

**Slovak University of Agriculture in Nitra**

**FACULTY OF AGROBIOLOGY AND FOOD RESOURCES**

**FACULTY OF BIOTECHNOLOGY AND FOOD SCIENCES**

**Scientific Conference of PhD. Students  
of FAFR and FBFS, SUA in Nitra**

-

**Proceedings of abstracts**



**on the occasion of the Science and Technology Week  
in the Slovak Republic**



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**Scientific Conference of PhD. Students of FAFR and FBFS, SUA in Nitra  
- Proceedings of abstracts**

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## **Preface**

The conference Proceedings is a collection of selected reviewed abstracts submitted to and presented at the Scientific Conference of PhD. Students hold on November 10, 2016 in Nitra, Slovakia. Submitted full scientific papers were reviewed and the best papers were selected for publication in Journal of Central European Agriculture (JCEA, Vol. 17, No. 4).

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).

Main aim of the Conference was to provide a platform for presentation of research findings, exchange of experiences and knowledge sharing. Presentations focused to the following basic and applied science results:

- Agrobiotechnologies
- Animal production
- Applied and molecular biology
- 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

Committees hope, that the Conference provided a meeting place for creative discussion and new ideas for collaboration; and that the scientific programme have contributed to increasing your knowledge, improving your 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*

## The establishment of *in vitro* cultures of *Rubus* spp. cultivars 'Loch Ness' and 'Reuben'

Júlia HUNKOVÁ

*Supervisor:* RNDr. Alena Gajdošová, CSc.

*Rubus* genus belongs to the most well-known group of small fruits consisting of several hundreds of species and hybrids. Both raspberries and blackberries contain high levels of vitamins, various antioxidants and other compounds (flavonoids, polyphenols) which are beneficial for human health. The demand for this fruit is continuously growing, especially in the food industry for various products as juices, jams, syrups, etc. However, commercial large-scale propagation still remains a problem. Micropropagation as a method for producing large amount of clone-identical plant material from mother plant could present one solution. With this method, planting stock for wide range of cultivars could be obtained and delivered to the market for further applications. 'Loch Ness' and 'Reuben' belong to the thornless cultivars of blackberries. They regularly provide high yield of fruit and are both frost- and disease-resistant. Although numerous reports about *Rubus* spp. micropropagation have been published, each cultivar responds and regenerates differently under *in vitro* conditions. That is why further experiments are needed for optimizing each step of this process in order to develop an effective multiplication system. The aim of this work was to develop protocols for micropropagation of selected *Rubus* spp. cultivars, namely 'Loch Ness' (*Rubus fruticosus* L.) and 'Reuben' (*Rubus eubatus*). *In vitro* cultures of these two cultivars were established from dormant buds collected in February 2016. Sterilization of material from contaminants was performed by agitation for 1 minute in 70 % ethanol and for 4 minutes of 0.1 % HgCl<sub>2</sub>. For shoot initiation, MS medium containing 8 g.l<sup>-1</sup> phytoagar, 30 g.l<sup>-1</sup> sucrose, 1 mg.l<sup>-1</sup> BAP, 0.1 mg.l<sup>-1</sup> IBA and 0.1 mg.l<sup>-1</sup> GA<sub>3</sub> was used. After 4 weeks of cultivation, BAP concentration was decreased to 0.5 mg.l<sup>-1</sup> and IBA concentration was increased to 0.2 mg.l<sup>-1</sup>. All explants were incubated in growth chamber at 25°C day/night temperature and 16h photoperiod. The shoot proliferation ability of these cultivars was evaluated by number of shoots formed per explant calculated after three subcultures (i.e. multiplication coefficient Q). 'Loch Ness' cultivar formed higher number of shoots than 'Reuben', resulting in Q = 3.48. Rooting was achieved on MS medium containing 1 mg.l<sup>-1</sup> IBA after 6 weeks of incubation. All plants formed visible roots and 90 % of them had survived acclimatization phase. Achieved data can contribute to further optimization of various *Rubus* spp. cultivars micropropagation.

**Key words:** *Rubus*, micropropagation, shoots multiplication, genotype

**Acknowledgement:** This scientific work was co-funded by project VEGA 2/0140/14 and project Research Centre AgroBioTech built in framework of European Community project Building Research Centre „AgroBioTech" ITMS 26220220180.

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## The remediation process of different soil types polluted by oil and oil products

Zafarjon A. JABBAROV

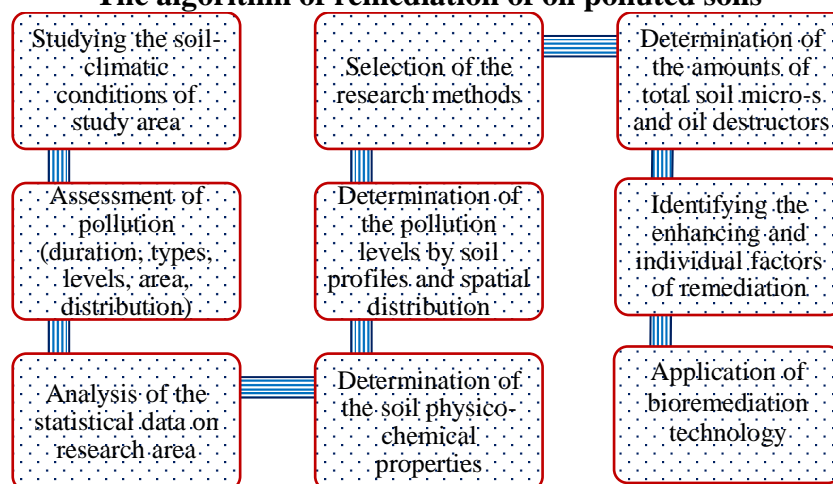
*Supervisor:* Dr. Tokhtasin Abdrakhmanov

The soil types and their main properties are different according to soil-climatic and spatial distributions features. In Uzbekistan, there are certain soil types and seasons which adequately separated from each other.

Nowadays, it is observable than in the same progress with oil industry the soil, pollution by oil and oil products is enlarging. Thus, our research focuses on to enlight of the oil pollution characteristics and mechanisms of remediation in different regional soil types of Uzbekistan.

In the study, in five soil types of two regions the bioremediation based on laboratory lysimeters were conducted. In soil types are light sierozems (by WRB, 2006), takyrs (by WRB, 2006), sandy desert soils (by WRB, 2006), grey-brown soils (by WRB, 2006) and sierozem-meadow soils (by WRB, 2006). According to our results, the analysed levels of oil pollution in light sierozems at 31-88 g/kg, in takyrs within 28-68 g/kg, in sandy soils at 43-241 g/kg, in grey-brown soils at 13-37 g/kg, and from 370-141 g/kg were in sierozem –meadow soils. We have developed an algorithm and stages of remediation as follows (picture 1).

### The algorithm of remediation of oil polluted soils



The main order in remediation is taken by the algorithm, thus it is essential to have are relevant data on soil-climatic conditions, levels and duration of pollution of soils of the research area.

Remediation technology requires special individual attention to each soil types, their physico-chemical properties, application of bacteria strains as an association, addition of mineral fertilizers, clover and sugar-maize phytomeliorative plants as well. The above mentioned treatments were implemented as preparation, agrotechnical, biological, analytical, and phytomeliorative stages. The duration and direction were set according to soil types, and the purification levels were determined.

The pollution soils were purified within 81-96 percent individually by soil types, and the soil fertility is improved after second year of remediation.

**Keywords:** soil, oil and oil products, pollution, remediation

**Acknowledgement:** The study were implemented within the project funding by the grants A-7-005 and Erasmus Mundus CASIA 1 we thank to all of the project coordinators and partners for their kind help and support.

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## Comparison of growth characteristics and proteolytic activity of *Lactobacillus fermentum* CCM 7192 and *Lactobacillus zymae* CCM 7241

Kvetoslava KAČMÁROVÁ, Peter SOCHA, Blažena DRÁBOVÁ, Dana URMINSKÁ

Supervisor: doc. RNDr. Dana Urminská, CSc.

*Lactobacillus* is the most representative strain in a group of lactic acid bacteria, which can be used for the preparation of sourdough bread. Lactobacilli are able to improve bread volume and texture, make flavour precursors or degrade celiac active peptides. They mainly produce lactic acid during the fermentation process. Proteolysis in dough depends on chemical or microbial acidification of dough. The first step is primary proteolysis, activated by cereal proteinases in acidic conditions, which cleave proteins into the smaller peptides. During the secondary proteolysis, peptides are degraded into di-, tripeptides or amino acids inside the intracellular matrix of lactobacilli. Some species of lactobacilli produce specific peptidases capable to hydrolyze hard-cleavable prolin-rich peptides. In this study we selected some lactobacilli species according to their general proteolytic aswell as prolyl-specific activity. These species can potentially be used for sourdough fermentation resulted in a dough with reduced allergenic potential to celiac disease. Growth characteristics and proteolytic activities of intracellular crude enzyme preparations derived from *Lactobacillus fermentum* CCM 7192 and *Lactobacillus zymae* CCM 7241 were analyzed. The growth of lactobacilli was studied by measuring the optical density (OD) at  $\lambda$  600 nm, pH value of MRS medium and dry cell weight. For determination of proteolytic activity specific substrates based on *p*-nitroanilides: Phe-*p*NA, Ala-*p*NA, Leu-*p*NA, Met-*p*NA, Pro-*p*NA and Lys-*p*NA were used. Results were expressed in units of activity per volume of solution ( $\mu\text{kat}\cdot\text{dm}^{-3}$ ). The exponential growth phase of *L. fermentum* CCM 7192 and *L. zymae* CCM 7241 were observed between 16<sup>th</sup> and 20<sup>th</sup> hours and 28<sup>th</sup> and 32<sup>th</sup> hours, respectively. Specific enzymatic activities for all *p*-nitroanilide substrates were detected and confirmed in both species of lactobacilli. *L. fermentum* possessed the highest activity of lysin-specific peptidase ( $821 \mu\text{kat}\cdot\text{dm}^{-3}$ ), while phenylalanine-specific peptidase showed the lowest activity ( $93 \mu\text{kat}\cdot\text{dm}^{-3}$ ). Peptidase complex of *L. zymae* reached different values. Activity of leucine-specific peptidase ( $1241 \mu\text{kat}\cdot\text{dm}^{-3}$ ), was the highest. On the contrary, proline-specific peptidase ( $26 \mu\text{kat}\cdot\text{dm}^{-3}$ ), was the lowest among all peptidases. Very interesting finding was identification of prolyl-specific activity in enzyme preparations of both species. It is very important prerequisite for successful cleavage of prolin-rich peptides for producing of gluten-low or gluten-free cereal products. Selected *Lactobacillus* strains will be used for fermentation experiments to produce a bread with low gluten content.

