical for the examined samples from feeding system based on pasture + supplementary feeding system and significantly higher proportion of polyunsaturated fatty acids was characteristic for the samples from silage feeding system. The influence of the feeding system on the monounsaturated fatty acids content was not confirmed. In milk samples from both feeding systems very different n6/n3 fatty acids ratio was detected (1.36 vs. 9.12).

**Key words:** *dairy cow, milk, fatty acids, feeding system, Slovak Spotted breed*

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SECTION  
*Applied and molecular biology*

## **Characterization of $\beta$ -1,3-glucanase originated from carnivorous plant species**

Monika FRÁTRIKOVÁ

*Supervisor:* Ing. Jana Libantová, CSc.

Carnivory in plants evolved as an adaptation strategy to nutrient-poor environments. Thanks to specialized traps, these plants can gain nutrients from various heterotrophic sources including small insects. Digestion in traps requires a coordinated action of several hydrolytic enzymes that break down complex substances into simple absorbable nutrients. Among these, several pathogenesis-related proteins including  $\beta$ -1,3-glucanases have previously been identified in digestive fluid of some carnivorous species. Currently, modern biotechnology methods enable to deploy the genes for hydrolytic enzymes with high enzymatic activity in plant transgenesis aimed to increase resistance to various phytopathogens. Since carnivorous plants have shown to be perspective sources of hydrolytic enzymes, in our research, we pay attention to isolation and characterization of  $\beta$ -1,3-glucanase gene(s) from carnivorous genus *Drosera*, which is characterized by high activity of hydrolases, especially in the digestion and defense processes. Previously we isolated the fragment of  $\beta$ -1,3-glucanase gene from carnivorous plant species using PCR with degenerate primers. Subsequently the Genome Walking method (involving the PCR with specific and adaptor primers) and sequencing were utilized for identification of flanking sequences to partial DNA sequence of isolated  $\beta$ -1,3-glucanase gene. Following the complete gene amplification and re-sequencing the bio-informatical analyses (involving CLUSTALW alignment, BLASTn and BLASTp searches) revealed that isolated sequence for  $\beta$ -1,3-glucanase exhibits high similarity to  $\beta$ -1,3-glucanases of carnivorous plants available in Gene Bank. Next *in silico* analysis showed, that genomic clone of  $\beta$ -1,3-glucanase has 1132 bp and contains one intron separating the signal peptide from remaining part of gene. The DNA sequence of  $\beta$ -1,3-glucanase after predicted splicing and translation corresponded to a protein with high similarity to plant  $\beta$ -1,3-glucanases belonging into the glycoside hydrolase family 17. The GlycoEP program enabled us to determine the presence of two potential N-glycosylated amino acid residues in the matured protein. Potential phosphorylation sites were determined through the NetPhos program. The absence of the C-terminal signal sequence responsible for the vacuolar localization indicates extracellular targeting of analysed  $\beta$ -1,3-glucanase.

**Key words:** *hydrolytic enzymes,  $\beta$ -1,3-glucanase, carnivorous plants, Drosera*

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**Antioxidant/antiradical activity of 1-*O*-galloyl-2,3-hexahydroxydiphenoyl-4,6-valoneyl- $\beta$ -D-glucose (PC-2)**

Ulugbek GAYIBOV, Esokhon KOMILOV, Rakhmatilla RAKHIMOV

*Supervisor:* Tahir Aripov

Presently, epidemiological, biological and clinical investigations have demonstrated an important role of cell membrane oxidation in the progress of atherosclerosis, cataract and some forms of cancer caused by free radicals. Such natural antioxidants as vitamin C, E and  $\beta$ -carotene are significant factors for prophylactics of these diseases. Biological activity of many plant polyphenols can be considered as a result of their antioxidant activity (AOA).

Here, we investigate the antioxidant (AOA) and antiradical activity (ARA) of polyphenol compound 1-*O*-galloyl-2,3-hexahydroxydiphenoyl-4,6-valoneyl- $\beta$ -D-glucose (PC-2). The effect of PC-2 on LPO in mitochondrial membranes depends on its concentration, i.e. with its increasing in the incubation medium, the percentage of inhibition became higher. Complete inhibition of liver mitochondrial swelling, i.e. of the LPO process was observed at 10  $\mu$ M of PC-2. At the same time, the concentration that caused the half-maximal inhibition of the LPO ( $IC_{50}$ ) process for PC-2 was  $6.08 \pm 0.06 \mu$ M. Thus, in the experiments it was shown that PC-2 possesses antioxidant properties.

Determination of lipid peroxidation process on mitochondrial membranes is a classical method for studying AOA of biologically active compounds. However, this method does not allow one to directly assess the contribution of each of these effects to the overall antioxidant activity of the compounds. In this respect, it is useful to determine ARA of compounds with the help of free radicals that carry free valence on their molecules.

ARA of above-mentioned compound has been studied by measuring the change in optical density of stable free radical 2,2-diphenyl-1-picrylhydrozyl (DPPH) in the ethanol solution after adding investigated compound. When polyphenol compound PC-2 is added to the alcohol solution of DPPH, the color of the solution changes, which indicates the transition of DPPH into a non-radical form. By analyzing kinetic curves of DPPH reduction with PC-2 it has been shown that investigated polyphenol has ARA.

From the experimental data it follows that the studied compound has an ability to quench free radicals. To estimate the antiradical activity we calculated  $t_{50}$  parameter, in which the time to reduce 50% of the initial concentration was used. 50% concentration of DPPH stable radicals in the reaction with the studied compound reached 9,6 sec., reaction rate constant ( $K 10^{-3}, c^{-1}$ ) equaled 5,3, and the half-maximal inhibition concentration ( $IC_{50}$ ) was 7.2 micromole.

Thus, antioxidant screening of new polyphenol compound PC-2 demonstrates high AOA and ARA measured by different methods. Correlation coefficient between AOA and ARA is  $r = 0.89$ .

**Key words:** *polyphenol compounds, antioxidant activity, mitochondria*

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**Characteristics of bacteria from soil and selected parts of sunflower  
(*Helianthus annuus* L.)**

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*Supervisor:* doc. Ing. Jana Maková, PhD.

Profitable bacteria colonizing the root region are also called PGPB (Plant Growth Promoting Bacteria). Their presence can be observed not only in the root area but also in the above-ground parts of plants (phyllospheric bacteria). They also occur inside the plant like endophytic bacteria. The aim of our study was analysis of microbial settlement of sunflower (*Helianthus annuus* L.) and soil by monitoring biochemical features. We have also focused on the diversity of microorganisms in relation to the plant growth phase by using next generation sequencing (NGS). The plants were cultivated in locality Koliňany. Samples were taken in three different phases of plant growth (BBCH 15, BBCH 55, and BBCH 85). Samples were separated to following groups: soil from outside the root system, soil from the rhizosphere, rhizoplane and root, stem and leaf (endophytic bacteria). Bacteria recovered from samples were screened to production of indole-3-acetic acid (IAA), production of siderophores using CAS tests, ability to dissolve phosphates, and to antifungal activity. DNA isolated from all samples was used for 16SrRNA gene sequencing on Illumina MiSeq. The obtained sequences were processed, clustered, and identified to phylum level. Diversity of microbial assemblage was evaluated. From soil and selected parts of sunflower we recovered 40 bacterial strains, which we further tested. All bacterial strains produced phytohormone indole-3-acetic acid (IAA) and 36 strains showed positive production of siderophores. From the total number of isolates, 16 bacterial strains achieved the low phosphate solubilization index ( $SI < 2.00$ ), 1 strain achieved the middle – class index ( $2.00 \leq SI < 4.00$ ), 1 strain achieved the high phosphate solubilization ( $SI > 4.00$ ) and 22 strains didn't have the ability to dissolve phosphates. Antifungal activity expressed by 100% inhibition of growth of mycelium of phytopathogenic fungi *Sclerotinia* sp. had 18 strains, *Rhizoctonia* sp. had 1 strain and none of the bacterial strains had a 100% effect on the growth of mycelium fungi *Fusarium* sp. Single isolate (*Bacillus pumilus* identified by MALDI-TOFF mass spectrometry) showed properties for perspective use in agriculture. Totally 235 321 sequences of 16S rRNA was recovered by next generation sequencing. Diversity of bacteria from soil outside the root system, the soil from the rhizosphere and rhizoplane samples was similar in all three phases of growth. It was shown that bacteria from root, stem and leaf were different in BBCH 85 (phylum *Actinobacteria* dominated) in comparison to bacteria in BBCH 15 and BBCH 50 (phylum *Proteobacteria* dominated). Significant difference of plant microbiome can be attributed to partial decay of plant tissues during ripening.

**Key words:** *sunflower, diversity, biochemical characteristics, metagenomic analysis*

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**Analysis of performance test parameters and relative breeding value of cold blood horses in Slovakia**

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*Supervisor:* prof. Ing. Marko Halo, PhD.

At the time of plant and machinery cold blood horses have their irreplaceable role. It is important to improve breeding of cold blood horses and assessing its performance. The aim of this thesis was to evaluate the performance tests and the estimated pedigree value of cold blood horses in Slovakia in the years 2014 - 2017. From performance test results were analyzed evaluation of type, exterior, performance and the final mark. Monitored indicators were relative breeding value, age of the horse, breed, gender and breeder. The results were evaluated by using single-factor Anova and dependence by linear regression. In overall, 41 horses were analyzed. The effort of breeders to improve the quality of cold blood horses was confirmed by the number of mares (30 mares) with graduated performance test and reached by relative breeding value at the level of 8.3 points. Monitored indicators of performance test (mark for type, exterior, performance and final mark) had a statistically significant effect ( $P < 0.05$ ) on the relative breeding value. The correlation coefficient  $r = 0.65$  was confirmed by the interrelationships between the relative breeding value and final mark from the performance test. The influence of the breed on the relative breeding value (RBV) was statistically significant ( $P < 0.05$ ). Average RBV of 3-year old horses was at the level of 8.23 points, 4-year 8.3 points, 5-year and 7-year 8.31 points, and 8-year-old horses reached 8.35 points. However the effect of age in our analysis did not have a statistically significant effect ( $P > 0.05$ ), it can be concluded that with age increases the level of RBV. In the monitored period the highest relative breeding value (8.58 points) reached Norwegian mare India. The lowest value of the pedigree (7.35 points) reached cold blood stallion Cyrano du Moulin. In the breeding of cold blood horses the most successful breeders were Forests SR, s. e., Dobšiná horse breeding center with 22 tested horses in the monitored period. Horses from the breeding environment of the Muráň area reached an average RBV of 8.29 points and in performance test achieved an average score of 8.41 points. In conclusion, it is possible to evaluate breeding of horses in Slovakia based on the analysis of performance parameters and it is very good tool for increasing of the performance level.

**Key words:** *cold blood horses, relative breeding value, performance test*

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**Action of sc-gsc-63 on active time of recalculation of plasma and aggregation of thrombocytes**

Nozim KHOSHIMOV, Madina MUSAEVA, Guli RAIMOVA, Abbas TURAEV

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In order to influence the process of formation of a thrombus in the bloodstream and its regulation, various anticoagulants are used. Anticoagulants have a pronounced effect on all phases of blood coagulation, so the study of their activity in the case of blood clotting disorders is very important. In this respect, sulfated polysaccharides are unique compounds that affect individual links of the hemostasis system. The establishment of strict specificity of these compounds to the diverse factors of hemostasis will allow us to reasonably develop recommendations on the possible use of them in the treatment of different degrees of thrombogenic danger and hemorrhagic complications in the case of a violation of the hemostatic system. The solution of this task is extremely demanded, as it will allow to reconsider and clarify the fundamental ideas about the regulation of blood clotting, and will also have practical implications for the therapy of thromboses. Modified sulfated polysaccharide was used in the work with linear compounds obtained by the method of heterogeneous sulfation. The effect of modified sulphated cellulose SC -GSC-63 on activated plasma recalcification time (APRT) and platelet aggregation was studied. Under the influence of CC-GSC-63, depending on the dose (10-100  $\mu\text{M}$ ), the time for recalcification of coagulation of the plasma is prolonged. The elongation of time under the influence of the drug CC-GSC-63, indicates a general tendency to hypocoagulation, and may be due to the inhibition of factor III and most other plasma clotting factors (other than factors VII and XIII). If we take into account that calcium ions participate in the process of activation of both internal and external ways of blood clotting, the effects of these drugs may be due to its influence on both ways of blood coagulation. In a study of the effect of sulphated cellulose SO-GSC-63 on platelet aggregation, it was shown that SC-GSC-63 at a concentration of 10  $\mu\text{g}/\mu\text{l}$  significantly changed the dynamics of the first phase and almost completely inhibited the second phase (90%), ADP-induced platelet aggregation. The pronounced inhibitory effect of SC-GSC-63 is manifested when platelet aggregation is induced by ADP. In this case, the inhibitory effect of sulfated cellulose is enhanced in the presence of  $\text{Ca}^{2+}$  ions (0.05-1.5 mM). Since SC-GSC-63 exhibits the most inhibitory activity in ADP-induced platelet aggregation, it is probably due to inhibition of cyclooxygenase activity of platelets and suppression of their aggregation. These properties of these components are of some interest and require further detailed study of the physicochemical characteristics and mechanisms of their action, which will eventually allow them to be used as a heparin-like preparation.

**Key words:** *hemostasis, anticoagulant, platelet aggregation, sulfated polysaccharide*

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**The antiarrhythmic effect of the ajacine, a diterpene alkaloid isolated  
from *Delphinium bitermnatum***

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*Delphinium sp.* (*Ranunculaceae*) is a medicinal plant commonly used in the countries of Asia including China for the treatment of cardiac diseases. In the present work, we investigated the antiarrhythmic activities of the ajacine, a diterpene alkaloid isolated from *Delphinium bitermnatum*. Isometric tension forces were recorded using a force transducer (Type F30/Model D–79232; Hugo Sachs Elektronik, Germany). In the experiments, modified the physiological Krebs–Henseleit solution containing (in mM): 118 NaCl; 4.7 KCl; 2.5 CaCl<sub>2</sub>; 1.2 MgSO<sub>4</sub>; 1.1 KH<sub>2</sub>PO<sub>4</sub>; 5.5 glucose and 25 NaHCO<sub>3</sub>; pH 7.4 were used. This Krebs–Henseleit solution which was continuously bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub> and kept at a temperature of +36±0.5°C by means of water heating system controlled by temperature controller U8 (Bulgaria), and flowed in and out of the organ bath at a rate of 3–5 ml/min with the peristaltic pump LKB Bromma (Sweden). This study was carried out in the Laboratory of Cell Biophysics of Institute of Bioorganic Chemistry of Academy Sciences of the Republic of Uzbekistan on physically fit, adult, albino rats in both sexes (female and male) obtained from *the vivarium* in the Laboratory of Pharmacology. During the experiments, while working with experimental animals, International principles of the Council for International Organizations of Medical Sciences and the rules of human attitudes towards animals were completely followed (2012). For studying of antiarrhythmic effect of ajacine used acetylbenzoylaconitane (aconitine)–induced arrhythmias (*tachycardia*). Aconitine, also known as acetylbenzoylaconitane, is a C<sub>19</sub> norditerpenoid alkaloid (C<sub>34</sub>H<sub>47</sub>NO<sub>112</sub>), and was dissolved in chloroform. Data were analyzed by OriginPro v. 8.5 SR1 (EULA, USA). Pooled data are given as means ±S.E.M. of observations (*n*). Statistical differences of the data were calculated by Student's *t*–test where appropriate. Values were considered significantly different when *p*<0.05. In the experiment arrhythmia occurred after ~10–15 min addition of aconitine (1 μmol/L) to the incubation condition. It is found that aconitine can bind to the voltage–gated Na<sup>+</sup>–channels and modify the channel kinetics, and consequently induce arrhythmias (265±5 beats/min). In the experiment ajacine, at concentration of 100 μmol/L reduced the rate of aconitine–induced arrhythmias in rat papillary muscles up to 9±2 beats/min (*n*=3–5; *p*<0.05). These data indicate that the ajacine, a diterpene alkaloid (100 μmol/L) can effectively antagonize aconitine–induced arrhythmias, and the effect can be explained by its modulations parameters of action potential or can be blocks Na<sup>+</sup>–channels of cardiomyocytes.

**Key words:** *Delphinium bitermnatum*, *ajacine*, *papillary muscle*, *antiarrhythmic effect*

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**Effect of oral supplementation of algae and polyphenols on antioxidant status and the motility of rabbit spermatozoa**

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*Supervisor:* prof. Ing. Norbert Lukáč, PhD.

In recent years, many studies have been focused on natural substances that could have effect on health of an animal. Algatan® is natural extract used as a dietary supplement and it consists exclusively of natural products. Its main components are polyphenols of marine and freshwater algae and plant polysaccharides. The effect of this supplement on reproduction has not been reviewed in the past, that is a reason why we decided to test its effect on the reproduction potential of male rabbits. Main subject of this article is to determine the effect of Algatan® in two different concentrations – those being 0.3% and 0.6% on selected reproductive characteristics of male rabbits (New Zealand white breed, n = 14) during the 60 days long dietary experiment. The rabbits were divided into three groups: one control group (C) and two experimental groups (T1, T2) and semen was collected with pre-warmed artificial vagina. After 60 days long dietary supplementation, we observed that sperm concentration in both experimental groups have risen in comparison with the control group (C =  $0.647 \pm 0.175 \cdot 10^6 \cdot \text{mL}^{-1}$ , T1 =  $0.699 \pm 0.345 \cdot 10^6 \cdot \text{mL}^{-1}$ , T2 =  $0.871 \pm 0.387 \cdot 10^6 \cdot \text{mL}^{-1}$ ). Results of the CASA analysis report that dietary addition of Algatan® affected spermatozoa motility, however with no statistical significance. Assessments showed enhanced motility and progressive motility in both experimental groups supplemented with combination of algae and polyphenols in comparison with the control (motility: C =  $85.09 \pm 7.526\%$ , T1 =  $87.21 \pm 8.245\%$ , T2 =  $89.38 \pm 8.023\%$ ; progressive motility: C =  $74.28 \pm 12.6\%$ , T1 =  $79.07 \pm 13.89\%$ , T2 =  $81.28 \pm 11.37\%$ ). While monitoring ferric reducing ability of plasma (FRAP), we found out that in T1 group FRAP was  $106.3 \pm 64.98 \mu\text{mol Fe}^{2+} \cdot \text{g}^{-1} \text{ TP}$ , in control group it was  $75.59 \pm 54.34 \mu\text{mol Fe}^{2+} \cdot \text{g}^{-1} \text{ TP}$  and ferric reducing ability of plasma was lowest in group, which feed was supplemented with 0.6% Algatan ( $69.75 \pm 26.35 \mu\text{mol Fe}^{2+} \cdot \text{g}^{-1} \text{ TP}$ ). Activity of glutathione peroxidase also shown us some changes – after two months we observed an increase in GPx activity in both experimental groups in comparison with the control group and it was more significant in T1 group (C =  $23.28 \pm 12.33 \text{ U} \cdot \text{g}^{-1} \text{ TP}$ , T1 =  $41.76 \pm 28.01 \text{ U} \cdot \text{g}^{-1} \text{ TP}$ , T2 =  $35.79 \pm 16.64 \text{ U} \cdot \text{g}^{-1} \text{ TP}$ ). In both experimental groups, we determined an increase in activity of superoxide dismutase (SOD) (C =  $0.296 \pm 0.194 \text{ U} \cdot \text{g}^{-1} \text{ TP}$ , T1 =  $0.443 \pm 0.268 \text{ U} \cdot \text{g}^{-1} \text{ TP}$ , T2 =  $0.346 \pm 0.150 \text{ U} \cdot \text{g}^{-1} \text{ TP}$ ) in comparison with control group. In conclusion, our studies suggest that Algatan® may be potentially useful for enhancement of sperm motility and protection against oxidative stress.

**Key words:** *Algatan, rabbit, semen, motility, SOD, GPx, FRAP*

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### **Preparation and biotechnological efficiency of magnetically modified active inclusion bodies**

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Insoluble protein aggregates, widely known as inclusion bodies (IBs), are commonly observed during protein overexpression or under stress conditions such as thermal, pH or oxidative stress. These protein aggregates can be formed in the cell cytoplasm and/or periplasm and can be found in both prokaryotic (*Escherichia coli*, lactic acid bacteria) and eukaryotic (yeast, microalgae, insect, mammalian cells etc.) cells. Although usually considered as waste byproducts of protein production, recent studies have revealed that IBs can retain their biological activity comparable with those of soluble proteins. Pertaining to their preparation the ease of their purification, separation and the high stability of IBs are their main advantages compared to soluble proteins. IBs can be directly recovered by centrifugation, however nowadays there are several approaches that simplify separation. In our study we present their preparation and application of magnetically modified IBs, which can be easily separated by a magnetic separator, as a new alternative for the convenient recovery of IBs from the reaction mixture. We used magnetic modification procedure at low temperatures to create “magnetic IBs” that showed excellent operational stability. Capillary electrophoresis was used to analyze all samples. Three protein examples were magnetically modified: green fluorescent protein (GFP), sialic acid aldolase (SAA), and UDP–glucose pyrophosphorylase (GalU). IBs of GFP was used for the microscopic visualization of the magnetic modification procedure. In the next stage, to recycle the active IBs by a magnet SAA-IBs were chosen as a model enzyme. When the reaction integral of biotransformation cycle was set to 4 hours and the fixed magnetized SAA-IBs were supplied with 1 mL of the reaction mixture the efficiency was not changed markedly, even after 14 measured reaction cycles. For a better demonstration of the IBs magnetization procedure we switched to another enzyme: IBs of GalU. This enzyme catalyzes the reversible production of UDP–glucose from Glucose-1-P and UTP. Magnetically modified inclusion bodies of UDP–glucose pyrophosphorylase were recycled 50 times. In comparison, inclusion bodies of the same enzyme were inactivated during ten reaction cycles if they were recycled by centrifugation. In the final experiment, to demonstrate the performance of magnetically modified IBs, SAA-IBs were lowered five times and the volume of the reaction mixture was increased ten times. From our results we assume that biotransformation by IBs adsorbed on magnetic iron oxide particles could be run with good performance and operational stability.

**Key words:** *inclusion bodies, magnetic modification, recycling in biotransformation, Sialic acid aldolase, UDP-glucose pyrophosphorylase*

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## **Detection of the effect of taurine on the structure of rabbit testes**

Ján KOVÁČ

*Supervisor:* Ing. Eva Tvrdá, PhD.

Energy drinks are among the most frequently consumed beverages around the world. Such drinks contain stimulating endurance and vigilance substances in the form of caffeine, guarana, and an organic acid called taurine. Taurine (2-aminoethansulfonic acid, TAU) is considered to be a conditionally essential amino acid in humans and primates. TAU is abundant in numerous tissues, and can be synthesized by the central nervous system, liver, kidney, retina and mammary gland. TAU is known mainly because of its positive effect on the organism such as osmoregulation, neuromodulation, membrane stabilization, improvement in sperm motility and maintenance of calcium homeostasis but it also believed to act as an antioxidant and energy stimulant. The aim of this work was to analyze the effect of TAU on the microscopic structure of rabbit testes, focusing on individual parameters of the seminiferous tubules. We used 5 months old sexually mature male rabbits, which were divided into four experimental groups. Rabbits from the first experimental group (E1) received taurine at a dose of 321.675 mg/rabbit/day, animals from the second experimental group (E2) received 643.35 mg TAU/rabbit/day and rabbits from the third experimental group (E3) received taurine at a dose of 965.025 mg/rabbit/day. Taurine was administered to the rabbit for four weeks dissolved in drinking water. To compare the results of our experiment a control group of rabbits (KK) was created without any TAU administration. We analyzed the following morphometric parameters: the diameter of the seminiferous tubules ( $\mu\text{m}$ ), the height of the epithelium and the diameter of the lumen of the seminiferous tubules ( $\mu\text{m}$ ), the area ( $\mu\text{m}^2$ ) and the perimeter ( $\mu\text{m}$ ) of the seminiferous tubules. Based on our results, we have found that the oral administration of TAU caused a significant increase ( $P < 0.05$ ) of the diameter (E1, E2, E3), height of epithelium (E3), lumen thickness (E1), area (E3) and perimeter (E3) of the seminiferous tubules when compared to the control group (KK). Based on these findings we can conclude that oral administration of taurine at the highest dose (965.025 mg/rabbit/day, group E3) caused the most significant changes in the structure of the rabbit seminiferous tubules. We consider that administration of taurine induced increasing values of the studied parameters in the seminiferous tubules, which indicates a positive effect of taurine on spermatogenesis.

**Key words:** *taurine, testes, morphometry*

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**Identification of autochthonous *Cotoneaster* species by using a flow cytometry method – preliminary results**

Samuel KŠIŇAN

*Supervisor:* Ing. Ľuba Ďurišová, PhD., doc. Ing. Pavol Eliáš, PhD.