**Key words:** *Lactobacillus zymae*, *Lactobacillus fermentum*, enzymatic activity, sourdough fermentation

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SECTION  
*Animal Production*

## **Impact of lameness and somatic cells count on the production and composition of ewe's milk**

Štefan BARANOVIČ, Vladimír TANČIN, Michal UHRINČAŤ, Lucia MAČUHOVÁ,  
Jozef PALKOVIČ

*Supervisor:* prof. Ing. Vladimír Tančín, DrSc.

Lameness and high somatic cell counts in milk are two very serious problems on the farms. The aim of the study was to evaluate the impact of lameness and somatic cells count and order of entry into the milking parlour on the milk production and its composition. We examined also the relationship between lameness and SCC and their impact on the order of entry. The experiment was carried at the farm, located in northern Slovakia. The farm keeps sheep crossbred of Improved Valachian and Lacaune. Milking was performed two times a day in milking parlor 1x24. Samples of milk were taken during two periods by evening milking: May, July. In May, 214 samples from random sampling ewes with milk yield minimum 300 ml per milking were collected. In July milk samples, milk yield and lameness from same ewes as in May were recorded. The order of entry into the milking parlour in milking row was also recorded. Number of milking rows was recorded 23 together. Ewes was divided by lameness (non-lame, slightly lame, strong lame), by SCC (A1= to  $2 \times 10^5$  cells, A2= from  $2 \times 10^5$  to  $4 \times 10^5$  cells, A3= from  $4 \times 10^5$  to  $7 \times 10^5$  cells, A4= from  $7 \times 10^5$  to  $10 \times 10^5$  cells A5= over  $10 \times 10^5$  cells\* $\text{ml}^{-1}$ ) and by the order of entry of ewes into the milking parlour – four groups (in first group ewes milked in 1-5 row were included, second 6-11, third 12-17, fourth 18-23). In this study non-lame sheep had in July the highest milk yield ( $356 \pm 11.36$  ml) compared with slightly ( $317 \pm 22.83$  ml) and strong ( $319 \pm 28.68$  ml) lame ewes. Lameness in July affected the change the order of entry in July compared with the order of entry recorded in May. Only 16.8 % in May and 8.4 % in July of ewes had SCC in milk over  $7 \times 10^5$  cells\* $\text{ml}^{-1}$ . Production of lactose was influenced by SCC in the month May ( $P < 0.0006$ ) and July ( $P < 0.0001$ ). In both cases, the production of lactose was lower in groups with higher SCC. The high SCC had negative impact also on proteins content and non-fat dry matter. In both months, we recorded a higher milk yield in sheep entering the parlor in the first three groups (first, second, third) in comparing with a milk yield of sheep entering the last group (fourth). Sheep entering into milking parlour early had higher SCC as sheep entry into milking parlour later.

**Key words:** *ewes, lameness, SCC, order of entry*

**Acknowledgement:** This study was funded by the projects: KEGA 006SPU-4/2014, VEGA 1/0292/14

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## **The results of piglet rearing in two different systems of sows keeping**

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The aim of the study was to analyze the indicators of piglets rearing. Observations included a total of 162 piglets from 14 sows of Polish Landrace breed. 10 sows were kept in yoke pens with 119 piglets. 4 sows were kept in traditional pens with 43 piglets. During the rearing period were controlled: the number and body weight of piglets: at 1, 2, 3, 7, 21 and 28 days of piglets age, the growth rate from 1 to 28 day of age, mortality from 1 to 28 days of age and the sex of the piglets. The average number of piglets born alive per litter of sows in traditional pens amounted to 10.75 and 11.80 in yoke pens. At 28 days of age were 10.50 piglets of sows kept in a traditional pens while sows kept in yoke pens on average 10.30 piglets. A lower mortality (2.5%) was observed in the group kept in traditional pens while higher mortality in the group kept in yoke pens (9.84%). The average birth weight of the litter in group of sows kept in yoke pens was 20.97kg, at 21 days was 71.16kg and 28 days was 91.89kg. In the group kept in traditional pens noted higher results, respectively: 1,56kg, 76.06kg and 101,73kg. From 1<sup>st</sup> to 28<sup>th</sup> days of average daily gains obtained 291g of piglets from traditional pens. Piglets kept in yoke pens obtained 252g daily gain. The differences between two analysed groups of piglets noted as significant in the trait of body weight. The body weight of piglets in the 3<sup>rd</sup> week was higher in the group kept in traditional pens (7.24kg vs 6.71kg) while in 4<sup>th</sup> week of their life higher results obtained piglets from the second group (9.69kg vs 8.84kg);  $P \leq 0,01$ .

**Key words:** *piglets rearing, farrowing crates, growth, pens*

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## Effect of ageing time on integrin degradation and the level of drip loss in turkey breast muscle

Magdalena GÓRSKA, Dorota WOJTYSIAK

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Integrins are a family of transmembrane adhesion proteins. An integrin molecule is composed of two subunits called  $\alpha$  and  $\beta$ , each of which has a large extracellular domain, a transmembrane fragment, and a short cytoplasmic sequence. The main function of integrin is to bind extracellular matrix proteins and skeletal muscle cell membrane. In addition, integrin as a membrane receptor is involved in signal transduction and cell response to microenvironmental signals, by relaying information about the structure and composition of the cell environment. Integrin degradation has a significant effect on the quality and microstructure of meat. *Postmortem* integrin degradation has been the subject of several studies, mainly in pork, where the mechanisms of *postmortem* integrin degradation are not completely understood. Therefore, the aim of the study was to determine the effect of ageing time on integrin degradation and the level of drip loss in turkey breast muscle. The study was conducted with 25 16-week-old turkey hens slaughtered at average 9.5 kg body weight. Fragments of (*musculus pectoralis major*) sampled from the left side of turkey carcasses were investigated at 45 min, 24 h and 72 h of refrigerated storage (+4°C). Degradation products of integrin were conducted using Western immunoblotting. The immunoblots were electronically scanned and then analysed using the densitometric method (ImageJ ver. 1.33U, National Institutes of Health, USA). The intensity of standard bands was also estimated. The intensity of all bands was divided by the intensity of the standard band on each gel. The obtained ratio was used for statistical analysis. Drip loss was measured in duplicate samples. After thorough weighing ( $e=0.001g$ ), samples were placed in sealed containers. After 24 h of storage, samples were removed from their individual sealed containers, towel dried, and weighed again. The samples were then placed in new sealed containers and stored for additional hours. Following 72 h of storage, samples were again towel dried and weighed. The results obtained indicated that ageing time of meat had a significant effect on rate of integrin degradation and the cumulative increase in drip loss during 72-h cold storage of meat, however the highest level of integrin degradation and level of drip loss was found during the first 24 h of storage. It was concluded, more degradation of *postmortem* integrin may contribute to the formation of drip channels between the cell body and cell membrane of muscle fibers, which increases the level of drip loss from turkey meat.

**Key words:** *ageing time, integrin degradation, drip loss, breast muscle, turkey meat*

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## **Functional foods with the participation of animal products in nutrition of selected consumers**

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The aim of the study was to determine the contribution of functional foods including animal products in nutrition of the consumers being students. The survey-study included 45 students representing the University of Technology and Life Sciences in Bydgoszcz. Respondents completed anonymous forms taking into account the specific questions and information focusing mainly on the knowledge and the use of functional food with special emphasis on animal products. The advantages of health-promoting food are appreciated by young people who are interested in their health, and the concept of functional food was known among examined students. The impact on knowledge of aspects of functional food had the young age of the respondents, their education and the field of the study. These criteria were associated with the awareness of consumers about the value of products they eat every day. Functional food was very popular among students, and the choices made when shopping were associated with nutrient content of the products and their positive impact on health. The views of consumers to genetically modified foods was generally negative. The influence on it may have a fear of the harmful effects on the human body and the environment. The vast majority of respondents read labels and learned about the content of the composition of the products they buy during the acquisition of food, and the biggest impact on the purchasing decision was the nutritional value and mineral content. Meat and dairy products have enjoyed a great popularity among the consumers surveyed, and the content of nutrients and their energy value had a major impact on the decision to impose a certain type of meat and meat products into the daily diet. Among the fermented dairy products the most popular was yogurt, especially the type, which had a fiber supplement. Students covered by the study used in the nutrition functional food obtained from raw materials of animal origin such as meat, eggs, milk and honey.

**Key words:** *functional food, animal products*

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## The influence of breeding on the social behaviour of cats

Weronika PENAR

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Commonly known opinion is that the cats, unlikely the dogs, are loners, and others representatives of their species treat as a rivals or the sexual partners. This view is correct only for cats living in the wild and having a extensive hunting grounds. In the cities and suburbs, these animals live in a larger density. The smallest breathing space have cats living under the care of a human. Having no choice, they have adapted to the situation, enriching their social life and developing their herding behaviour.

The main purpose of the study was define the optimal factors affecting social behaviour of cats on the example of Siberian, Cornish Rex, Devon Rex, Persian and Exotic Cats. Additionally attempt to determine the possible relationship between „time of domestication“ cats breed and the occurrence of herd behaviour.

Research was based on the interviews conducted among the owners of cats bred in selected breeding. Selection of breeders was deliberate – chosen only breeders with extensive experience with cattery and a lot of success exhibition. Interviews were informal, were conducted in an atmosphere of relaxed conversation. They were based only on a standardized list of the topics. Most of the questions had an open character. In total, 74 interviews were conducted, including 15 breeders of Persian / Exotic cats, 18 breeders of cornish rex / devon rex, 23 breeders of Siberian cats, and 18 with the owners of multiracial catteries.

The answers of surveys showed that herding behaviour can be observed in all domestic cats. However not with the same intensity. Most herding behaviour developed in Siberian cats. Siberian cats is a natural, new breed which still has huge adaptability. In all analysed breeds of cats owners observed the presence of a strong hierarchy with alfa and omega cat, but only in Siberian cats is a huge willingness to cooperate within a herd. As the only one learned how to use calming signals, so well understood by dogs.

Cornish rex and Devon rex were bred as faithful companions of their owners. Years of breeding were targeted in the direction of ability to create large empathy with the human, but no with other animals. These cats being forced to live in a herd "learning" mutual coexistence with other individuals. Their social behaviour slowly developing.

The smallest herd behaviour observed between Persian cats. For years breeding disturbed their adaptability and influenced on their social behaviour.

**Key words:** *cats, social behaviour, breed, breeding*

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## **Dynamics of changes in the composition of milk of Icelandic mares during lactation**

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Mare's milk is the only food during the first 2 -3 weeks of foal's life, providing all essential nutrients for young organism. Till 12 hours after foaling mare produces colostrum containing immunoglobulins which enhances the immune system and stimulates gastrointestinal tract of newborn foal. The growth and maturation of the foal depend inter alia on the content of protein, fat, lactose and mineral elements. The composition and quantity of the milk produced by the mare is associated with the stage of lactation, mare age and the type of feed consumed. The aim of the study was to determine the dynamics of changes in the composition of milk of Icelandic mares during the first stage of lactation. Material for the analysis was collected from 8 Icelandic mares over five years of age, kept in the Icelandic horses stud Punktur in Dębowy Gaj. All mares were kept on pastures in identical conditions throughout the studied period of lactation. Milk samples (about 0.06 dm<sup>3</sup>) were collected during the first 15 days after foaling every 2 days, and from 16 to 36 of every seven days. Samples were analyzed in the Milko Scan FT120 apparatus for the content of protein, fat, lactose, dry matter and density. Then, the means and standard deviations for each test component within the individual days of lactation were calculated. The average level of protein in the milk of Icelandic mares during the studied lactation period was 2.98%. The highest level (3.61%) was noted for the 5th day of lactation and proved to be highly significantly higher than that observed for 36th day of lactation (2.23%). The average fat content during the lactation period reached 1.98%. Fat content reached its lowest level (1.82%) on day 7 of lactation and on 9 - the highest (2.2%) but these differences were not statistically significant. The average level of lactose in the milk of lactating Icelandic mares was 6.21%. The lowest level of lactose was observed on 3rd day of lactation (5.8%) and proved to be highly significantly lower than in 36 day (6.63%). The average level of dry matter in the milk was 11.83%, while on day 9 of lactation it reached the highest value (12.36%) and proved to be highly significantly higher than on day 29 (11.38%). Additionally it was observed that the average density of milk of Icelandic mare during lactation was 1037.30 g/dm<sup>3</sup>. On day 7 of lactation this component reached its highest level (1038.56g/dm<sup>3</sup>) and was highly significantly higher than on 36 day (1036.00 g/dm<sup>3</sup>). Analysing the results, it was found that the level of protein, fat, dry matter and density in the milk of mares Icelandic decreased during studied period of lactation, while lactose showed an upward trend.

**Key words:** *composition, icelandic mare's, lactation*

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## **The effect of the humic acid and garlic powder on growth performance and carcass characteristic of broiler chicken**

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The objective of present study was to investigate the effect of humic acids and humic acids and garlic powder (*Allium sativum* L.) combination on performance parameters and carcass characteristic of broiler chicken. A total of 120 Ross 308 broiler chicken were divided into 3 groups (n=40) in two repetition. The control group of chickens was fed with complete feed mixtures without any additives. First experimental group was fed a diet containing 1% of humic acid. Second experimental group was fed a diet containing 1.8% of humic acid and 0.2% of garlic powder (*Allium sativum* L.). At the end of the first experiment was average body weight (values in the order of the groups: 1796.4±188.06; 1999.1±355.83 and 1958.6±201.16 g±SD) significantly higher ( $P\leq 0.05$ ) in both experimental groups compared to control group. In first experimental group was weight of gizzard (values in the order of the groups: 34.86±5.22; 43.12±9.35 and 38.92±7.04 g±SD) significantly higher ( $P\leq 0.05$ ) compared to control group. At the end of the second experiment was average body weight (values in the order of the groups: 1791.4±218.38; 1972.64±196.03 and 1777.3±266.73 g±SD) significantly higher ( $P\leq 0.05$ ) in the first experimental group compared to control group. Carcass weight was significantly higher ( $P\leq 0.05$ ) in the first experimental group (values in the order of the groups: 1367.2±114.10; 1489.4±158.44 and 1402.7±124.48 g±SD) compared to control group.

**Key words:** *broiler chicken, humic acid, garlic (Allium sativum L.), performance parameters, carcass parameters*

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### ***In vivo* digestibility of corn silage nutrients in young horses**

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The aim of this study was determine *in vivo* digestibility of the corn silage nutrients in young horses. The experiment was performed on four Slovak warmblood young horses with average age  $2.5 \pm 0.5$  years, which were fed twice daily at 07.00 and 19.00 h. The young horses were kept in individual boxes without bedding. A 7-day adaptation period was followed by a 5-day experimental period. Horses were fed only with corn silage. Young horses had *ad libitum* access to fresh drinking water. All the diets, feed remnants and excrement samples were collected. Excrements were collected immediately after excretion. The content of nutrients in the diets, feed remnants and excrement samples were analyzed in Laboratory of Quality and Nutritive Value of Feeds. Dry matter was determined after drying at  $103^{\circ}\text{C}$ , nitrogen (N) was analyzed by the Kjeldahl method and crude protein was calculated as  $\text{N} \times 6.25$ , crude fat was extracted with petroleum ether, ash was determined after dry ashing at  $530 \pm 20^{\circ}\text{C}$ . Organic matter (OM) content was calculate:  $\text{Dry matter} - \text{Ash}$ . Dry matter, crude protein, crude fat, organic matter digestibility (in %) was calculate:  $(\text{Nutrient in feed} - \text{Nutrient in excrement} / \text{Nutrient in feed}) \times 100$ . *In vivo* digestibility of dry matter 62%, crude protein 56%, crude fat 72% and of organic matter 65% of corn silage in young horses was found.

**Key words:** *corn silage, nutrients, digestibility, in vivo method, young horses*

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## **The influence of piglets weight at birth on the effects of rearing, fattening results and value of carcasses**

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The aim of the research was the influence of body weight piglets at birth on the results of rearing and fattening later. Assessment included 110 piglets from birth through suckling, fattening until slaughter of animals. The piglets were from 10 litters from sows hybrid breed of Polish Large White and Polish Landrace (PLW x Landrace) x Pietrain. The animals were divided into three groups, depending on their body weight at birth: Group I below 1.20kg Group II from 1.21 to 1.7 kg and a group III above 1.7kg. During the tests was monitored body weight from birth to slaughter. Evaluated the slaughter value of carcasses: hot carcass weight, dressing percentage and conformation. The highest losses during rearing and fattening was observed in Group I (20.69%) and the lowest in group II (1.96%). Differences in body weight observed between the I, II, III groups throughout the rearing period as well as during fattening. The highest hot carcass weight gained in Group III (98.17kg) to Group I (94.244kg). There were no significant differences in the effect of birth weight piglets meat content in the carcass (Group I 57.71% 57.61% Group II, Group III 57.25%).

**Key words:** *piglets rearing, fattening performance, slaughter performance*

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## **Influence of estrus to changes in the locomotion activity and rumination in the Holstein dams**

Mária RUŠINOVÁ

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The aim of the work is focused on the evaluation of the locomotion activity and rumination in heifers and Holstein dairy cows during estrus. We have analyzed the locomotion activity and rumination using the Heatime RuminAct device on 99 dams (68 dairy cows and 31 heifers) and we evaluated a total of 213 estrus cycles during the reference period of 3 days before estrus, 3 days after estrus and on the day of the estrus occurrence. The data was analyzed using the DataFlow™ II program. Besides of this, it evaluates the influence on age and parity (heifers, primiparous and multiparous cows) and daily milk yield (high – with daily production over 35.4 kg and low milk production cows, with daily milk production under 35.4 kg) on the locomotion activity and rumination changes. The locomotion activity of cows was expressed in the units of locomotion activity in 24 hours (u/24h).

During the reference period, 3 days before estrus the cows showed locomotion activity 603 u/24 h., with an increase in the locomotion activity on the day of estrus of 876/24 u/24h (+45.3%) and during the reference period 3 days after estrus the level of locomotion activity decreased to 566 u/24 h, what is the similar locomotion activity to the reference period before estrus. In term of evaluation the influence of the parity on the locomotion activity during estrus we registered the highest locomotion activity in the group of heifers and primiparous cows (929, 934 u/24h respectively), where the multiparous cows achieved an average locomotion activity of 775 u/24h during estrus time.

The daily milk production also strongly influenced the locomotion activity. During estrus time, the high production cows showed a lower locomotion activity -5.4% on average. Ruminations of dams were expressed in minutes per 24 hours (min/24h). The analyses of impact the rumination to estrus cycles showed that, the reference period 3 days before estrus found an average rumination of 554 min/24h. On the day of estrus was statistically significant decrease in rumination time to 492 min/24h (-62 min/24h, -12.6% respectively) and in the reference period of 3 days after estrus, the averages of rumination increase on 548 min/24h. For parity, the most significant decrease occurred in the time of rumination (from 537 to 462 min/24h) at category of heifers (-75 min/24h, -16.2% respectively), and at least in multiparous cows (-50 min/24h, -9.9% respectively).