Some of the phylogenetic relationships among taxa within *Rosaceae* family are poorly understood. Recent studies proved, that the classification of some taxa included in *Rosaceae* family is relatively problematic. The *Cotoneaster* genus belongs to *Spiraeoideae* family. It includes approximately 300 species. The *Cotoneaster* genus is often associated with polyploidy and hybridization. That causes a serious problem, when it comes to determination of these taxa, because some of the differences between them cannot be recognized morphologically. Flow cytometry (FCM) is a cytogenetic method. In the field of botany, flow cytometry is mainly used in taxonomy. The most common application of flow cytometry in the field of botany is to determine the amount of nuclear DNA and ploidy level. Nuclear DNA determination is a very effective way to detect closely related taxa in taxonomically complicated groups. The aim of this study was to estimate genome size of species within *Cotoneaster* genus by using a flow cytometry method. The collection included samples from 7 different localities. Overall, 16 individuals were analysed in this study and the analysis of each individual was repeated three times. Samples of two different tetraploid species (*Cotoneaster integerrimus* and *Cotoneaster melanocarpus* agg.) were collected in several localities within the Western Carpathians in northern Hungary (Börzsöny Mts – Kóspallag; Cserhát Mts – Bér, Hollókő; Bükk Mts – Alsóhámar, Bélapátfalva; and Zemplén Mts – Hejce). Samples of pentaploid *Cotoneaster tomentosus* were collected in Ružomberok (the Western Carpathians, Nízke Tatry Mts), Slovakia. *Zea mays*, with nuclear genome size 5.43 pg, served as an internal standard. Nuclei were stained with propidium iodide (PI). Genome size of samples ranged from 2.22 to 3.21 pg. Flow cytometry revealed, that there is a significant difference between a genome size of pentaploid *Cotoneaster tomentosus* and tetraploid species. A mean value of *Cotoneaster tomentosus* genome size was 3.13 pg. Flow cytometry analysis also showed a significant difference between *Cotoneaster integerrimus* and *Cotoneaster melanocarpus* agg. A mean value of *Cotoneaster integerrimus* genome size was 2.42 pg and mean value of *Cotoneaster melanocarpus* agg. was 2.36 pg. This study proved the efficiency of flow cytometry method, however, for a better identification of studied species a further research is needed.

**Key words:** *determination, Cotoneaster, hybridization, flow cytometry*

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### **Spasmolytic effects of flavonoids on isolated rat's ileum**

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Gastrointestinal disorders the most common problems affecting general population and synthetic drugs are present, however, with low efficacy and high side effects. In this study, seven flavonoids, *viz.* genkwanin, kumatakenin, penduletin, xanthomicrol, apigenin, quercetin 3,4'-dimethyl ether and cirsimaritin were evaluated on ileum contractions of rats. The rat ileum smooth muscle preparations ~1–1.5 cm long were mounted in 20 mL tissue baths containing Tirode solution (mM): NaCl–139.9; KCl–2.68; CaCl<sub>2</sub>–1.8; MgCl<sub>2</sub>–1.05; NaH<sub>2</sub>PO<sub>4</sub>–0.42; glucose–5.55; NaHCO<sub>3</sub>–11,9 (*pH*=7.4). This physiological solution which was continuously bubbled with 95%–O<sub>2</sub> and 5%–CO<sub>2</sub> and kept at a temperature of 36±0.5°C by means of water heating system was controlled by temperature controller U1 (Russia). The signals obtained were given from the transducer SI–KG20 to amplifier and sent to a computer with analogue–digital converter Lab–Trax–4/16 (WPI, USA) software (iWorx LabScribe2; iWorx Systems, Inc., USA). Data were analyzed by OriginPro v. 8.5 SR1 (EULA, Northampton, MA 01060–4401, USA). The isolated rat ileum smooth muscle preparations were contracted with BaCl<sub>2</sub> (10 mmol/L), cumulative concentrations of flavonoids (1–100 µmol/L) were added after the contraction reached a stable plateau. Flavonoids relaxed in a concentration–dependent manner (1–100 µmol/L) ileum smooth muscle preparations pre–contracted with BaCl<sub>2</sub>, with respective the maximal effected concentration (100 µmol/L) of genkwanin, kumatakenin, penduletin, xanthomicrol, apigenin, quercetin 3,4'–dimethyl ether, and cirsimaritin, the isometric developed force of rat ileum preparation was decreased from 74.23±4.5%, 78.34±3.3%, 86.58±5.1%, 84.15±4.9%, 61.47±6.2%, 76.43±2.6%, and 62.18±4.6% in comparison with the control group respectively (*p*<0.05; *n*=3–4). It was reported that BaCl<sub>2</sub> causes smooth muscle membrane depolarization and opens the voltage–dependent Ca<sup>2+</sup><sub>L</sub>–channels resulting in a Ca<sup>2+</sup> influx and intracellular Ca<sup>2+</sup> release. In conclusion, results suggest that Ca<sup>2+</sup><sub>L</sub>–channels were involved in the flavonoids spasmolytic effect on rat ileum. Therefore, spasmolytic effect of flavonoids isolated from *D. komarovii* confirms its benefits for the treatment of gastrointestinal disorders.

**Key words:** *isolated rat ileum, flavonoids, spasmolytic effects*

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### Vasorelaxant effect of the alkaloid penduletin on isolated rat aortic smooth muscle

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The aim of the present study was to determine the vasorelaxant effect of penduletin, a flavonoid isolated from plants *Dracocephalum komarovii* Lipsky (*Lamiaceae*) in the rat isolated aorta smooth muscle. The experiments were performed in preparations, which are ~3–4 mm wide rings isolated from aortic albino rats (~200–250 g) and placed in a special chamber (5 mL) was perfused with solution Krebs–Henseleit following composition (mmol/L): NaCl – 118.6; KCl – 4.8; CaCl<sub>2</sub> – 2.5; MgSO<sub>4</sub> – 1.2; KH<sub>2</sub>PO<sub>4</sub> – 1.2; NaHCO<sub>3</sub> – 20, glucose – 10 (pH=7.4). During the experiments, while working with experimental animals, International principles of the The Council for International Organizations of Medical Sciences and the rules of human attitudes towards animals were completely followed (2012). Force contraction was recorded using a FT.03 (Grass–Telefactor, USA). In the experiments, pretreatment with penduletin (3–75 μmol/L), attenuated the vasorelaxant effect of the isolated rat aorta from 85.7±5.3% in comparison with the control group ( $p<0.05$ ;  $n=3-5$ ). Studies have shown that in incubation to inhibit potential-dependent Ca<sup>2+</sup>-channel – verapamil ( $IC_{50}=0.01$  μmol/L) the relaxant effect of penduletin ( $IC_{50}$ ) decreases to 32.4±4.7% of control values. The results of experiment demonstrate that the relaxant effect of penduletin on isolated rat aorta smooth muscle is not completely connected with Ca<sup>2+</sup>-channels. Pretreatment with L-NAME (100 μmol/L), an inhibitor of NO-synthase, and the blocker of cyclooxygenase – indomethacin (10 mmol/L) in the intact endothelium attenuated the relaxant effect of penduletin (75 μmol/L). The results of these experiments suggest that the effect relaxant of penduletin endothelium-dependent and may be mediated by its interaction with the NO-synthase and its activation. And also, in the experiments pre-incubation of aortic rings with chlorpromazine (CPZ), a Ca<sup>2+</sup>-CaM complex inhibitor, significantly increase the activity of penduletin, which is showed as a left shift in the vasorelaxant effect the Schild plot curves. This effect suggests a possible Ca<sup>2+</sup>-CaM complex inhibition by penduletin. The present study demonstrates that penduletin has showed a endothelium-dependent relaxant effect on rat isolated aorta smooth muscles that can be explained with the block of Ca<sup>2+</sup>-channels, and may inhibition of Ca<sup>2+</sup>-CaM complex. In conclusion, our studies suggest that penduletin may be potentially useful for the development of therapeutic treatments for cardiovascular diseases.

**Key words:** *isolated rat aorta, penduletin, relaxant effect, NO-synthase, Ca<sup>2+</sup>-CaM complex*

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SECTION  
*Human nutrition*

## **The utility value of Tokaj essences**

Zuzana EFTIMOVÁ

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The cultivation of grape-vine in the Tokaj vineyard, the production of Tokaj wine and essences regulate by act no. 313/2009 coll. and specification of products for protected designation of origin. Here only the varieties Furmint, Lipovina, Muscat Yellow allowed to cultivate. These varieties are able to ripen in these climatic and soil conditions. In years, when it is a warm and dry autumn with early fogs, the varieties are ripe and, after attack by the noble mold *Botrytis cinerea* Persoon, they will create cibebas. Cibebas are used for the production of essence and Tokaj wine selections to which they supply a specific botrytis bouquet. The essence is produced by fermentation of the cibebas. The aim of our work was to determine total polyphenols, flavonoids, phenolic acids and antioxidant activity in the Tokaj essences samples collected in 1999, 2000, 2006, 2007, 2009, 2013, 2015 years by TOKAJ & CO, Malá Tŕňa. In all analyzed Tokaj essences, we found the highest total polyphenols ( $386.88 \text{ mg.L}^{-1}$  expressed in gallic acid equivalent) in the sample of 2006 year. The sample of essences from 2013 year had the lowest value of total polyphenols ( $200.22 \text{ mg.L}^{-1}$  expressed in gallic acid equivalent). We determined the highest content of flavonoids ( $177.66 \text{ mg.L}^{-1}$  expressed in quercetin equivalent) in the essence sample of 2000 year. We measured the values of total polyphenols and the lowest amount of flavonoids ( $44.09 \text{ mg.L}^{-1}$  expressed in gallic acid equivalent) was in the essence sample of 2006 year. The highest content of phenolic acids of  $116.68 \text{ mg.L}^{-1}$  (expressed in coffee acid equivalent) was determined in the sample from 2000 year and the lowest amount of these substances was  $55.01 \text{ mg.L}^{-1}$  in the essence of 2013 year. We measured an antioxidant activity (reducing force) in essence samples by the phosphomolybdenum method. We found that the highest reducing force was ( $1245.43 \text{ } \mu\text{mol.L}^{-1}$  Trolox) in the sample of 2007 year. Essence sample of 2013 year had the lowest reduction power of  $837.57 \text{ } \mu\text{mol.L}^{-1}$  Trolox. Our results showed that the lowest antioxidant activity was determined by the ABTS method and was measured in the essence sample of 2013 year which correlates with the lowest measured values of total content of polyphenols, flavonoids and phenolic acids. We have found that the phosphomolybdate method for measuring the Tokaj essences antioxidant activity is inappropriate because the essences contain a high concentration of carbohydrates, alcohol and other oxidizable substances. And they have a reducing potential and produce false positives results.

**Key words:** *cibebas, Tokaj wine, Tokaj vineyard, polyphenols, flavonoids, phenolic acids antioxidant activity*

**Acknowledgement:** This work was created with the financial support of the European Community under the project ITMS 26220220180 "Building the AgroBioTech Research Center".

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### **Comparison of different methods of the assessment of body composition**

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Daniel MAGULA

*Supervisor:* MUDr. Daniel Magula, CSc.

The main aim of the present study was to compare 2 methods used for the body composition assessment – air displacement plethysmography (ADP) and bioelectrical impedance analysis (BIA). The difference in the assessment of body composition between these 2 methods was observed in the selected parameters, such as fat mass (FM), fat free mass (FFM) and resting metabolic rate (RMR). The selected parameters were compared with each other. On the study participated 22 healthy subjects ( $n = 22$ ), 11 women ( $n = 11$ ) and 11 men ( $n = 11$ ). The age range of the participants was from 19 to 67 years. All subjects were measured at InBody 720 and InBody S10 (Biospace, Seoul, Korea) and BOD POD (Cosmed, CA, USA). The collected data were evaluated by using suitable standard mathematical and statistical methods. For the evaluation of data was used MS Office Excel and statistical software STATISTICA Cz ver. 10. All data are displayed as the mean of the measured parameters  $\pm$  standard deviations (SD). The differences between ADP and BIA were not significant. It was noted a significant ( $p < 0.05$ ,  $p < 0.001$ ) gender differences in the observed parameters by both methods (%FM, %FFM, BMR, SMM, SLM, VFA, BMI). The %FM in all subjects measured by ADP was  $28.13 \pm 9.83\%$ . The %FM in all subjects measured by BIA was  $24.13 \pm 9.83\%$ . The value of %FFM measured by ADP in all subject was  $28.13 \pm 9.83\%$ . The value of %FFM measured by BIA in all subject was  $75.18 \pm 9.17\%$ . The average of RMR in all subjects was calculated to the value of  $1490.77 \pm 421.60 \text{ kcal.day}^{-1}$  in ADP and at BIA the average value of basal metabolic rate (BMR) in all subjects was  $1605.45 \pm 352.31 \text{ kcal.day}^{-1}$ . Comparing the selected parameters, the results show that ADP tends to overestimate the parameter of FM (kg) and %FM. On the other hand, BIA tends to overestimate the parameters of FFM (kg), %FFM and BMR.

**Key words:** *air displacement plethysmography, bioelectrical impedance analysis, body composition*

**Acknowledgement:** This work has been supported by the **Support of technologies innovation for special bio-food products for human healthy nutrition** project implemented under the Operational Programme Research and Development financed by European Fund for Regional Development (ITMS 26220220115) and AgroBioTech Research Centre built in accordance with the project **Building „AgroBioTech" Research Centre** (ITMS 26220220180).

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SECTION

*Multifunctional agriculture, environment and rural development*

**Closed loop agriculture: Environmental aspects of waste products application in sweet sorghum cultivation for biofuels**

Lilianna GŁĄB, Józef SOWIŃSKI, Bernard GAŁKA

*Supervisor:* Prof. Józef Sowiński

In the last 50 years, agriculture has become more resource intensive, dependent heavily on the synthetic nitrogen and phosphorus fertilizers, oil derived agrochemicals and fossil fuels. Recently, in Europe much attention has been paid to the developing a circular economy in agriculture. This approach requires the adoption of closed loop systems which work towards the goals of improved economic and environmental sustainability. Closed loop agriculture strategy aims to using of minimal amount of external inputs and reducing negative discharges to the environment. Sustainable management of bio-based waste materials and by-products plays a pivotal role in implementing circular economy and achieving more resource efficient society in Europe. The use of sewage sludge and biogas digestate as soil amendments to improve fertility and plant growth providing an added value to the residue, which is in line with European policy. In Poland sweet sorghum can be considered as an alternative to maize and promising co-substrate for anaerobic digestion based on livestock manure. Small-scale anaerobic digestion technology that can be applied on farm provides not only local energy, but also nutrients recovery throughout agricultural use of digestate. This practice is a sustainable system of decentralized energy and fertilizer production. However, the agricultural use of waste materials can generate environmental problems related to higher levels of heavy metals, which could be absorbed by plants. Given this, a field experiment was carried out in Agricultural Research Station belonging to the Institute of Agroecology and Plant Production of Wrocław University of Environmental and Life Sciences in the Lower Silesia region of southwestern Poland. During the field experiment four fertilization treatments in sweet sorghum production for biofuels were tested: urea, solar dried sewage sludge, biogas digestate and control without fertilization. Sorghum biomass was used for ethanol and methane production by ethanol fermentation and batch anaerobic digestion. The research sought to: (1) determine macro- and microelements (including heavy metals) contents in soil amended with waste materials, (2) evaluate the effect of application of digestate and sewage sludge on chemical composition of sorghum biomass, (3) evaluate the content of macronutrients and microelements in stillage and digestate – by products, which were obtained after the ethanol fermentation and anaerobic digestion using sweet sorghum as feedstock. Sewage sludge application has significantly increased the content of Zn and Mn in sorghum biomass. Digestate application has contributed to increasing content of Fe in biomass compared to the other treatments. Digestate obtained after methane fermentation was very rich in potassium (8.57-11.36% s.m.). Fertilization treatments significantly affected macroelements content in stillage, however concentration of macronutrients in stillage was lower as compared to digestate.

**Key words:** *circular economy, nutrients circulation, closed loop, sweet sorghum, digestate, sewage sludge, heavy metals*

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## **Influence of sous vide heat treatment on microbiological quality of pork meat**

Paweł HANUS

*Supervisor:* Maciej Kluz, PhD, prof. Ing. Miroslava Kačániová,

Pork meat can be a breeding ground for bacterial growth, the chemical composition supports the development of microflora, including pathogenic microflora. The further development of undesirable microflora is influenced by treatments from slaughtering itself, through storage and processing. Pollutants can occur from various sources, poor technical condition of the equipment as well as microflora that occurs on the surface, skin of animals and also in the digestive system.

The research included the influence of sous vide thermal treatment on microbiological quality of pork meat and survival of *Listeria monocytogenes* bacteria. The samples were vacuum packed and then cooked at 55 °C, 60 °C and 65 °C for 5, 15, 30 and 60 minutes. Ten grams ( $\pm$  0.5 g) samples were placed in PA/PE film bags and 100  $\mu$ l of *L. monocytogenes* biomass was added, the samples were vacuum packed and incubated at 37 °C for 18 hours. The meat samples were cooked in a water circulator at different times and temperatures. Samples were tested on day 1 and after 3 days. The number of colony forming units (cfu/g) was determined and identified by using MALDI-TOF MS Biotyper (Bruker, Germany). Meat microflora was identified by mass spectrometry.

Microbiological contamination of meat was checked by number of colony forming bacteria units (log CFU/g). This parameter was significantly different depending on temperature and time, which can be observed. On the first day after thermal treatment the number ranged from 1.33 log CFU/g to 6.65 log CFU/g. In the samples was detected microflora such us: *L. monocytogenes*, *Listeria ivanovii*, *Bacillus cereus*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus hominis*.

The study shows that the sous vide method is an effective method and can be used to protect the microbiological products. It is extremely important for raw material if stay fresh and with the high quality. The treatment should be carried out in a strictly controlled manner, which can be a difficulty at home.

**Key words:** *sous vide*, *heat treatment*, *Listeria monocytogenes*, *food safety*

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### **A composition for the effective melioration of saline soils**

Ilyos HUDOYNAZAROV, Nodirali NORMAKHAMATOV

*Supervisor:* acad. Abbaskhan Turaev, DSc.

The aim of the present study was the creation of a composition having interphase affecting activities with salts in saline soils in Central Asia. The composition was developed mechanically mixing four compounds, which consists surface-active substance with anionogenic nature, a polyfunctional copolymer of synthetic and natural polymers, and tertiary amine. The copolymer structure was chosen during its synthesis by the selecting of the scientifically-based manner of polymer chain and functional groups, their proportions and evaluating of their effectiveness.

In order to having high ion-exchange capacity the ionogenic copolymer was synthesized on basis of acrylic acid, maleic anhydride. The copolymer and its physical and chemical analyzes were performed that the molecular weight of the copolymer carboxylic acid was within 2000-5000 Da.

The composition was easily and instantly water-soluble, biodegradable as well. For the further standardization, it has been developed method of physic-chemical analysis of each ingredient in it. Aromatic parts with carbon-containing rings in the copolymers and anionic hydrophilic functional groups (sulphonic and carboxyl) were determined by UV-spectroscopic and potentiometric titration methods respectively.

Based on the obtained copolymer, the ionogenic polymer composition (Biosolvent) was developed and its structure was analyzed qualitatively and quantitatively, functional groups and molecular weights were determined.

Laboratory and field experiments of Biosolvent on leaching effect on the saline soil showed the reduction of:  $\text{HCO}_3^-$  by 25%;  $\text{Cl}^-$  by 35–42%;  $\text{SO}_4^{2-}$  by 13–16%;  $\text{Ca}^{2+}$  by 21–28%;  $\text{Na}^+$  by 21–23%, and increasing of the  $\text{Mg}^{2+}$  by 63–68%;  $\text{K}^+$  by 24–28% respectively to the control washing (water-leaching), as well as decreasing of the soil bulk density by 11%.

The effect of Biosolvent on the soil microflora has been studied. The results showed that the polymer composition had no adverse effect on the overall dynamics of eleven agronomically important microorganisms (ammonifying bacteria, oligonitrophyles, microorganisms involved in phosphorus metabolism, fatty acid bacteria, nitrogen fixing bacteria, actinomycetes, cellulose-decomposing microorganisms), but increasing the number of nitrificants instead.

The results showed that this composition can be used to remove “toxic” salts from a medium and strongly saline soils and as a desalinate-conditioner to optimize the micro-aggregation structure of the soil.

**Key words:** *saline soil, leaching effect, soil microflora, micro-aggregation structure*

**Acknowledgement:** The research was performed under the project K7-002 financially supported by National Program for applied researches of Uzbekistan.

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**The effects of different rates of biochar and biochar in combination with N fertilizer on soil organic matter and soil structure parameters**

Martin JURIGA

*Supervisor:* doc. Ing. Vladimír Šimanský, PhD.

In 2017, during the vegetation season of corn, the study was conducted on experimental site of SUA in Nitra (Dolná Malanta) on a silty loam Haplic Luvisol as a part of biochar experiment. The aim of study was (1.) to quantify the extent to which individual rates of biochar: 0, 10 and 20 t.ha<sup>-1</sup> (B0, B10 and B20) and biochar with N fertilizer at the rate of 240 kg.ha<sup>-1</sup> affected soil organic matter (SOM) and soil structure parameters, (2.) to evaluate the changes of dynamics of SOM and soil structure parameters to relate on application rates of biochar and biochar in combination with N and (3.) to determine the interrelationships between SOM and soil structure parameters. The results showed that the application rate 20 t ha<sup>-1</sup> of pure biochar (B20) had the highest effects on evaluated parameters SOM and soil structure. In comparison to the control (B0), the biochar dose of 20 t ha<sup>-1</sup> statistical significantly increased the soil organic carbon content (C<sub>OX</sub>) by 29%. In B20 treatment, the extractability of humic substances carbon (C<sub>HS</sub>), humic acids carbon (C<sub>HA</sub>) and fulvic acids carbon (C<sub>FA</sub>) decreased by 18%, 16% and 20%, respectively than B0 treatment. At the same time, the higher rate of pure biochar significantly increased content of the water-stable macro-aggregates (WSA<sub>ma</sub>), the mean weight diameter of water-stable aggregates (MWD<sub>w</sub>) and the index of water-stable aggregates (S<sub>w</sub>) by 10%, 37% and 11%, respectively compared to the B0. In B20, during the vegetation season of corn, we also observed statistical significant changes of dynamics in follows parameters: C<sub>HA</sub>, C<sub>L</sub>, C<sub>FA</sub>, C<sub>HA</sub>:C<sub>FA</sub> and Q<sub>HA</sub>. The C<sub>HA</sub> stability statistically significant varied in all treatments. In addition, significant changes in dynamics of soil structure parameters were observed in B20 treatment. These dynamics the best expressed the quadratic polynomial model. Content of C<sub>L</sub> positively correlated with WSA<sub>ma</sub> (r=0.384, P<0.05), MWD<sub>w</sub> (r=0.427, P<0.05) and S<sub>w</sub> (r=0.393, P<0.05). At the same, C<sub>L</sub> negatively correlated with WSA<sub>mi</sub> (r=-0.384, P<0.05) and K<sub>v</sub> (r=-0.466, P<0.01). Higher content of C<sub>HA</sub> resulted in higher MWD<sub>d</sub> and K<sub>v</sub> values. Between Q<sub>HA</sub> and MWD<sub>d</sub> (r=-0.576, P<0.001) and K<sub>v</sub> (r=-0.559, P<0.01) negative correlations were determined.

**Key words:** *biochar, fertilization, soil organic carbon, aggregate stability*

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## **Development of waste cooking oil for the production of energy carriers**

Michał KAMIŃSKI

*Supervisor:* prof. dr hab. inż. Leszek Romański

Frying is one of the most popular methods of heat treatment of food products of animal and plant origin before human consumption. In the world, palm and rapeseed oils are most often used for frying. At the turn of 2017/2018 a total of 99.27 million metric tons of these types of oil in the world was used for food purposes. Such a large amount of used oil generates a significant stream of waste, which should be properly managed.

Increasing attention to environmental protection requires the introduction of strong and specific waste management methods. An important part is played by the ecological awareness of the society. Currently, the extraction of frying oil in Poland is carried out by specialized companies only from catering companies. Unfortunately, in the case of domestic sources of waste oil, there are no regulations concerning the treatment of precipitation. Most often this type of waste is poured into the sewage system, which reduces its capacity.

The paper presents the possibility of processing used frying oil through the use of transesterification reaction. The process consists in adding an appropriate type of catalyst (acid or alkaline) and alcohol (usually methyl alcohol) to the oil. As a result of mixing and temperature action, final products are obtained, i.e. biodiesel, glycerine fraction and soap sludge. All of these products can be used for energy generation without polluting the environment. The experiment was carried out in five repetitions in the configuration shown in the table below. The main aim of the experiment was to determine the efficiency of transesterification reaction depending on the temperature of the process.

Type of alcohol	Oil weight [g]	Type of catalyst [-]	Process temperature [°C]	Molar ratio of alcohol [-]	Mass ratio of catalyst [%]
Methyl alcohol	200.0	KOH	30°C	3:1	0.5%
			40°C	6:1	1.0%
			50°C	9:1	1.5%

The analysis of laboratory results shows that the amount of biodiesel produced by transesterification increases with the temperature of the process (for example: molar ratio of alcohol - methanol 3:1, mass ratio of catalyst - 0.5% KOH, process temperature: 30°C - 47.92%, 40°C - 76.34%, 50°C - 77.47%). With the increase in the amount of alcohol, catalyst and temperature in the process, total production costs increase.