In assessing the impact of milk production at the level of rumination in the categories of high producing dairy cows compared to the reference period of 3 days before estrus we recorded reduce in rumination about Mária Rušinová-64 min/24h and low production cows by -58 min/24h (-11.5%). Based on the results of work and undertake statistic analysis, we can conclude that one of the characteristic behaviors of cows during estrus is to increase locomotion activity and reduce rumination time and therefore this paper presents original results of the locomotion activity and rumination time of dams using the Heatime RuminAct system on the farms.

**Key words:** *dairy cows, estrus, locomotion activity, rumination, Heatime RuminAct*

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## Safety assessment of selected disinfectants and pest control agents used in poultry houses

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*Supervisor:* Marcin Wojciech Lis

A good disinfectant and pest control agent should be characterized by high effectiveness (biocidal effect) and broad-spectrum activity while displaying no toxicity towards animals, humans and the environment. Therefore, it seemed desirable to assess toxicity of four selected disinfectants and pest control agents using the Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM), as an alternative to the Draize test.

Aqueous solutions of Viron FF, Amino-Steril, Oxydion and Hydro-Clean (at concentrations of 1%, 0.5%, 0.25% and 0.125%) Viron FF (mixture composed of glutaraldehyde, didecyldimethylammonium chloride, quaternary ammonium compounds, benzyl-C12-C16-alkyldimethyl and chloride), Oxydion (containing stabilized peracetic acid with hydrogen peroxide), Amino-Steril (17.5% quaternary ammonium compounds) and Hydro-Clean (hydrogen peroxide) were applied directly onto the surface of pre-prepared chorioallantoic membrane of 8-day-old chick embryos (n = 10 embryos / preparation / concentration). Water at room temperature was administered to the control group (n=10). Toxicity was assessed using 27-point scale based on degree of congestion, hemorrhage and coagulation of membrane blood vessels observed at fixed time intervals of 0.5, 2 and 5 minutes [Luepke, 1985]. The results were analyzed by the two-way analysis of variance and Tukey's test using the SigmaStat 3.5. The study revealed differences in toxicity of tested agents ( $P \leq 0.05$ ). Aqueous solutions of chemicals at concentrations of 1% (recommended by producer) were characterized by toxicity: strong- Hydro-Clean (9points), moderate- Oxydion (8points), Amino-Steril (5points) and poor- Viron FF (3 points).

Other observations included reduction of toxicity followed by decrease in concentration of the active substance ( $P \leq 0.05$ ). Moreover, noted no reactions for Viron FF (concentrations of 0.25%) while moderate reaction for others mixtures (concentrations of 0.125%).

The study indicates that test proposed by Luepke could be utilized as a rapid and reliable alternative to the Draize test for testing chemical substances. In use chemicals be careful and do not contact with tissue.

**Key words:** *chorioallantoic membrane, HET-CAM*

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## The impact of a mixture of silanes and siloxanes (Dergall) used to disinfect poultry houses on chicks hatchability

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A good disinfectant and pest control agent should be characterized by high broad-spectrum efficacy and no toxicity to animals, humans and the environment. The above mentioned criteria seem to be matched by Dergall a formulation designed to red poultry mite (*Dermanyssus gallinae*) control in poultry houses. It is a mixture of various silicon polymeric compounds, and its efficacy is based on purely physical mechanism of action on parasites. Dergall when sprayed in the presence of animals thoroughly covers surface of the skin and feathers of hens and laid eggs. Therefore, we have found worthwhile the assess a possible toxicity to laying hens and farm workers, as well as possible negative impact on the embryo development in treated eggs.

In the first experiment, the toxicity of the formulation Dergall (ICB Pharma, Poland) has been assessed with hen's egg test on the chorioallantoic membrane (HET-CAM), as an alternative to the Draize test. Aqueous solutions of Dergall (0.3%; 0.6%; 1%; 3%; 6%; 25%; 50% and 100%), were placed on a previously prepared chorioallantoic membrane of the living eight-day chick embryo (n=10 embryos/ concentration). The control group (n=10) was treated with water. In the assessment of toxicity was used a 27-point scale based on degrees of congestion, hemorrhage and coagulation of blood vessels' membrane after 30 seconds, 2 and 5 minutes [Luepke, 1985].

Possible impact on the egg hatchability was assessed on the eggs of reproductive flock Ross 308 that were treated with aqueous solutions of Dergall at the concentration of 0; 0.3%; 0.6%; 3%; 6% (10 repetitions, n=10 embryos/concentration/repetition). The control group was not treated. After drying the eggs were incubated in a standard conditions (temperature 37.8°C, RH 50%). The eggs were candled at E7, E14 and E19 and the unfertilized or dead eggs were rejected. All rejected eggs were weighed to assess the incubation-related loss of the weight. Eggs were weighed before incubation and on the 19<sup>th</sup> day of incubation (E19) to assess the incubation-related loss of weight. The hatched chick were also weighted and grade of healing of the navel was scored from 0 (completely healed) to 5 (inflammation of the navel).

The results were analyzed by two-way analysis of variance and Tukey's test using the SigmaStat 3.5.

In the first experiment a reduction of toxicity related to decrease of concentration of the active substance ( $P \leq 0.05$ ) was observed. The application of Dergall concentrate (100%) caused moderate reaction (5 points). while no reaction was observed at a concentration of 3%.

In a second study, the highest hatchability of (mean and SD) 96.3±2.40% and 95.7% was observed in control and group sprayed by 3% Dergall solution, respectively, while the lowest (84.4±10.11%) was observed in the group sprayed with 6% Dergall solution ( $P \leq 0.05$ ). Decrease of hatchability was mainly caused by mortality of embryos in the first week of incubation. There were no differences between groups in terms of egg weight loss during incubation (7.81±2.88%,  $P > 0.05$ ), weight of one day chicks (48.4±3.57g,  $P \leq 0.05$ ) and score of healing the navel (1.2±1.03 point, median 1 point).

The experiments have proved that the application of Dergall at the concentration of 3% and lower should not cause any negative reaction to animals and humans or decrease the hatchability of eggs (recommended by the manufacturer concentration is 0.6%).

**Key words:** *chorioallantoic membrane, Dergall, HET-CAM*

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## Apoptosis-like changes in boar semen cooled in different extenders and temperatures

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The quality of boar semen is affected by many factors, such as the extender composition and storage temperatures. Liquid storage of semen compromises sperm function, and commercial extenders have been used to protect the sperm functional viability during cooling from 16°C to 20°C. This study investigated the apoptosis status of boar spermatozoa following cooling of semen in different extenders and temperatures. Sperm rich fractions, collected from 5 boars, were diluted in 4 extenders: Androhep® Plus (AHP), Androstar® Plus, ASP (Minitübe), Safecell® Plus, SCP (IMV Technologies) and TRIxcell® Plus, TCP (IMV Technologies), and cooled for 2h at 17°C and then for 24h at 10°C. Total motility (TMOT, %) and progressive motility (PMOT, %) were analyzed with the computer-assisted sperm analysis (CASA) system (HTR-IVOS 12.3, Hamilton Thorne Biosciences). The sperm mitochondrial function (%) was analyzed with the JC-1/PI assay, whereas the Vybrant Apoptosis Assay Kit (YO-Pro/PI) was used to analyze the percentages of three subpopulations of spermatozoa categorized as live (YO-Pro-1-/PI-), apoptotic (YO-Pro1+/PI-) and necrotic (YO-Pro-1+/PI+). No marked changes in either sperm TMOT or mitochondrial function were observed between the storage periods or among the extenders. Sperm PMOT was significantly lower in the ASP-extended semen compared with the other extenders cooled for a 2-h period. Prolonged cooling for a 24-h period caused a slight reduction in sperm PMOT, being significantly lower in the TCP-extended semen. The proportions of apoptotic-like changes in spermatozoa (YO-Pro1+/PI-) were not significantly differed between the cooling periods, regardless of the extender type. It was found that the percentages of live spermatozoa (YO-Pro-1-/PI-) were markedly higher in the SCP-extended semen cooled for 2h compared with that cooled for 24h. Furthermore, the proportions of spermatozoa with necrotic changes (YO-Pro-1+/PI+) markedly increased in the SCP extender following prolonged semen cooling. The results of this study indicate that cooling of extended semen at either 17°C or 10°C does not significantly affect the apoptosis status and mitochondrial function of boar spermatozoa. Such findings will have significant relevance in the pre-cooling procedure of boar semen prior to cryopreservation.

**Key words:** *boar, spermatozoa, semen, cryopreservation, apoptosis*

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## Densitometric and morphometric parameters of tibial bone in chickens

Joanna WOJCIECHOWSKA

*Supervisor:* Dorota Wojtysiak

Currently, mechanical and structural characteristics of the bone system are in the centre of attention. High body weight in poultry and the imbalance in the growth of muscle mass and bone cause distortion and fracture of bones. This is the result of an inadequate adaptation of the armature in relation to a relatively high body weight. Incorrect load of the skeleton causes the structural deformation and damage occurring especially often in the femoral bones and tibial bones. Studying densitometric features of the tibiotarsal bones with the use of DEXA is one of the methods allowing the determination of the structure of bones. There are many methods of intravital evaluation of the skeletal system in poultry, e.g. radiography, digital fluoroscopy, quantitative computed tomography or microcomputed tomography microCT. The aim of the study was finding the specific structure of tibial bones of adult domestic chicken males. The research was conducted on 10 males of a domestic chicken in Department of Animal Science at Agriculture University in Krakow. The bones were prepared and measured with STALCO s-11115 caliper (with accuracy of 0.01 mm). There were analysed parameters such as: bone mass, bone length, width of the proximal epiphysis, width of the distal epiphysis and width of the diaphysis. Densitometry was also performed to determine Bone Mineral Density (BMD) and Bone Mineral Content (BMC). Densitometry studies were performed in IMED24 Medical Centre in Krakow. The results of the measurements were subjected to statistical analyses using Statistica program.