**Key words:** *waste cooking oil, transesterification, waste development, biodiesel*

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## **The influence of harrowing on the density of spring triticale and field bean mixture**

Agnieszka ŁAGOCKA

*Supervisor:* dr hab. inż. Piotr Sobkowicz

Mixtures of leguminous plants with cereals are cultivated to produce concentrated, protein-rich animal feed. The problem is the lack of registered herbicide for both components of the mixture. In the experiment, attention was pointed to the regulation of weed infestation by mechanical method with the use of a harrow. It is important that the treatments do not have a negative impact also on the crop cover. The paper presents the results of research from 2016-2018, the aim was to determine whether the depth and date of harrowing have an impact on the density of spring triticale and faba bean mixture. The 1-factor field experiment was established using the random block method on the Agricultural Experimental Institute Swojec of the Wrocław University of Environmental and Life Sciences. The factor investigated was the method of protecting the mixture against weeds. The harrowing procedures were completed in 3 different dates and at 2 depths. On one of the objects an increased standard of seed sowing was applied, on the other one a herbicide Basagran was applied. This herbicide was registered for mixtures a few years ago. Planting density was measured in 5 terms: after each harrowing (phase of 2-3 leaves, beginning of tillering, full tillering phase), in the full vegetation phase, and in the full mix maturity phase. The highest number of triticale plants per m<sup>2</sup> in the mixture maturity phase was observed on plots where the sowing standard was doubled. On the opposite side, the smallest number of this plant per m<sup>2</sup> was characterized by a mixture, in which one shallow harrowing was carried out in the first term and two deep ones in the third term. Compared to the number of plants in the unprotected mixture there was 45% more and 32% less, accordingly. One shallow harrowing in the full tillering phase of triticale had a positive effect on the triticale density, which was 11% higher than in the unharrowed triticale mixture. The triticale density was higher than the unprotected object also after two deep harrowing in the last term, however, it was not statistically proven. The treatments conducted had a lower effect on the number of faba bean plants on the examined objects. The highest faba bean density was also observed in the case of increased seeding standards. Compared to the unprotected mixture, the increase was 93%. Significantly higher density was found after one shallow harrowing in the first term, it was 40% higher. A smaller number of cultivated plants was noted in the mixture with a single deep harrowing in the third term, however, this difference was not statistically significant.

**Key words:** *mixture, spring triticale, field bean, density*

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**Digital agriculture / Precision agriculture - Digital agriculture's farm software platform**

Ondrej PINČEK

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Digital agriculture has proven itself as not only one of the most exciting new frontiers in the advance of technology and science, but also as a central element of the solution to one of agriculture's – and humanity's – most pressing issues: the need to feed a growing population while minimizing impact on the environment. With news popping up almost daily about new applications, sensors, imaging platforms, even autonomous “robotic” equipment, the ag tech space can be confusing for even the most tech-savvy farmer, whether they are farming 4000 ha of corn in Iowa, or 2 hectares of tomatoes and onions in India. Given all of these new technologies, it is important to clarify terms like “digital ag” and “precision ag.” Digital ag is the use of data and advanced computational techniques to make more informed decisions about managing our crops and agronomic operations. It encompasses all the information in the ag ecosystem, including data about the crop itself, equipment data, environmental data, operational data, and even market and logistics data. Precision ag is about executing our agricultural plan as precisely as possible and “farming by the seed.” It is often executed by specialized equipment, encompassing how we plant, manage, and harvest that seed, as well as how we manage the soil between plantings with processes like tillage, crop rotation, cover cropping, poly cropping, etc. This specialized equipment can often then provide the data we need in digital ag to do better analysis, such as a “seed firmer” device on a precision planter giving us a high resolution soil map of the field. Digital and precision agriculture combined aren't just about higher yields, but also about being as efficient as possible, producing more with less, and in a sustainable and reliable fashion. There is no question that digital ag will continue to expand in the years to come, providing farmers with increasingly powerful tools to improve harvests and profitability while reducing food security concerns and enabling more sustainable agricultural practices around the globe. But, at the same time, there are significant questions that remain as to how quickly digital tools can develop, and how quickly the digital ag industry, farmers and the entire agriculture ecosystem can adapt to make the most of them. Digital farming is a tool to address challenges, answer questions, alleviate fears and advance change, we can expedite the transformation of agriculture into a data insights-driven enterprise. At every step along the way, to help to address one of society's most pressing challenges in feeding a growing population. The results showed that precision ag can help in managing those inputs in an environmentally friendly way. By using site-specific knowledge, precision ag can target rates of fertilizer, seed and chemicals for soil and other conditions. One example is that spatial management of N can reduce overall N application, and reduce N on sensitive areas, while maintaining profitability. Another example is that spatial management of insecticides and herbicides can reduce overall applications of those chemicals by applying them only where the problem exists. Precision ag can be part of an environmentally benign economically viable system. If the need for external inputs is accepted, information is also needed for proper spatial allocation of the external inputs. Most of the papers reviewed indicate that precision ag can contribute in many ways to long-term sustainability of production agriculture, confirming the intuitive idea that precision agriculture should reduce environmental loading by applying fertilizers and pesticides only where they are needed, when they are needed. Precision ag benefits to the environment come from more targeted use of inputs that reduce losses from excess applications and from reduction of losses due to nutrient imbalances (K deficiency reducing N efficiency, for example), weed escapes, insect damage, etc. Other benefits include a reduction in pesticide resistance development. One limitation of the papers reviewed is that

only a few actually measured directly the environmental indices, such as leaching with the use of soil sensors. Most of them estimated indirectly the environmental benefits by measuring the reduced chemical loading. One key research need is field measurement of the environmental impact of precision agriculture technologies. Other results indicated that auto-swath technology could result in a 4.3% average savings on input costs for a farm with a payback of around two years. If the savings due to GPS guidance were included, the total cost savings could be in the 20 to 30% range. In case of Variable Rate Seeding soybeans, previous seeding rate of 437 500 seeds/ha. Current rate of 396 000 seeds/ha. These numbers significantly influence cost per seeds. Variable-rate fertilizer placement and auto-steer have enabled to save 15% on fertilizer costs. That's 7% from auto-steer and 8% from variable-rate. Fertilizer costs for spring were 180 € per ha, for a total savings of 26 € per ha. Over a couple ha the savings are 22 550 €, that helps pay for precision ag upgrades, while also reducing fertilizer runoff.

**Key words:** *digital ag/agriculture, precision ag/agriculture, future farming, forward farming, Climate FieldView, climate change, more with less,*

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**Analyses of dominant species in some biocorridors at the border of Pannonian region on  
territory of West Slovakia**

Ján SOTÁK

*Supervisor:* prof. RNDr. Tibor Baranec CSc.

Shrubby species in agricultural landscape are much more important for soil protection of some wind and water erosion. A specific type of vegetation formed as biocorridors. Urbanization and intensive agricultural activities are the main reasons for landscape fragmentation. Biocorridors are effective conservation tools that provide linkage between fragmented habitats. Based on the results of our assessment, vegetation is classified as *Ulmeto - Fraxinetum carpineum* forest type and *Saliceto – Populetum* forest type at localities. Skeleton of the ecological corridors comprises non-native hybrids of Canadian poplar (*Populus × canadensis*) and *Robinia pseudoacacia* tree species. The shrub undergrowth is represented considerably and comprises particularly *Prunetalia spinosae* shrub populations with dominance of sloe hybrids (*Prunus × fruticans*). Autochthonous sloe species (*Prunus spinosa*) is gradually replaced with its hybrids. Phytocoenoses of ecological corridors comprise also species that penetrate from adjacent agrobiocoenoses and pose a potential threat to autochthonous populations.

Autochthonous blackthorn taxa (*Prunus spinosa* agg.) is gradually replaced with its hybrids (*P. × fruticans*, *P. × fetchneri*, *P. × dominii*) in *Prunetalia spinosae* shrubby populations, which form dominant component of phytocoenoses of bio-corridors in marginal zones of agrobiocoenoses. Blackthorn hybrid (*P. × fruticans*) is a dominant taxon of this phytocoenoses. Methods of floristical analyses were applied to evaluate the occurrence of populations these taxa in bio-corridors at Svätý Jur, Modra and Pezinok localities. Based on the results of our assessment of blackthorn populations was approximately the same at studied localities. Similar character of habitats, as well as the same growth form of individuals may be the reason. The spatial structure of populations is similar, probably, because vegetative propagation is main way of propagation. Frequency of size distribution of blackthorn individuals was asymmetric.

**Key words:** *bio-corridors, Prunus spinosa* agg., *floristical analyses, agricultural landscape, W Slovakia*

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SECTION  
*Plant Production*



**Application effect of selected plant biostimulants on the quantitative and qualitative parameters of sweet pepper (*Capsicum annuum* L.) in the organic farming system**

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*Supervisor:* doc. Ing. Alena Andrejiová, PhD.

Sweet pepper is one of the most cultivated vegetables on the world. Fruits of peppers are important sources of health promoting substances, especially vitamin C content, which significantly increasing during maturation. The aim of this study was to evaluate the effects of plant biostimulants Agriful and Trichomil on selected quantitative and qualitative parameters of pepper fruits. To the small plot experiment were included 2 hybrid varieties: Kapirex F1 and Esperansa F1. The experiment was carried out in the unheated plastic tunnel in the Botanical Garden of the SUA in Nitra in 2016 and 2017. We observed three variants: control (without application of biostimulants); Agriful variant (5 times application of Agriful at 50 ml for 10 l of water) and Agriful + Trichomil variant (5 times Agriful at 50 ml for 10 l + 2x Trichomil at 50 ml for 10 l). Fruit harvest was gradual in full botanical maturity. We examined the effects of biostimulant application on quantitative and qualitative parameters of pepper fruits. Differences in fresh pepper fruit length, weight, total yield and contents of refractometric dry matter, total carotenoids (spectrophotometrically) or vitamin C (HPLC method) were recorded. The fruits were also classified according to the commercial quality standards for fresh sweet pepper into quality classes (Extra class, 1st and 2nd quality classes and substandard). Our results showed that genotype and growing season had a significant effect on the observed parameters: fruit length and weight. Application of biostimulants had a positive effect on the yield of fruits and fruit quality. Especially after Agriful application we recorded more than 20% increase in total yield while only a minimum of fruits were in substandard class. However, vitamin C or total carotenoids content were not affected by the variant.

**Key words:** *Sweet pepper, Agriful, Trichomil, biostimulants, carotenoids, vitamin C*

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## Application of hyperspectral parameters for phenotyping of wheat varieties

Lenka BOTYANSZKÁ

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Feeding the world's growing population is a challenge, one that is compounded as climate change has an impact on crop yields and food supplies. FAO estimates that world food production must rise by 60% to keep pace with demographic change. The most important food crops are more prone to abiotic stresses due to high yield and quality losses. Climate change puts this at risk, for every degree Celsius that the global thermostat rises, there will be a 5 to 15% decrease in overall crop production. Non-invasive and fast phenotypic methods in the field play a major role in the analysis of genotype-phenotype-environment interactions. To understand the emerging and unknown threats to global food security, phenotyping research and knowledge are necessary. Non-invasive and rapid methods play an important role in the study of phenotyping. Hyperspectral imaging, like other spectral imaging, collects and processes information from across the electromagnetic spectrum what can be used to identify the onset and intensity of plant drought stress. Hyperspectral analysis was used as an alternative technology to characterize the different properties of crop canopies. From a sampling standpoint, hyperspectral imaging refers to the acquisition of images in continuous spectral bands over a specified wavelength range. To examine this reliability under dry conditions, we tested the set of 35 wheat varieties grown in pot experiment trials in two variants. First variant was control variant and second was drought stress variant. The control variant had a fully compensated evapotranspiration compared to the stressed variant that received only 30% of the control variant. During the summer season, the plants were monitored 3 times weekly using fast non-invasive methods based on proximal sensing, such as VNIR polyspectral and hyperspectral measurements. The traits of wheat varieties were correlated with 132 hyperspectral indices developed to estimate different properties. The selected varieties provided relatively high diversity in all observed traits. Our results demonstrated that estimations based on spectral reflectance were reliable and some of the numerous parameters derived from measurements were able to recognize drought effects, even in the early stages of drought stress, with significant variance. Chlorophyll content had a relatively good correlation with measured data of hyperspectral indices. Hyperspectral data have also shown favorable correlations with some growth indicators. The results also confirmed that hyperspectral analysis may serve as a valuable and reliable tool in the evaluation of wheat varieties measurements in the conditions of environmental stresses.

**Key words:** *phenotyping, hyperspectral analysis, crop properties, wheat varieties*

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## Study of the mechanism of effects of glycyrrhizic acid on volume regulation in thymocytes

Diyor FAYZIEV, Ozoda KHAMIDOVA, Sarvinoz RUSTAMOVA

*Supervisors:* DSc. Ranokhon Kurbannazarova, Petr Merzlyak and acad. Ravshan Sabirov.

Licorice (*Glycyrrhiza glabra*) belongs to legumes and contains triterpens, saponins, flavonoids, isoflavonoids, chalcones and other biologically active compounds in their roots. Glycyrrhizic acid is a major component of the licorice root. Although its numerous biological activities are well known, no studies have been performed on the effects of this compound on the volume-dependent cellular processes. The aim of the present study was to investigate the effects of glycyrrhizic acid in the form of mono ammonium salt (MASGA) on the volume regulation of thymocytes. Changes in thymocyte volume were estimated from light transmission of the suspension in transmitted light at 610 nm. The study was conducted with the cell suspension (final concentration  $10^7$  cells/ml) on a MKMF-1 microcolorimeter at 25 °C.