The average weight of tibial bone was 19.8 g. Average values of other parameters were: the average length of the tibial bone – 111.7 mm, the average width of the proximal epiphysis – 28.86 mm, the average width of distal epiphysis – 21.04 mm, the average width of diaphysis – 9.37 mm, the average Bone Mineral Density – 0.15 g/cm<sup>2</sup> and the average Bone Mineral Content – 0.40 g.

Proper functioning of the skeleton play an extremely important role in efficient poultry farming. Current farming conditions based on intensive feeding and nutrition as well as genetic selection of birds focus mainly on attainment of fast growth rate expressed as body weight. Introducing birds of high productive parameters to breeding predisposes to frequent and unexplained skeletal and health problems. High body weight in poultry as well as lack of balance in the growth of the muscle mass and bones lead to deformities and bone fractures. Given the scale of bone pathologies in poultry it is necessary to recognise diseases and to assess optimally the skeletal system of these animals.

**Key words:** *chicken, densitometry, morphometry, tibial bone*

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SECTION  
*Applied and molecular biology*

## Formation of artificial bilayer membranes of glycyrrhizic acid and its mixtures with asolectin

Diyor D. FAYZIEV, Khabibulla A. YULDASHEV

*Supervisor:* prof. Davron N. Dalimov, Bakhrom N. Babaev and Ravshan Z. Sabirov

Glycyrrhizic acid (GA) is the main component of the licorice root having a chemical structure of five-membered  $\beta$ -saponins of amiran type. It consists of a hydrophobic aglycone, 18- $\beta$ -H-glycyrrhetic acid, and diglucuronic hydrophilic parts.

The unique structure of this natural saponin molecule confers GA a wide range of biological activities and unique physical and chemical properties. It is known that amphiphilic molecules exhibit surface active properties, tend to aggregate and form micelles in solution gels. In recent years, the interest to this natural compound has increased because of its ability to form water-soluble inclusion compounds – "supramolecular complexes" with hydrophobic drugs, as well as crystal structures, in which the molecule packaging resembles a package of lipid molecules in natural and model membranes. Based on these, we aimed to evaluate the capability of pure GA to form bilayer lipid membrane (BLM)-like structures under conditions in which natural lipids form them spontaneously.

As a control membrane-forming lipid we used 2% solution of soybean asolectin in n-octane that formed stable lipid bilayer membranes with a capacitance of  $\sim 150$  pF. When a voltage ( $\pm 50$  mV) was applied the current across the membrane did not change and remained at a low control level throughout the experiments (600 s, n=5).

In order to increase the degree of ionization and, thus the amphiphilic properties of the molecule, in the present study we employed GA in a form of monoammonium salt. All our attempts to form BLM of pure GA were unsuccessful: we were unable to detect any bilayer structure with a measurable capacity in either of 10 experiments. In the next series of experiments, we tested BLMs consisted of equal amounts of GA and asolectin (1:1 by weight), dissolved in n-octane. Under these conditions, the BLM with a capacitance of  $\sim 150$  pF could be successfully formed. The GA at a content of 50% did not affect the electrical conductivity of artificial BLM, but significantly reduced the "lifetime" of the membrane to  $274 \pm 33$  s (n=7).

Reducing the concentration of GA in the above-mentioned conditions down to 33.3% (2:1); 25% (3:1) and 10% (9:1), respectively, also had no effect on the electrical conductivity of BLMs. In these experiments, the "lifetime" of the membrane containing 33.3% GA was  $428 \pm 7$  seconds (n=3), whereas at a lower content of GA membranes remained stable throughout the experiments (600 s).

In conclusion, we have demonstrated that GA alone is not able to form a bilayer structure similar to that of BLM with measurable capacitance. Introduction of GA into the membrane forming-solution does not affect the electrical conductivity of artificial BLMs, but reduces the "lifetime" of the membrane at a content of more than 25%.

**Keywords:** *glycyrrhizic acid, supramolecular complex, bilayer lipid membrane*

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## **The use of multiple correspondence analysis to explore associations among fungiform papillae density, caffeine detection threshold, coffee intake and age of young and elderly persons**

Tomáš FEKETE

*Supervisor:* doc. Ing. Radoslav Židek, PhD.

Ageing is accompanied by diminishing of fungiform papillae (FP) number on the tongue, which is associated with impaired taste sensitivity. As a result, food preferences may also be changed, which may have unfavourable fallout on health of people. We have used multiple correspondence analysis (MCA) to analyse the age of subjects, PF density, caffeine detection threshold and frequency of coffee intake in order that we revealed potential relationships among these variables. Analysed data were obtained in previous experiments. Two groups of subjects – young ( $n = 39$ ) and seniors ( $n = 38$ ) differed significantly each other by the age, the FP density and the caffeine thresholds (*Mann-Whitney*;  $p < 0.01$ ;  $\alpha = 0.05$ ). In order to acquire categorised – qualitative variables, both the FP and the age values were assigned to intervals 0 – 5, 5 – 10 ... 60 – 70 and 18 – 24, 25 – 34 ... 75 – 84, respectively. The possible detection thresholds for caffeine were as follows: stock solution –  $2.55 \text{ mmol.L}^{-1}$ ,  $1.39 \text{ mmol.L}^{-1}$  (D1),  $1.13 \text{ mmol.L}^{-1}$  (D2),  $0.90 \text{ mmol.L}^{-1}$  (D3),  $0.71 \text{ mmol.L}^{-1}$  (D4),  $0.57 \text{ mmol.L}^{-1}$  (D5),  $0.47 \text{ mmol.L}^{-1}$  (D6),  $0.38 \text{ mmol.L}^{-1}$  (D7),  $0.29 \text{ mmol.L}^{-1}$  (D8). The frequency of coffee intake was extracted from food frequency questionnaire. Possible answers of coffee intake were as follows: never, 1x p/d, 2x p/d, 1x p/w, 3-4x p/w, 5-6xp/w, 1x p/m and 2-3x p/m. All the computational work, including the graphical presentations, was performed using XLSTAT (Addinsoft, 2014) package program. The result of analysis was correspondence map, where both the variables and the observations were displayed on two axes – factors. The factors reflect “how close is the representation to the reality”. Specific values such as squared cosines, contributions and test values suggested which variables are important for particular axe. In this case, the first two axes accounted for total of 45.69 % variability, especially among age and caffeine thresholds values. From positions of vectors and their distances can be concluded that young subjects were frequently aged 18 – 24 years and perceived bitterness of caffeine very well (threshold D7). The close position of vectors suggested high correlation. On the other hand, seniors were aged 65 – 74 years, had 0 – 5 FP per  $\text{cm}^2$  of the tongue and most of them perceived just stock solution or the highest diluted solution of caffeine (D1). The first two axes did not completely displayed differences between FP density and frequency of caffeine intake. The 2<sup>nd</sup> and 3<sup>rd</sup> axes revealed that young subjects had from 20 to 40 FP per  $\text{cm}^2$  of the tongue and consumed coffee either never or 1x per month. Consumption of coffee by seniors was more heterogeneous compared to youths, which was also indicated by orthogonal position of vectors.

**Key words:** *MCA, age, fungiform papillae, caffeine, threshold, coffee, senior, youth*

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## Effect of gossypol and its derivatives on functional parameters of rat liver mitochondria

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*Supervisor:* prof. Takhir Aripov, DSc.

Gossypol, a triterpenoid aldehyde, is a naturally occurring compound extracted from the cotton plant and other plants of the genus *Gossypium*. Gossypol has been known for over a hundred years and the dichotomy between its toxicity and its unique biological activities places gossypol in an unusual position. And one of points for researchers is using gossypol's binaphthyl structure, functionality, and unique biological activities to make gossypol a potentially important value-added natural product. The compound has been shown to reveal bacteriostatic, antifungal, antitumor, antifertility and anti-insect activities. Being biologically active, gossypol has received significant attention as a potential medicinal product and has been extensively studied over the past two decades. This work focuses on the study of influence of gossypol and its mono-derivatives (monoaminogossypol, MAG; monoanilinogossypol, MANG) on proton transportation across mitochondrial membranes and their effect on  $\text{Ca}^{2+}$ -dependent mitochondrial permeability transition pore in vitro.

Experiments on mitochondrial permeability transition pore (mPTP) showed that gossypol and its derivatives in dose-dependent manner can inhibit pore opening. Mitochondrial membrane is slightly permeable to some anions and cations, like  $\text{NH}_4^+$ , but not permeable to chloride, sulphate, as well as to protons. Penetration of  $\text{NH}_4^+$  does not cause mitochondria swelling as  $\text{H}^+$  or  $\text{OH}^-$  are transported outside the cell thus protecting mitochondria from increased osmotic pressure. When both cation and anion ions penetrate into mitochondria the swelling process increases as increases osmotic pressure.  $\text{NH}_4^+$  penetrates as neutral molecule of ammonia. We propose that in presence of gossypol and MAG cations and anions penetrate into mitochondria causing significant mitochondria swelling. It was shown that antiradical and membrane activity of gossypol derivatives were determined by the structure of the substituent and that gossypol and its derivatives were partially localized in the lipid bilayer and possibly induced formation of a new interdigitating phase.

It is known that gossypol in comparison to its derivatives (MAG and MANG) possesses higher membrane and antiradical activities. Our results also support the data that the presence of carbonyl groups in the gossypol structure might introduce significant contribution into molecular mechanisms of this polyphenol activities.

**Keywords:** *gossypol, gossypol derivatives, mitochondria, mPTP*

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## Calcium-dependent vasorelaxant effect of cynaroside on isolated rat aortic rings

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*Supervisor:* prof. Takhir Aripov, PhD., DSc.

Flavone-derived compounds are a large group of natural compounds with a great number of properties present in vegetables, fruits, and aromatic plants. High dietary intake of fruits, vegetables, and whole grains, is strongly connected with a lower risk of developing cancer, cardiovascular disease, and other chronic diseases with high prevalence. A recent study on the effects of flavonoid intake on cardiovascular disease mortality found that in particular flavone consumption was associated with the greatest reduction in cardiovascular diseases in women (other flavonoids classes used in the study were anthocyanidins, flavan-3-ols, flavanones, and flavonols) and that flavone consumption was associated with the lowest risk of fatal ischemic heart disease, in both men and women. Cynaroside (luteolin-7-O- $\beta$ -D-glucopyranoside) – is a natural flavone, one of the bioactive compounds purified from aerial part of *Ferula varia* (Schrenk) Trautv. (Apiaceae). In the present study, the concentration-dependent vasorelaxant effect of cynaroside on isolated smooth muscle preparation was studied and specific molecular mechanisms of action are discussed.

In preliminary studies, flavone cynaroside under normal conditions in a wide range of concentrations (up to 50  $\mu$ M) had no effect on the tone of rat aorta preparations. These data suggest that at rest cynaroside does not cause the activation of the contractile apparatus of the preparation of rat aorta. However, in further experiments, we found that cynaroside effectively relaxes rat aorta preparations, pre-incubated in hyperpotassium solution (50 mM). In these conditions, the EC<sub>50</sub> for cynaroside was 28.2  $\mu$ M. It is known that KCl-induced reduction of smooth muscle cells (SMC) aorta is associated with activation of potential-dependent Ca<sup>2+</sup>-channels of plasma membranes of SMC. At the same time, the increase of K<sup>+</sup> concentration changes the membrane potential and causes depolarization that activates potential-dependent Ca<sup>2+</sup>-channels and leads to an increase of (Ca<sup>2+</sup>)<sub>i</sub>, which in its turn causes a contraction of SMC. Taking this into account and analyzing the data obtained, it can be assumed that the vasorelaxant mechanisms of cynaroside may be due to inhibition of Ca<sup>2+</sup> flow into the cytosol of SMC by blocking potential-dependent Ca<sup>2+</sup>-channels of sarcolemma. To test this hypothesis, we performed a special series of experiments using a calcium-free Krebs solution. In presence of verapamil the relaxant effect of cynaroside increased significantly. Summarizing the data, cynaroside does not cause the contraction of smooth muscle cells. Oppositely it possesses significant relaxant effect. One of molecular mechanisms may be induced by blocking L-type channels (in KCl-induced contraction) with micromolar affinity. However taking into account versatile process of SMC contraction further research is needed.

**Key words:** *cynaroside, flavone, potential-gated channels, vasorelaxant effect*

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## Apoptosis in the chicken postovulatory follicles

Anna M. GDULA, Anna HRABIA

*Supervisor:* dr. hab. eng. Anna Hrabia, prof. UR

The avian ovary is an organ undergoing dynamic morphological changes during the ovulatory cycles. In the ovary of a laying hen numerous primary follicles embedded in the stroma as well as: white, yellowish and yellow preovulatory follicles (F7-F1) and postovulatory follicles (POF1-5) can be distinguished. Unlike mammals, the follicles in birds after ovulation rapidly terminates the metabolic activity and fails to form a corpus luteum. Postovulatory follicles are regressed via the process of apoptosis within approximately 6 days after the extrusion of the oocyte. Apoptosis is a form of cell death which allows the controlled removal of unwanted cells and remodeling in numerous tissues. In this process, two main pathway can be found: extrinsic and intrinsic. Each of these activities has own initiator caspases which in turn activate executioner caspases, leads to the cleavage of numerous cellular proteins and cell death. However, the signals and molecular mechanisms regulating this process in chicken POF are not fully elucidated. Recent reports assume that factors such as: cytokines, macrophages and NO (produced by nitric oxide synthase) might participate in the regression of POFs, but probably caspases play a crucial role in this process. Therefore, the current study was undertaken to determine activity of various caspases involved in apoptosis (initiator caspases: -2, -8, -9 and the most important effector: caspase-3) in the chicken POFs. Additionally, the number of apoptotic cells during POF regression was determined by counting TUNEL-positive cells per 100 cells. The experiment was carried out on 25-week-old Hy-Line Brown laying hens (n=12) decapitated 2 h after ovulation. From the ovaries, five POFs were isolated and weighed. Tissue samples were subjected to measurement of caspase activity by fluorometric assay kit. The other POFs were embedded in paraffin and apoptotic cells were examined by TUNEL methods. The POFs lost their weight from  $440 \pm 24$  mg to  $76 \pm 9$  mg during regression. The activity of all tested caspases increased gradually (approximately 80-150% compared to control) reaching the highest level in POF3 follicle, and next slowly decreased to the value as in POF1 follicle. Results of the TUNEL analysis also indicate a significant elevation in the percentage of apoptotic cells along with follicle regression; the highest number of TUNEL-positive cells was in the POF5 follicle. The results obtained demonstrating that the regression of chicken POF occurs via the caspase-mediated apoptosis, and the process might be initiated by the external or internal pathway.

**Key words:** *caspases, apoptosis, postovulatory follicles, ovary, chicken*

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## Methods for utilization byproduct from biodiesel production using metabolic engineering of yeast *Ogataea (Hansenula) polymorpha*

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*Supervisor:* Andriy Sibirny, PhD, Professor

Biodiesel is produced by a transesterification reaction using vegetable oils or animal fats and an alcohol. This process inevitably generates large amounts of glycerol as a by-product. The availability of crude glycerol is predicted to increase in the next years because of the tremendous growth in the production of biodiesel worldwide. To maximize the full economic potential of the biodiesel process, it is important to convert crude glycerol into useful chemicals, e.g. ethanol. Glucose fermentation is efficiently carried out by microorganisms, at the same time, there are few microorganisms that are capable of converting glycerol to ethanol. Therefore, the tools of metabolic engineering along with classical selection should be used for construction of the recombinant strains with improved of ethanol production. Thermotolerant yeast *Hansenula polymorpha* is able to convert glycerol as well as glucose and xylose, to ethanol. However, the yield of ethanol from glycerol by wild type strain is too low to and need for substantial improvement before introduction of those strains for production of ethanol on industrial scale.

The aim of this work was to improve production of ethanol from glycerol using thermotolerant methylotrophic yeast *H. polymorpha*.

In this study, vectors for multicopy integration of both *PDC1* (encodes pyruvate decarboxylase) and *ADH1* (encodes alcohol dehydrogenase) genes under control of strong constitutive promoter have been constructed. Obtained recombinant *H. polymorpha* strains (with overexpression of both *PDC1* and *ADH1* genes) revealed increased specific activities of both Pdc1 and Adh1 and ethanol production. In addition, the use of optimization of fermentation conditions resulted in the recovery of increasing ethanol production from glycerol up to 5 g/l. The next step is overexpression of genes encoding enzymes, which are responsible for catabolism of glycerol in yeast cells. In *O. polymorpha* there are two ways for glycerol catabolism. We suggest that overexpression of 4 genes (*GPD1*, *GCY1*, *GUT1*, *DAK1*) will further increase in ethanol production from waste glycerol. The newest data of glycerol conversion to ethanol by recombinant strains will be discussed.

**Key words:** *biodiesel, glycerol, ethanol, Ogataea (Hansenula) polymorpha*

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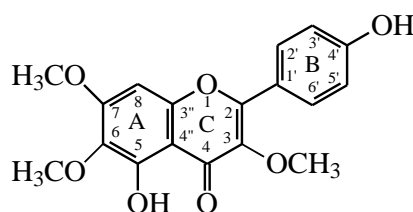
## The inotropic effects of 4',5-dihydroxy-3,6,7-trimethoxyflavon in isolated rat papillary muscle

Shunkor S. KHUSHMATOV, Zokir O. TOSHMATOV, Kamila A. ESHBAKOVA

*Supervisor:* prof. Pulat B. Usmanov

Nowadays, in pharmaceutical industry researches find cure against many various diseases, the direction of tendency is observed towards the growth of interest to search and create medical products based on bioflavonoids.

The aim of the present study was to determine the inotropic effect of 4',5-dihydroxy-3,6,7-trimethoxyflavon in the rat myocardium. 4',5-dihydroxy-3,6,7-trimethoxyflavon was isolated from plants *Dracocephalum komarovii* Lipsky (Lamiaceae), collected from the Tashkent region of Uzbekistan (Fig. 1).



**Figure 1.** Chemical structure of 4',5-dihydroxy-3,6,7-trimethoxyflavon (C<sub>16</sub>H<sub>7</sub>O<sub>16</sub>; F.w. 344 g/mol)

*Dracocephalum komarovii* Lipsky (Labiatae) is endemic species, growing only in territory of Republic Uzbekistan. *Dracocephalum komarovii* Lipsky – a perennial semishrub that grows in the vicinity of ~2300–3600 m above sea level in the western Tien Shan mountain system. This is called «*buzbosh*» in Uzbekistan, and the local people use the aerial parts in a tea to treat various diseases such as inflammatory diseases and hypertony. 4',5-dihydroxy-3,6,7-trimethoxyflavon also displays antioxidant activity [Heo et al., *Amyloid*. 2001.8(3):194–201].

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 Electrophysiology 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 Helsinki Declaration and the rules of human attitudes towards animals were completely followed.

In the experiments, the 4',5-dihydroxy-3,6,7-trimethoxyflavon did not show inotropic effects at low concentrations (1–3 µmol/L), and positive inotropic effects of 4',5-dihydroxy-3,6,7-trimethoxyflavon started to appear at concentrations 4 µmol/L. And 4',5-dihydroxy-3,6,7-trimethoxyflavon (4–10 µmol/L) showed dose-dependent positive inotropic effects in rat papillary muscle contractility. Studies have shown that in incubation to inhibit potential-dependent Ca<sup>2+</sup>-channel – nifedipine (0.01 µmol/L) the positive effect of 4',5-dihydroxy-3,6,7-trimethoxyflavon decreases to 27.3±4.7% of control values. The results of experiment demonstrate that the positive inotropic effect of 4',5-

dihydroxy-3,6,7-trimethoxyflavon on papillary muscle is not completely connected with potential-dependent  $\text{Ca}^{2+}_{\text{L}}$ -channel cardiomyocytes.