In isotonic conditions (normal Ringer's solution, 290 mOsm/kg H<sub>2</sub>O), the volume of thymocytes remained constant for 15 min (n=5). When we studied the effect of different concentrations of MASGA, there was no significant effect at the concentrations of up to 200 μM in the isotonic medium (n=5).

Next, the effects of MASGA on volume regulation of thymocytes under hypoosmotic stress conditions (147.1 mOsm/kg H<sub>2</sub>O) was studied. Under these conditions, the cellular volume first passively increased and then, during the incubation time of 15 minutes, the volume decreased to the level of  $78.9 \pm 3.3\%$  (n=15). This phenomenon is called regulatory volume decrease (RVD). When the effects of different concentrations of MASGA on the RVD were studied, we observed two-phases. Up to 150 μM concentration, the RVD decreased to a level of  $56.1 \pm 1.5\%$  of the control value (n=8), and then, above 200 μM, the RVD increased to a level of  $69.8 \pm 2.5\%$  of control. The dose-dependency curve of the first phase was approximated with a Hill equation with a half-maximum effect at  $104.8 \pm 2.1$  μM (n=8).

In order to reveal the mechanism of the inhibitory effect of MASGA on the RVD, the cationic permeability of the membrane was increased using Gramicidin D (GrD) and all monovalent cations in the medium were replaced with large organic cation, *N*-methyl-D-glucamine (NMDG). Under these conditions, the regulatory volume decrease mainly depends on the anionic permeability of the plasma membrane. In control, the RVD of thymocytes equalled  $108.7 \pm 6.6\%$  (n=5) indicating that at 15 min cell volume became even less than the initial value. When MASGA was added into the experimental NMDG-GrD medium, the RVD value decreased depending on the MASGA dose, and at 200 μM the cell volume regulation system was suppressed down to  $84.8 \pm 2.5\%$ . When obtained results were approximated using Hill equation, half-maximum efficiency was  $62.7 \pm 6.5$  μM and Hill coefficient equalled  $3 \pm 0.7$ , respectively (n=5-7). The obtained results demonstrated that MASGA in isotonic medium does not significantly affect the cellular volume. Under hypotonic conditions, MASGA exhibited moderate inhibitory effect on RVD, and this process may occur by blockage of the volume-activated anionic channels.

**Keywords:** *glycyrrhizic acid, thymocytes, RVD, gramicidin.*

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**Phytochemical screening of five extracts of aboveground parts of *Tanacetum vulgare* L.**

Sabina GAYIBOVA, Eva IVANIŠOVÁ

*Supervisor:* prof. Takhir Aripov, academician

*Tanacetum vulgare* L. (tansy) from *Asteraceae* family has been widely applied in traditional and complementary medicine for preserving such cases as helminthiasis, hypertension, weakness, inflammation, flatulence and many others. Keeping above valuable features of *T. vulgare* the present study was carried out to analyze a qualitative content of *Tanacetum vulgare* L. stems and leaves harvested in locality Kľačno, Slovakia (395 meters above sea level). Extraction of biologically active compounds from plant sources depends mostly on the type of solvent used during the procedure of extraction. Thus several solvents namely water, methanol, ethanol, chloroform, and hexane were used and the extraction procedure of dried and powdered plant samples took 24 hours on a rotary shaker at room temperature. Benedict's test was used to identify reducing sugars, Salkowski test to identify terpenoids, Ferric chloride test to identify tannins, alkaline reagent test to identify flavonoids. Preliminary phytochemical analysis by Salkowski test did not reveal any presence of terpenoids in all the extracts. Methanolic and ethanolic extracts of Tansy were very beneficial as they contained reducing sugars, flavonoids, and tannins. On the contrary, water extract contained only reducing sugars. Chloroform and hexane extracts were not observed to possess any of analyzed compounds that were quite controversial to some other researches pointing that chloroform is a suitable solvent for extracting terpenoids and flavonoids. All the phytochemicals detected in this study are widely utilized, mostly for their pharmacological characteristics and antioxidant capacity that is very important for maintaining and restoring health. Thus flavonoids contained in medicinal plants are of interest not only as potential antioxidant drugs, but also as biologically active compounds, which can exert in total herbal remedies, contributing to the successful treatment of any disease, cause or effect which are violations in the system of antioxidant protection of the organism. Tannins are used mainly as binders, anti-inflammatory and haemostatic agents. Solutions of tannins bind to skin proteins, forming a waterproof film. This is the basis for their medical use in the form of astringents, since the film formed on the mucous membranes prevents further inflammation, and applied to the wound, they coagulate the blood and therefore act as local hemostatic agents. Terpenoids – hydrocarbons of varying complexity – are usually a mixture of simple terpenoids, their ketones and alcohols, and derivatives of phenylpropane and benzoic acid providing antimicrobial, antifungal, anti-inflammatory effects, they stimulate the digestive function, also successfully used as antispasmodic agents. Thus *Tanacetum vulgare* could be a good source of botanical compounds and determining suitable solvents and a procedure for extraction is of further investigations.

**Key words:** *qualitative analysis, solvents, medicinal herbs*

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## Evaluation of physiological traits of genetic resources of wheat by using phenomic approaches

Erik CHOVANČEK

*Supervisor:* doc. Ing. Marek Živčák, PhD.

The green revolution associated with a better understanding of crop biology was bringing higher yields of crops in a global scale. Recently, it is clear that new approaches are needed to face the future challenges. Thanks to the technological progress in non-destructive methods, the phenomics was introduced as a new scientific discipline providing huge datasets of the characteristics of plants. Phenomics is expected to contribute significantly to future progress in crop improvement, including breeding of new varieties of crops with higher yields, better adaptability to drought, high temperature and other environmental and biotic constraints. For that reason and to make the particular methods useful for the final users, development and testing the methods in various conditions and different growth phases is crucial. In our work, we have employed RGB imaging, spectral reflectance records and hyperspectral imaging to describe 35 genotypes of wheat and gain data on growth of plants, chlorophyll fluorescence and amounts of photosynthetic pigments in the leaves. RGB imaging basically scans the plants from three sides while using specific light intensity and quality. The gained data are subsequently processed and provide information about leaf area, volume of biomass, and morphological features. The fluorescence imaging uses the actinic light excitation to gain emission of fluorescence from plants, providing the data about the functional state and activity of the plant photosynthetic apparatus. Spectral reflectance methods use different electromagnetic spectra to acquire more profound information about the leaf traits in experimental plants. Our results have shown that there is sufficient genotypic variability in measured traits, reflecting the diversity of genetic resources of a testing wheat collection. Comparing the outdoor and indoor measurements, we found that spectral reflectance parameters and fluorescence excitation ratio were more reliable when done in laboratory conditions compared to the field conditions. Comparison of the results of non-invasive methods with standard analyses of leaf traits and growth parameters has shown various level of correlations, which enabled to identify reliable and promising parameters, which may serve as reliable indicators of selected traits. Thus, our results confirmed that the non-invasive methods can be used for assessment of important phenotypic traits and can be used in the next experiments to assess the environmental plasticity of wheat genotypes in conditions of drought and higher temperatures. To analyze the open tasks more deeply, in the next period we focus on measurements using more analytical, slow non-invasive methods, that may provide important novel information on the biological background of the plant environmental responses as well as on the limits of the methods.

**Key words:** *phenomics, RGB imaging, spectral reflectance, hyperspectral imaging*

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***In vitro* cytotoxic activity on cancer cell lines of lectin-like proteins from *Cuscuta europaea***

Komola A. KAKHOROVA

*Supervisor:* Z. S. KHASHIMOVA, D.Sc.

Lectins of vegetable origin are well known for their cytotoxic effects. Cytotoxic and anticancer activities of lectin-like proteins (LLP) of the phytoparasites are not well established. *Cuscuta europaea* evoked our interest due to necessity of highly cytotoxic agent efficient against cancer cells for further production of a tumor-addressed therapeutic.

The aim of this work is to study cytotoxic activity of LLP of the dodder, *Cuscuta europea*, in interrupting lines of cell cultures.

We isolated the LLPs from the seed of dodder growing on the meadow grasses by extraction with saline (0.14 M NaCl, pH 7.7). The total fraction ( $\Sigma$  fraction) followed by gradual salt in gout with ammonium sulfate. We labeled supernatants as S<sub>20</sub> and S<sub>50</sub> and the precipitates as LLP<sub>20</sub> and LLP<sub>50</sub> which were subsequently dialyzed against distilled water and used for the further work. Protein content was determined by Lowry method. The content of total carbohydrates was determined by the anthrone-sulfuric acid method. The hemagglutinating activity of the obtained proteins was studied with 2%-suspension of erythrocytes. It was found that the fractions of LLP<sub>20</sub> and LLP<sub>50</sub> exhibited a high hemagglutinating activity. Carbohydrate specificity was determined by the method described earlier. It was shown that the LLP<sub>50</sub> fraction showed distinct specificity for glucose and mannose.

To assess cytotoxic effect of the proteins melanoma B-16 and U937 cells were disseminated in 96- well plates in amounts of 20,000-30,000 of cells/ml in 100  $\mu$ l of DMEM with 10% fetal bovine serum to be cultivated at 37°C in CO<sub>2</sub> incubator. In 24 hours the proteins in the doses of 100, 10 and 1  $\mu$ g/ml in 100  $\mu$ l of the medium were added to cells to be cultivated for 24 hours, to be subsequently mixed with 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) for viable cells to be found. After one-hour incubation the medium was decanted with care to add DMSO and incubated for 20 minutes; optical density of the solution was measured at 620 nm.

On B-16 cells, the highest cytotoxic activity was found at protein dose of 100  $\mu$ g/ml by the total fraction ( $\Sigma$  fraction) and fractions of LLP<sub>20</sub> and LLP<sub>50</sub>: 96, 97 and 93%, respectively. On U937 cells, the total fraction and fractions LLP<sub>20</sub> and LLP<sub>50</sub> showed the cytotoxic activity at the protein of 100  $\mu$ g/ml: 100, 86 and 93 percent, respectively. Similar results of suppression of cell growth were obtained by counting the cells with trypan blue.

Thus, we isolated and characterized LLP from *Cuscuta europaea*, as well as demonstrated cytotoxic effect of LLP<sub>20</sub> and LLP<sub>50</sub> on B-16 and U937 cells.

**Key words:** *Cuscuta europaea*, cytotoxic activity, lectin-like glycoproteins

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## The analysis of endophytic bacteria in amaranth

Matúš KYSEL

*Supervisor:* doc. PaedDr. Ing. Jana Žiarovská, PhD.

Metagenomics is a modern analytical method for identifying uncultivated microbial species, studying mutual phyto-microbial interactions, monitoring health care of the population, or monitoring environmental quality. Microbial communities are the most reliable source of information on host plants, populations and ecosystems. We can deliver quantitative and qualitative results with high accuracy and specificity by combining these two comprehensive information resources together.

Regeneration of microbial composition could be predicted and reveal a role of microorganism in the process of trait fixation, which were applied on *Amaranthus cruentus*, L. represented by 2 varieties (Ficha and Pribina) and 2 mutation lines (M1 and M7).

Seeds of the amaranth had radically changed in the endophytic composition after a gamma rays exposure by a dose of 175 Gy. This decrease represented approximately 80% of all identified endophytic sequences comparing the M1 mutant line (after irradiation) to the original variety Ficha (before irradiation). In terms of quantitative changes due to irradiation that managed the first flux of changes, the pair of bacterial orders *Burkholderiales* and *Enterobacteriales* have gained a competitive advantage. The rest of other major endophytic representatives *Bacillales*, *Cytophagales*, *Lactobacillales*, *Micrococcales*, *Pseudomonadales*, *Rhizobiales*, *Sphingomonadales* and *Xanthomonadales* have been exceeded more than twice by this competitive advantage. This did not occur immediately, but with the timeshifted second flux of quantitative changes when the original endophytome level has restored and followed by increase of about 40% (comparison of the original variety Ficha and the new variety Pribina (radiation mutagenesis)). The crucial factor is the moment of colonization indicating the outcome of the settlement by a given bacterial species and determining the composition of the endophytes. The "quorum sensing" strategy allows and controls the coordination of gene expression depending on the density of bacterial settlement for *Burkholderia*, *Pseudomonas* and *Enterobacteria*. Our recent studies showed that these bacterial orders contain pathogenic species capable of using this strategy not just to acquire a dominant position in the endophytic composition of the host plant but also to shift a recolonization and delay a trait fixation (the weight of a thousand seeds).

Final results show that the presence of bacterial endophytes is significant because of their metabolic versatility for host plant homeostasis and can even affect the fixation of the target sign. This knowledge is even more important if pathogenic bacterial species using the "quorum sensing" strategy are involved because the amaranth has a significant role in the plant production. Preventing their expression and uncontrollable spread requires them to be identified, to determine the colonization phase, the state in which the colonies are located, and, last but not least, the health status of the host plant.