In addition, the positive inotropic effect of 4',5-dihydroxy-3,6,7-trimethoxyflavon was almost completely disappeared in the presence of inhibitor of potential-dependent  $\text{Ca}^{2+}_{\text{L}}$ -channel – nifedipine (0.01  $\mu\text{mol/L}$ ) and inhibitor  $\beta$ -adrenoreceptor – ( $\pm$ )-propranolol hydrochloride (10  $\mu\text{mol.L}^{-1}$ ) under incubation conditions.

In conclusion, the present study demonstrates that 4',5-dihydroxy-3,6,7-trimethoxyflavon has showed a positive inotropic effect on rat papillary muscles that can be explained with the increase of  $[\text{cAMP}]_{\text{in}}$  and may depend on increase of  $[\text{Ca}^{2+}]_{\text{in}}$ .

**Key words:** *4',5-dihydroxy-3,6,7-trimethoxyflavon,  $\beta$ -adrenergic receptor, inotropic effect*

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## Effect of diterpenoid alkaloids 1-*O*-benzoilkarakolin and 14-*O*-benzoiltalatizamin on contractile activity of smooth muscle cells in aorta

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Medicinal plants distributed in the Central Asia provides abundant and precious medicinal resources for the Traditional Medicine in treating diseases. Alkaloids are unique secondary metabolites are synthesized in almost all plant cells, exhibiting high biological activity and due to their properties increasingly finding wide practical application in pharmacology and medicine. Also, nowadays, in chemical researches have extracted and identified many alkaloids from plants. However, their mechanisms of cardiovascular–pharmacological impact have not been studied yet. Therefore, the aim of the present study was the relaxant effect of 1-*O*-Benzoilkarakoline (1-*O*-BK) and 14-*O*-benzoiltalatizamine (14-*O*-BT), alkaloids from plant *Aconitum karakolirum* and 14-*O*-BT from *Aconitum nemorum* on the contractile of the rat aorta smooth muscle cells (SMC).

Studies were conducted on isolated rat aorta rings 3–4 mm wide. Contractile activity was recorded in isometric mode using FT–03 tension sensor (Grass, USA). The preparations were fixed in the organ bath and perfused with Krebs solution at +37°C. In preliminary experiments we showed that 1-*O*-BK and 14-*O*-BT in wide range of concentrations had no effect on smooth muscle cells contractility. However studied alkaloids exhibited strong dose–depending relaxing effect of aorta pre–contracted with KCl (50 mM) and noradrenaline (1 μM).

In the experiments, the alkaloid 1-*O*-BK (5.1 mM) decrease of caffeine–induced contractions up to 15.5±3.5% in comparison to control. Similar suppression of caffeine–induced contractions of aorta rings was also caused by alkaloid 14-*O*-BT, and in concentration of 52.3 mM it decreased contraction to 18.8±3.6% of control. Results of these experiments testify that the studied alkaloids 1-*O*-BK and 14-*O*-BT suppress the caffeine–induced contraction, and these results suggest that studied alkaloids relaxed the rat aorta by suppressing the Ca<sup>2+</sup> release from SR through IP<sub>3</sub>R pathway.

These data may serve as a basis for further detailed pharmacological mechanism of action of this compound.

**Keywords:** *smooth muscle cells, diterpenoid alkaloid, sarcoplasmic reticulum, relaxation*

**Acknowledgement:** The work was supported by project FA–F6–T083 (Academy of Sciences of the Republic of Uzbekistan).

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## Relaxant effect of diterpenoid alkaloid 1-*O*-benzoilkarakolin on aorta cells

Yulduz T. MIRZAYEVA

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Using the isolated rat aortic rings the relaxant effects of the diterpenoid alkaloid 1-*O*-benzoilkarakoline (1-*O*-BK) was studied. The purpose of this work was studying the relaxant action of 1-*O*-BK isolated from plant *Aconitum karakolirum*.

Studies were conducted on isolated rat aorta rings 3–4 mm wide. Contractile activity was recorded in isometric mode using FT–03 tension sensor (Grass, USA). The preparations were fixed in the organ bath and perfused with Krebs solution at +37°C.

It was shown earlier that diterpenoid alkaloid 1-*O*-BK exhibits significant relaxant activity towards smooth muscle cells of rat aorta pre-contracted by various constrictors (KCl and PE). In a wide range of concentrations 1-*O*-BK had no effect on muscular tonus of aorta rings indicating that the alkaloid has no effect on smooth muscle cells contractility.

In our experiments in standard Krebs solution aorta rings contraction was caused by phenylephrine and the force of tension was accepted as control and was taken 100%. In Ca<sup>2+</sup>–free Krebs solution containing 1 mM of EGTA addition of phenylephrine caused aorta rings contraction which was 70.2±4% in comparison to control.

We have found that 1-*O*-BK exhibits prolonged relaxant effect towards aorta rings pre-contracted by phenylephrine in Ca<sup>2+</sup>–free Krebs solutions. Thus at the minimum concentration of 30 μM 1-*O*-BK causes aorta rings relaxation to 21.5±4.5% in comparison to control.

These results suggest that studied alkaloid relaxed the rat aorta by suppressing the Ca<sup>2+</sup> entry into smooth muscle cells through both receptor–operated calcium channels. In general our results let to conclude that the shown effects might be caused by release of Ca<sup>2+</sup> ions from sarcoplasmic reticulum (SR).

**Keywords:** *smooth muscle cells, diterpenoid alkaloid, sarcoplasmic reticulum, relaxation*

**Acknowledgement:** The work was supported by project FA–F6–T083 (Academy of Sciences of the Republic of Uzbekistan).

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## Studying the effect of flavonoid luteolin on $\text{Na}^+/\text{Ca}^{2+}$ - exchanger in smooth muscle cells of rat aorta

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*Supervisor:* prof. Pulat B. Usmanov

The purpose of this work was studying relaxant effect of flavonoid luteolin from plant *Inula caspica*. The  $\text{Na}^+/\text{Ca}^{2+}$ -exchanger plays an important role in maintenance of  $\text{Ca}^{2+}$ -homeostasis in cell and therefore regulation of its activity is of a great value and is provided with a number of mechanisms.

Experiments were conducted on preparations in the form of rings (width of 3-4 mm) isolated from rat aorta. Contraction of aortic rings was registered in the isometric mode by means of FT-03 tension sensor (Grass, USA). Preparations were fixed in the organ bath perfused by Krebs's solution at 37 °C. Earlier we showed that flavonoid luteolin possesses significant relaxant effect towards aortic rings pre-contracted by hyper potassium solution or noradrenaline. To study the influence of luteolin on  $\text{Ca}^{2+}$  transport through  $\text{Na}^+/\text{Ca}^{2+}$ -exchanger of smooth muscle cells (SMC) we used standard protocols to estimate functional activity of a  $\text{Na}^+/\text{Ca}^{2+}$ -exchanger.

Additional confirmation of interaction of flavonoid luteolin with  $\text{Na}^+/\text{Ca}^{2+}$ -exchanger of SMC was received in experiments with a glycoside ouabain which in concentration of 10  $\mu\text{M}$  (in presence of 1  $\mu\text{M}$  of verapamil) caused the maximum reduction in contraction of aorta rings to 48.5±4%. Results showed that luteolin effectively suppresses the contraction of aorta rings induced by ouabain. Such effect of luteolin had dose-dependent character and at concentration of 5 $\mu\text{M}$  reduced the force of contraction of aorta rings to 41.5±3.8%, and at 15  $\mu\text{M}$  caused the maximum suppression to 79.5±4.2% in comparison to control. The effect of flavonoid luteolin on the rat aorta smooth muscle cells  $\text{Na}^+/\text{Ca}^{2+}$ -exchanger was studied. Obtained results suggest that studied alkaloid relaxed the rat aorta by suppressing the  $\text{Ca}^{2+}$  entry into smooth muscle cells both through voltage operated calcium channels and  $\text{Na}^+/\text{Ca}^{2+}$ -exchanger.

The results showed that the mechanisms underlying the relaxant effect of luteolin involve not only its interaction with voltage-dependent  $\text{Ca}^{2+}$ -channels, but also its effect on transport of  $\text{Ca}^{2+}$  ions through a  $\text{Na}^+/\text{Ca}^{2+}$ -pump of SMC.

**Keywords:** *flavonoid,  $\text{Na}^+/\text{Ca}^{2+}$ -exchanger, rat aorta, smooth muscle cells*

**Acknowledgement:** The work was supported by state project FA-F6-T083 of Academy of Science of Uzbekistan.

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## **Expression of egg-specific protein mRNAs in the chicken oviduct during tamoxifen-induced pause in laying**

Joanna SOCHA, Anna HRABIA

*Supervisor:* dr. hab. eng. Anna Hrabia, prof. UR

The hen oviduct is of special interest to commercial egg producers, because disrupted activity or pathological changes directly affect egg quality. During the reproductive cycle, the avian oviduct undergoes dynamic morphological and functional changes. After about one year of egg production, a pause in laying occurs in hens and is accompanied by regression and subsequent rejuvenation of the reproductive system. Although, numerous studies revealed the morphological changes which occur in the oviduct during regression, alterations in synthesis of egg-specific protein in the oviduct remain to be elucidated. Therefore, in the study expression of some egg-specific protein mRNAs in two sections of the chicken oviduct (magnum where is produced the most of egg white and shell gland where calcification of the eggshell takes place) during a pause in egg laying induced by tamoxifen (TMX; estrogen receptor modulator) was examined. The experiment was carried out on Hy-Line Brown laying hens housed under a 14L:10D lighting schedule and provided with water and commercial food *ad libitum*. Hens were randomly divided into two groups: control (n=6) and experimental (n=6). The control birds were injected subcutaneously with vehicle (ethanol) and the experimental one with TMX at a dose of 6 mg/ kg of body weight. Hens were injected daily until a pause in egg laying occurred. Chickens were killed on day 7 of experiment and the oviductal magnum and shell gland were isolated. In tissue of the magnum the mRNA of ovalbumin, and in the shell gland the mRNA of ovocalyxin-32, ovocalyxin-36 and ovocleidin-116 expression was evaluated by quantitative real-time PCR with TaqMan chemistry. TMX injections lead to gradual decline in laying of eggs from day 5 of the experiment and all hens stopped egg laying on day 6. The weight of the oviduct in the control and TMX-treated chickens amounted to  $56.6 \pm 2.8$  g and  $47.9 \pm 0.8$  g, respectively. Treatment of hens with TMX (1) significantly decreased the relative expression (RQ) of ovalbumin mRNA as well as ovocleidin-116 mRNA, (2) tended to increase in ovocalyxin-36 mRNA level, and (3) did not affect the ovocalyxin-32 expression. The results obtained indicate that gene expression of some egg proteins changes during pause in laying induced by TMX. Furthermore, relationship between estrogen action and egg specific protein gene expression is suggested.