**Key words:** *amaranth, metagenomics, endophytes, bacteria*

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## **Influence of plant growth stimulators Florone and Fertisilin on selected yield-forming indicators**

Ján MAREK

*Supervisor:* doc. Ing. Ivan Černý, PhD.

The aim of the field poly-factorial experiment was to test the effect of two plant growth stimulators Florone and Fertisilin on selected yield-forming parameters (number of plants, number of heads, head diameter, weight of head, weight of thousand achenes) and seed yield of sunflower. All afore mentioned indicators were observed and analysed on sunflower hybrid Carrera, which has a high yield potential and can be cultivated with ClearField plus technology. The experiment was implemented in vegetal season of the year 2018 at the research fields of the Plant Biology and Ecology Centre of the Faculty of Agrobiolgy and Food Resources of the Slovak University of Agriculture in Nitra – Dolná Malanta. Biostimulator Florone is a product on free amino-acids basis with added NPK and microelements such as B and Mo. Stimulator Fertisilin contains in addition to microelements, a silicic in the form of ortho-silicic acid, which is fully accessible and assimilated by plants. Both biostimulators were applied, depended on variant, in three terms (phase of 6-8 leaves, beginning of flowering, and both terms). Dosing for stimulator Florone was  $0.4 \text{ l.ha}^{-1}$  and for Fertisilin was  $0.7 \text{ l.ha}^{-1}$ . The influence of biostimulators on parameters such as number of plants (60 409), number of heads (60 518) and weight of thousand achenes (81.41 g) was statistically non-significant. The influence of biostimulators on head diameter (245.71 mm), weight of head (166.87 g) and seed yield ( $3.97 \text{ t.ha}^{-1}$ ) of sunflower was statistically significant. By using of statistical variance analysis ( $\alpha = 0.01$ ), was not determined influence of application of plant growth stimulators on number of plants, number of heads and weight of thousand achenes. The influence of biostimulators effect was confirmed by variance analysis ( $\alpha = 0.01$ ) on head diameter, weight of head and seed yield of sunflower. The highest values of head diameter (269.00 mm) and weight of head (207.20 g) were observed in variants with used biostimulator Fertisilin. The highest values of weight of thousand achenes (89.706 g) and seed yield ( $4.461 \text{ t.ha}^{-1}$ ) of sunflower were on variants with used biostimulator Florone.

**Key words:** *Sunflower, biostimulators, yield-forming parameters, yield*

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## Change of floristic composition and plant production of grassland by digestate application

Alena ROGOŽNÍKOVÁ

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The article presents the results of changes in floristic composition and production of grass phytomass in the cambisol soil type due to the surface application of nitrogen fertilizer of organic origin called as digestate. A bio-component substrate, a secondary product of biogas plants, is created by co-fermentation process of plant and animal biomass. Economic costs minimizing for maintaining soil fertilising properties in order to provide nutrition for aboveground and underground biomass production plays a very important role in the management system, especially during the shortage of feed for animal husbandry. The aim of the research was to evaluate the potential of increasing the growth of grassland production in the first and the second year of the year after a single application of digestate in conditions of sustainable and circular economy in agriculture, the output is the transfer of knowledge for practice. The digestate was applied once in the spring 2016 to the surface of the grassland research area. The area is located in a landscape-ecological complex of a polygenic hill-county and a low plateau foothill with a mosaic of deciduous trees, mixed forests and grasslands at an altitude of 480 m on a slightly sloping slope with north-eastern exposure. Field experiment of four variants with individual fertilization doses was based on a block method in three repetitions. The area of the experimental parcel was  $5 \times 2$  m ( $10$  m<sup>2</sup>). On the basis of the chemical analysis of the extinguished biocolor, the number of individual doses of the applied biochole equal to the net nutrients of nitrogen in  $\text{kg} \cdot \text{ha}^{-1}$  of N in variants V1 (non-fertilized variant  $0 \text{ kg} \cdot \text{ha}^{-1}$  of N), V2 ( $90 \text{ kg} \cdot \text{ha}^{-1}$  of N), V3 ( $120 \text{ kg} \cdot \text{ha}^{-1}$  of N) and V4 ( $150 \text{ kg} \cdot \text{ha}^{-1}$  of N). In the years 2016 – 2017, the stands were used by three times mowed, the first mowing being made at the beginning of the flowering stage of dominant grass species, the second one with a 7 – 8 week interval after the first, the third (depending on the state of the crop) 8 – 10 weeks after the second. Before each mowing, the floristic composition of the vegetation was carried out by the method of projected dominance according to Regal. In each case, the average sample of green matter was taken from the individual variants to determine dry biomass and production quality. The dry matter content was determined by gravimetric method, drying the sample at  $103 \pm 2^\circ$  C in a laboratory drier. Statistically significant differences were observed in the crops over the reference years and between the non-fertilized variant and the other variants. Statistically non-conclusive results were found between variants with doses of  $90 \text{ kg} \cdot \text{ha}^{-1}$  of N and  $120 \text{ kg} \cdot \text{ha}^{-1}$  of N.

**Key words:** *grassland, plant production, fertilization, digestate, sustainable management*

**Acknowledgement:** The work was supported by the National Agricultural and Food Centre – Grassland and Mountain Agriculture Research Institute (NPPC – VUTPHP Banská Bystrica) as part of a project: Complex Systems of Management of Grassland under the "New Model of Science and Research" in the Ministry of Agriculture and Rural Development of the Slovak Republic.

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## Applications of metagenomic approaches in plant biotechnologies

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Metagenome is a common name for genomes of individuals located in one place at one time and metagenomics is a field of biology interested in. NGS approaches not only makes new options how to get data for plant biotechnologies, but it allows data availability because of relative low cost and quick protocols. NGS provides amounts of data, e.g. origin, phylogenetic relationships, the presence or absence of species and more. Metagenomic approaches can be used for specialized research such as rapid pathogen identification, dendrogram design according to phylogenetic relationships and more. Pathogen identification by NGS used Adams et al. (2009) for unknown virus pathogen isolated from *Liatris spicata*. Nagaraj et al. (2017) used 16S rRNA metabarcoding to identify bacterial communities on reverse osmosis membranes of a full-scale desalination plant and design its dendrograms. Data can be also used as primary studies for making new hypothesis which can be verified by other methods. In this case, it is mainly about screening the settlement of different habitats. Knowledge about the settlement of extreme habitats offers information about the way of life, adaptation to stress conditions or products of new species which could be used in research in the future. Application of such information is also important in agriculture, mainly for rapidly changing climatic conditions or soil contamination. Nowadays, agriculture metagenomic research is interested in symbiotic relationships between plants and microorganisms in different conditions (drought, salt soil, heating) and try to find out how the relationships could affect plant vitality.

This approach was used on the lettuce to screen inside root microbiome. The study compared microorganisms of micromycetes of six varieties of lettuce (DarkGreen Romain, Pavane, Shinning Star, Sentry, Romana Larga Blanca, Bibb), which were grown under constant conditions: 200 Lux.m<sup>-2</sup>.S in a cycle: day 16 hours and night 8 hours; 18 - 20 °C; 60% humidity. Fungi microbiome was selected from other microbiome by ITS4 and ITS7 primers in selected PCR. Kit TrueSeq Illumina and its protocols were used to sequence whole fungi microbiome by sequenator MySeq Illumina. Sequences were grouped to OTU's and identified to a genus level in program Seed 2. Primary screening provided data of root microbiome and it was found that species distribution was affected by the variety as well as by the specific microenvironment was formed by the root. The largest differences were made by three genera of microscopic fungi: *Candida*, *Malassezia* and *Actinomucor*, which were considered to be odd-specific.

**Key words:** *plant biotechnology, metagenomics, fungi, lettuce, NGS*

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SECTION

*Technology, quality and safety of raw materials and foodstuffs  
of animal origin*

**Identification of animal species in Slovak traditional cheeses using LCD Array kits  
Meat 5.0**

Lucia BENEŠOVÁ, Jozef GOLIAN

*Supervisor:* prof. Ing. Jozef Golian, Dr.

Detection of species substitution have become an important issue in the food and farming industries. Important need is the development of simple, reliable, reproducible and repeatable tests that would be able to detect the presence of species in dairy products. One reason for counterfeiting may be that cow's milk is considerably cheaper than ewe's or goat's milk, but also changes in demand and supply. Traditional half-fat cheese that is produced in Slovakia is Oštiepok cheese. The aim of the work was the identification of 19 samples of oštiepok cheeses obtained from different regions of Slovakia. According to the label, they have been produced from the ewe's milk. We used innuPREP DNA Mini Kit (Jena Analyzer) to isolate DNA from samples. The correctness of the amplification (PCR product size) and PCR fragmentation efficiency were verified by electrophoresis. For the authentication, if the samples contain the impurity, the manufacturer of the undeclared animal component we used the Meat 5.0 LCD chip, which is suitable for testing raw, pasteurized or otherwise heat-treated food (meat and dairy) matrices. The chip was analysed using a scanner (PF 7250U, Chipron), which can analyse all reaction chambers at once. Samples were evaluated using ChipronV12 (SlideReader V12, Chipron). The analysis was repeated for each sample 2 times. The chip can detect the presence of <1% DNA, but this method does not serve for DNA quantification. Sheep DNA was presented in all 19 samples of oštiepok cheeses, but in 18 of the samples were presented another species of animals. Pig DNA were found in 13 samples, goat DNA were found in 11 samples, cow DNA were found in 3 samples, chicken DNA were found in 2 samples and camel DNA was found in 1 sample. We found that some species of animals such as cattle, goat, pig, chicken, and camel caught by the chip may be present in the products, since ruminant, swine and poultry are used to produce rennet's. Camel chymosin shows 70% higher milk coagulation activity than calf chymosin, DNA of the camel was found in one sample. DNA of other animal species could also be present in samples due to contamination and thus failure to observe the principles of good manufacturing practice or could be cases of food adulteration.

**Key words:** *authentication, oštiepok cheeses, LCD Meat 5.0.*

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**Carcass characteristics and quality of veal from bull calves of two different breeds**

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*Supervisor:* doc. Ing. Klára Vavrišínová, CSc.

Monitoring of selected carcass parameters and analysing of qualitative parameters of veal calves has become an object of concern. The aim of this experiment was to evaluate slaughter parameters and veal quality of calves from two different utility types: Holstein (H) and Slovak spotted breed (S). Experiment included 7 bulls of Holstein and 7 bulls of Slovak spotted breed. Calves of both groups were housed and fed in the same conditions. After 150 days were calves slaughtered in the slaughter house according to the standard methods, dressed and chilled for 24 hours. After chilling, dissection of the right carcass half was carried out. For physico-technological analysis were samples of *m. longissimus thoracis* (MLT) and top round (*m. semitendinosus*) taken. Energy value in 100 g of meat sample was calculated according to the basic equation for calculating the energy value of the food. Values of pH were measured 1 hour and 24 hours after slaughter. Bulls of Slovak spotted breed were heavier at the end of experiment ( $P \geq 0.05$ ). No significant differences in the carcass yield were revealed. Higher content of rumen and intestinal fat was observed in the H veal calves ( $P \leq 0.001$ ). Weights of head, skin and legs were greater in S bulls ( $P \leq 0.001$ ;  $P \leq 0.01$ ). Proportions of individual valuable cutting parts were heavier in Holstein calves, especially round meat, tenderloin as well as sirloin ( $P \geq 0.05$ ). Carcasses of Slovak spotted breed had higher MLD area ( $33.74 \text{ cm}^2$ ), results were not significant. Significant differences were found in the proportions of bones ( $P \leq 0.01$ ), but no significant proportion of meat from right carcass half ( $P \geq 0.05$ ), marrow bones ( $P \geq 0.05$ ) and separable fat ( $P \geq 0.05$ ). Statistical significant variety of the water content ( $P \leq 0.01$ ),  $\text{pH}_1$  ( $P \leq 0.001$ ) and  $\text{pH}_{24}$  ( $P \leq 0.001$ ) values as well as drip loss value ( $P \leq 0.01$ ) were found. In the Holstein veal calves were found higher values of  $\text{pH}_{24}$  in both muscles (MLT – 6.13,  $P \leq 0.001$ ; top round – 6.04,  $P \leq 0.001$ ). Lightness (CIE  $L^*$ ) of *m. longissimus thoracis* after 24 hours post mortem was similar in both breeds ( $P \geq 0.05$ ). In colour spectrum after 7 days after slaughter we observed darker and redder meat in the S male calves which had also higher electrical conductivity value ( $P \leq 0.001$ ). Leaner colour of MLT from Holstein calves were characterized by high  $L^*$  values ( $P \leq 0.01$ ), low  $a^*$  (-11.16,  $P \geq 0.05$ ) as well as  $b^*$  value (-12.66,  $P \leq 0.001$ ). Energy value of the meat was greater in the Holstein veal calves ( $447.46 \text{ g} \cdot 100 \text{ g}^{-1}$ ) consistent with the higher IMF content; however results were not significant ( $P \geq 0.05$ ).

**Key words:** *calves, veal quality, proportion of tissues, cutting parts, slaughter value*

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**Antihypoxic activity of the watersoluble protein fraction *Artemia cyst***

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Hypoxia is a typical pathological process that adversely affects human bioenergetics. Oxygen is necessary for the synthesis of a source of free energy-adenosine triphosphate. Under hypoxia, functions of all cells decrease, physical and mental performance, fast fatigue, depression, apathy, immunodeficiency etc. develop. Hypoxia initiates the accumulation of under-oxidized products - free radicals. The latter quickly form powerful destructive oxidative reactions, which can be removed by biologically active additives - antioxidants.

The aim of the work was to study the antihypoxic activity of the watersoluble protein fraction isolated from the Aral Sea *Artemia* cysts, proposed as a dietary supplement.

Antihypoxic activity was studied in models of cytotoxic hemic and normobaric hypoxia. Experimental studies were performed on 60 white male and female laboratory mice weighing  $30 \pm 2.0$  g, divided by 10 animals per group. The protein fraction was administered orally to experimental mice at doses of 20, 30, 40, 50 mg/kg, one hour prior to the onset of hypoxia modeling. Animals of intact group were administered distilled water orally in a bioequivalent volume.

In hemic hypoxia, the life span in an intact group of animals was  $11.6 \pm 1.0$  minutes. With the introduction of distilled water (control), the life expectancy increased to  $12.0 \pm 1.1$  minutes, and the antihypoxic effect accordingly was 13.5%. When water soluble protein fraction was administered at a dose of 20 mg/kg, the life expectancy increased to  $17.5 \pm 1.2$  minutes, and the antihypoxic effect correspondingly was 45.8%. The best indicator for antihypoxic activity was revealed when a protein fraction was administered at a dose of 30 mg/kg, which was  $19.5 \pm 1.2$  or 62.5%.

Study of the protein fraction at a dose of 40 and 50 mg/kg showed a decrease in the antihypoxic effect with respect to the dose of 30 mg/kg. In the experimental group of animals, using a dose of 40 mg/kg, the life expectancy was  $16 \pm 1.2$ , or 33%, and at a dose of 50 mg/kg -  $13.5 \pm 1.2$  or 16%.

When studying normobaric hypoxia, it was shown that the life span in an intact group of animals was  $13.4 \pm 1.0$  minutes. With the introduction of distilled water (control), the life expectancy increased slightly to  $15.0 \pm 1.2$  min, and the antihypoxic effect, respectively, was 12%. With the introduction of the watersoluble protein fraction at a dose of 20 mg/kg, the life expectancy increased to  $18.3 \pm 1.7$  minutes, and the antihypoxic effect was 36.6%, respectively. In the experimental group of animals which were administered a dose of 40 mg/kg, the life expectancy was  $17.1 \pm 1.4$  or 28%, and in a dose of 50 mg/kg,  $15.5 \pm 1.3$  or 15.7%. The best indicator for antihypoxic activity, as well as in case of hemic hypoxia, was also detected when the protein fraction was administered at a dose of 30 mg/kg, which was  $21.3 \pm 2.1$  or 59%.

Thus, it has been established, that at a dose of 30 mg/kg, water soluble protein fraction of *Artemia cyst* shows the best antihypoxic activity in both cases.

**Key words:** *Hypoxia, water soluble protein fraction, the Aral Sea Artemia cysts,*

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**Evaluation of effect of various buckwheat honey additions on sensory properties of yogurts during storage**

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Yogurt is fermented dairy product obtained by fermenting milk with lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The typical aroma of yogurt is generated from metabolites produced by yogurt culture. Lactic acid and a wide range of compounds are generated in the process of fermentation of lactose by lactic acid bacteria.

The aim of this study was to produce yogurts with the different additions of buckwheat honey and evaluate chosen sensory properties of yogurts with honey addition and compare them with control samples without addition of honey.

Three experimental samples of yogurts were produced with the various additions (1, 3 and 5% w/v) of buckwheat honey. As a control was used yogurt without honey addition. Semi-skimmed milk, skimmed milk powder and yogurt culture were used for yogurts production. Sensory analysis was performed by five-member committee of assessors who evaluated selected parameters by fivepoint scale. Honey aroma, undesirable aroma, sweetness taste, sour taste, bitter taste, firm consistency and washy consistency were evaluated. Evaluation was carried out on the 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>th</sup> day of refrigerated storage.

The honey aroma of yogurt samples with buckwheat honey addition increased during 21 days of storage. The honey aroma of yogurts increased gradually with increased addition of honey. During whole storage period, undesirable aroma was observed in all of yogurt samples with honey addition compared to control samples. In the control samples, undesirable aroma was detected only on the 1<sup>st</sup> and 7<sup>th</sup> day of storage. The yogurt samples with the lowest honey addition showed decrease of undesirable aroma during storage. On the contrary, undesirable aroma increased gradually in the yogurt samples with the highest honey addition during 21 days of storage. From the view of taste, sweetness taste of yogurt samples increased gradually with increased addition of honey. The sweetness taste decreased gradually in the yogurt samples with honey addition during storage. The sour taste of yogurt samples decreased with increased addition of honey. The bitter taste was detected in all of analysed samples. In the control samples without honey addition, the increase of intensity of bitter taste was observed during storage. From the view of consistency, the control samples showed the highest firmness during storage. The firm consistency decreased gradually in all of analysed samples during storage. On the contrary, the washy consistency of all analysed yogurt samples increased during storage.

**Key words:** *yogurt, buckwheat honey, sensory properties*

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SECTION

*Technology, quality and safety of raw materials and foodstuffs  
of plant origin*



**Effect of gamma-cyclodextrin- and 2-hydroxypropyl-beta-cyclodextrin-complexed  
cynaroside on aorta relaxation**

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*Supervisor:* prof. Takhir Aripov, academician

Some pharmaceutically beneficial plant substances are often poorly soluble in water that limits their consumption in the field of medicinal, veterinary, cosmetic, food products, etc. At the present methods for increasing the solubility of the sparingly soluble preparations or increasing the degree of their dissolution are abundant. One of the contemporary methods showing good results and being quite safe is using cyclodextrins as solubilizers. Due to their unique structure, cyclodextrins are able to form clathrate inclusion complexes of the "guest-host" type with different types of molecules: organic, inorganic, organometallic, etc. Numerous of poorly soluble in water natural compounds, namely polyphenols, encapsulated in a complex with cyclodextrins, was studied. The results showed good solubility in water, as well as an increase in antioxidant capacity. A great interest in polyphenols is associated with a wide range of their biological effects, including antioxidant anti-inflammatory, cardioprotective, anticarcinogenic effects. Cynaroside, which is 7-O-glucoside of luteolin – is a natural flavone, one of the bioactive compounds purified from various genres of plant families: Apiaceae, Lamiaceae, Asteraceae, etc. In recent years, several lines of evidence suggested that cynaroside while being non-toxic, reduces hepatotoxicity, repairs antioxidant system, normalizes carbohydrate, energy and lipid metabolism as well as improves flow of bile, etc. Therefore, scientific interest in flavones as therapeutic agents is rapidly increasing. We have previously investigated the complex between flavone cynaroside and gamma-cyclodextrin (cyn/ $\gamma$ -CD), and cynaroside and hydroxypropyl-beta-cyclodextrin (cyn/2-HP- $\beta$ -CD) prepared by co-evaporation and lyophilization methods. Spectrophotometric studies have shown that cynaroside water-solubility increases in the present of cyclodextrins. The aim of the present study was to investigate the effect of cynaroside and its cyclodextrin complexes on rat aorta relaxation. Aorta rings from white random-bred rats of both sexes were used. Briefly, the isometric tension was recorded from rat isolated aortic rings mounted between steel wires in an organ bath. Our preliminary study showed that pure cynaroside relaxed rat aorta rings pre-contracted by 50 mM KCl, with  $IC_{50} = 27.2 \pm 2.4$  microM.  $\gamma$ -CD and 2-HP- $\beta$ -CD were also studied on pre-contracted aorta rings and did not reveal any relaxation effect. Complex cyn/ $\gamma$ -CD prepared by co-evaporation and lyophilization methods were found to relax aorta rings with  $IC_{50} = 91,71$  microM and  $IC_{50} = 83.95$  microM, respectively. However, when recalculating to the pure cynaroside in the complex  $IC_{50}$  was 13.23 microM and 21.45 microM, correspondingly. Moreover, cyn/2-HP- $\beta$ -CD complexes prepared by above mentioned methods also showed lower  $IC_{50}$  (14.54 microM and 8.24 microM, respectively) when recalculating to pure cynaroside. These results demonstrate that the complex cyclodextrins-cynaroside shows higher bioavailability in comparison to cynaroside alone.

**Key words:** *water solubility, co-evaporation, lyophilization, vasodilatation, contraction*

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**Isolation and identification of water-soluble and alkali-soluble polysaccharide fractions  
from basidiomycetes**

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*Supervisor:* Acad., Prof., Dr. Abbaskhan Turaev

Polysaccharides are a complex group of structural and functional macromolecules widespread in nature, in a great variety of organisms such as plants and animals. These polymers are found in pericellular locations, on the external surface of cell membranes, and play a crucial role in recognition mechanisms, cell-cell communication, acting as a barrier between tissues and protection against pathogens. Some polysaccharides isolated from fungal species contain a large variety of polysaccharides such as pectin, chitosan, and glucose-rich polysaccharides that have a number of biological activities. In addition, these polysaccharides are natural resources to prepare bio- and nanomaterials by chemical modification.

In this report, the results on isolation and identification of water-soluble and alkali-soluble polysaccharide fractions from basidiomycetes were shown. The basidiomycete materials were finely powdered, then placed into a Soxhlet extractor, and extracted with the chloroform-ethanol system (1:4) for 72 h to eliminate the lipids and pigments. The residues were air dried and then extracted three times with 15 volumes of distilled water, 0.1 M HCl solution and 5% NaOH solution for 4 h at 100°C to obtain water-soluble polysaccharides. The aqueous fractions were centrifuged, the supernatant concentrated using vacuum evaporator and dialyzed against distilled water. The alkaline fraction was centrifuged, the supernatant neutralized, the yielded precipitate collected by centrifugation, suspended in water and dialyzed. The acidic and alkaline fractions, after dialysis, were concentrated using vacuum evaporator and freeze-dried to yield water-soluble and alkali-soluble crude polysaccharide fractions. The yield of the polysaccharide fractions was 10-15% by the weight. In the IR spectra of the polysaccharide fractions, the absorption bands corresponding to the polysaccharides and melanins were observed at 3430 cm<sup>-1</sup>, 2920 cm<sup>-1</sup>, 1630 cm<sup>-1</sup>, 1420-1380 cm<sup>-1</sup>, and 1200-750 cm<sup>-1</sup>. The paper chromatography results showed the polysaccharide fractions contain glucose, mannose, and galactose, indicating that fractions are composed of glucose-rich polysaccharides. In addition, the UV-VIS spectroscopy studies showed that the solution of the polysaccharide fractions have an absorbance at 300-400 nm, characteristic for melanins, and the fractions contain the melanin up to 40% by the weight.

In conclusion, water-soluble and alkali-soluble polysaccharide fractions were isolated from the basidiomycetes with a yield of 10-15%. The studies showed the polysaccharide fractions constitute glucose-rich polysaccharides and melanins. It seems the polysaccharides and melanins in the basidiomycetes found in a complex form.

**Keywords:** *basidiomycetes, polysaccharides, melanin, isolation, characterization*

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**Studying hot air drying of sweet potatoes with dual frequency ultrasound pretreatments and its influence on the quality and bioactive composition by HPLC-DAD and FTIR analysis**

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*Supervisor:* Ma Haile (PhD, School of Food and Biological Engineering)

The drying of sweet potatoes not only helps to prolong its storage life but the dried form reportedly enriched with high bioactive compounds than the regular sweet potatoes. The study aims to investigate the influence of dual-frequency ultrasound pretreatments (40 KHz and 60 KHz) at two different hot air-drying temperatures (70 °C and 80 °C) on the quality of dried product. Ultrasound pretreatment at 40 KHz with 70 °C maintained the phytochemical compounds in the dried sweet potatoes. Ellagic acid and chlorogenic acid were found as predominant phenolic acids using HPLC analysis, while identification of two new bioactive compounds quercetin-3-rhamnoside and quercetin 3-β-D-glucoside were the novel finding of the current study. A short new band appeared in FTIR in all treatments from 2164 to 2041 cm<sup>-1</sup> which refers to C = C alkenes functional group. The multivariate analysis showed great influence of USH3 and USH1 with positive relationship with most of bioactive compounds.

**Key words:** *dual-frequency ultrasound, drying, phenolic profile, antioxidants, carotenoids, SEM, FTIR*

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