**Key words:** *tamoxifen, egg proteins, ovoalbumin, ovocalyxin, ovocleidin, oviduct, chicken,*

**Acknowledgement:** Supported by DS-3243/KFiEZ

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## Preparation of plant transformation vector containing expression cassette of At3g50970 gene from *Arabidopsis thaliana* controlled by dCaMV 35S promoter

Mária ZIMOVÁ

*Supervisor:* Ing. Jana Moravčíková, PhD.

*Supervisor-specialist:* Mgr. Ildikó Matušíková, PhD.

Plants are continuously exposed to various environmental stresses such as low temperature, salinity or water deficit with a series of cellular and molecular changes. A part of plant response is the production of a wide range of proteins including dehydrins. Dehydrins belong to the group of LEA proteins that are accumulated in the late stages of the seed development and in vegetative plant tissues. These proteins play multiple roles including membrane protection, cryoprotection of enzymes and protection from reactive oxygen species. Dehydrins are characterized by the presence of conserved sequences known as Y, S and K segments. The K-segment is obligate for all groups of dehydrins. The structures of some dehydrins contain histidine-rich regions that have the potential to bind free metals; therefore, it could contribute to increased tolerance to heavy metals.

The gene At3g50970 (K<sub>6</sub> motif) from *Arabidopsis thaliana* belongs to the group of neutral/basic dehydrins. The gene plays important role in stabilization of a membrane during stress conditions. The gene regulates endogenous proline content which acts as an important osmolyte. The gene regulates the activity of the enzyme catalase which catalyzes dissociation of hydrogen peroxide preventing oxidative destruction of the cell.

The aim of this work is to study the effect of an overexpression of the dehydrin gene At3g50970 (*dh3*) in transgenic tobacco on the tolerance to abiotic stresses such as drought, osmoticum, or heavy metals; and their combinations.

The *dh3* gene was isolated from *A. thaliana* by PCR approach. Using techniques of recombinant DNA, we prepared two expression cassettes containing i) the *dh3* gene fused to the constitutive dCaMV 35S promoter and ii)  $\beta$ -glucuronidase (*gus*) reporter gene controlled by the light-sensitive Lha3.St1 promoter. The both cassettes were introduced into the plant transformation vector pBinPlus. The created plasmid pDH3 was transformed into *Agrobacterium tumefaciens* LBA 4404. We verified the stability of the plasmid pDH3 in *Agrobacterium* by restriction analysis after the re-transformation of *Agrobacteria* in *E. coli*. The plasmid was used for transformation of tobacco leaf explants. Regeneration of transgenic plants is in progress.

**Key words:** *abiotic stress, Agrobacterium tumefaciens, Arabidopsis thaliana, dehydrins, gene At3g50970, Nicotiana tabacum L.*

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SECTION

*Multifunctional agriculture, environment and rural development*

## Testing suitability of cellulolytic enzymes obtained from selected fungi for the hydrolysis of wood material

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The aim of this paper is testing possibility of use cellulolytic enzymes obtained from selected fungi using to hydrolysis of wood material. The influence of cellar fungus on the composition of poplar wood was tested along with suitability in the process of enzymatic hydrolysis of wood. It is currently very fashionable to use green energy. Also EU directives indicates that this type of energy is favourable. Many research centres conducts research on bioethanol obtainment from different materials. Faculty of Wood Technology in Warsaw University of Life Sciences performs studies about obtaining bioethanol from lignocellulosic material which is wood. The technology step which should be still developed is the enzymatic hydrolysis. However, commercial enzymes are expensive and quite difficult to access. That is why the attempt was made to obtain enzymes from common fungi. Cellulolytic enzymes were obtained from Turkey tail fungus *Trametes versicolor*, brown-rot fungus *Coniophora puteana* and black mould *Aspergillus niger* cultured on special media. Collected enzymes solutions were used to perform the enzymatic hydrolysis of cellulose and holocellulose. Those polysaccharides were obtained from common aspen wood (*Populus tremula*), according to Kürschner-Hoffer or chlorite method, respectively. Hydrolysis was carried out at various temperatures from 25 to 50°C and various pH from 4.0 to 5.5. Experiments were followed by determination of sugars yield using high performance liquid chromatography (HPLC). The activity of enzyme complexes derived from fungi was compared. In spite of relatively low effectiveness of hydrolysis, which can be probably connected to applied procedure of enzymes purification, the highest activity was found for enzyme complex obtained from *Aspergillus niger* fungus. The highest yield of glucose was obtained at the temperature of 50°C and pH of 4.5. In the case of *Trametes versicolor* fungus the highest yield was found at pH of 5.0 and the temperature of 50 °C. *Coniophora puteana* fungus showed the lowest activity at all, while the highest yield was observed at the temperature of 25°C only.

**Key words:** *hydrolysis, poplar wood, fungi, cellulolytic enzymes*

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## **Sodium cellulose sulfate as an antiviral agent for the tomato plant diseases induced by tobamoviruses**

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*Supervisor:* prof. Dr. Abbaskhan Turayev

Tobamoviruses include 35 species such as tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), pepper mild mottle virus (PMMoV) etc., and induce most common viral diseases in vegetable and cucurbitaceous plants. The viral diseases cause the plants to yellowing, stunting, uneven ripening of fruit and further reducing yield. Tobamoviruses possess a rod-shaped structure composed of single-stranded RNA encapsulated with coat protein. Tobamoviruses have very high stability, as instance TMV remains infectious for 50 years in unpreserved plant sap, the thermal inactivation point of the virus is 93°C for 10 min, and the dilution end point is  $1 \times 10^{-6}$ . In the course of exploring for antiviral compounds against tobamoviruses, it was found that natural sulfated polysaccharides, such as heparin, hyaluronic acid, alginate, chondroitin sulfate, carrageenans and fucoidan had inhibitory activity against TMV. Natural sulfated polysaccharides include a complex group of structural and functional macromolecules widespread in nature, in a great variety of organisms such as mammals and invertebrates. Sulfated polysaccharide analogues such as dextran sulfate, cellulose sulfate, lentinan sulfate etc. can also be prepared with modification of appropriate polysaccharides. Both natural and synthetic sulfated polysaccharides possess a broad range of biological activities.

Our recent studies showed that the sodium cellulose sulfate (SCS) exhibits potent inhibitory activity against tobamovirus infection in plants. In this study, antiviral effect of SCS on the infection of ToMV in tomato plants was investigated. SCS was prepared through heterogeneous sulfation of cellulose by using pyridine-chlorosulfonic acid complex, and characterized by physicochemical methods of analysis. The ToMV was separated from systematic infected leaves of *N. clevelandii* and purified by differential centrifugation. The tomato plants grown in the open field, with average height of 45 cm, were inoculated with ToMV suspension, and the viral disease symptoms were identified 10-15 days after inoculation. The tomato plants with ToMV symptoms were treated with 0.5 mg/mL aqueous solution of SCS, once every 10 days, 3 times in total. After 20-30 days of initial treatment, noticeable differences regarding plant development were observed in the plants treated with SCS, compared to control plants. According to the received results, tomato plants treated with SCS showed a decrease in the degree of ToMV infectivity by 71.5%, an increase in the crop yield by 24%, compared to control plants. In addition, tomato plants treated with SCS exhibited better crop quality and plant development than those of control plants.

In conclusion, a treatment with SCS inhibits ToMV infection in tomato plants and improves plant development and crop yield. SCS can be used as an antiviral agent for the tobamoviruses induced tomato plant diseases.

**Key words:** *tomato plants, tobamoviruses, sodium cellulose sulfate*

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## ***Trichoderma* as biological control agent protecting crop plants against downy mildew**

Justyna NAWROCKA, Monika NOWOGÓRSKA, Adrian WITCZAK, Monika SKWAREK

*Supervisor:* prof. Urszula Małolepsza, CSc.

The subject of this study is associated with the search for environmental friendly methods of crop plant protection by natural biocontrol agents. The newly selected, Polish strain of *Trichoderma atroviride* (TRS25) fungi seems to be an alternative to the use of harmful pesticides in cucumber crop cultivation. However, plant protection by this microorganism may depend on the form of *Trichoderma* application. Therefore, the aim of this study was to evaluate the influence of different forms of *Trichoderma* spore application on cucumber growth and health in the field conditions.

In the present study TRS25 was evaluated for its ability to promote seed germination and growth of cucumber plants (*Cucumis sativus* L.) cv. Iwa F1 as well as their protection against cucurbit downy mildew during three vegetation seasons. A comparative study was conducted concerning the effect of the tested strains on biochemical changes in phenolics metabolism, as hallmarks of the strengthening of plant protective barriers against pathogen. *Trichoderma* spores were used to directly treat cucumber seeds, and they were introduced with organic carrier into the soil before cucumber seeds sowing. Samples were taken from the plants at the one-leaf stage (five week old) and the early flowering stage (seven week old). When used directly or with the organic carrier, TRS25 improved cucumber seed germination and positively influenced biomass accumulation of cucumber plants mainly at the early flowering stage of the plants. Moreover, tested strain induced defence responses limiting cucurbit downy mildew severity. Biochemical and microscopic analysis showed that protection against the disease might be associated with enhanced deposition of callose and lignin which protect vascular bundles and the surface of shoots and roots of cucumber plants. Moreover, results revealed a key role of TRS25 in induction of salicylic acid mediated systemic response and in accumulation of phenolics in the form of phenylpropanoids, flavonoids and anthocyanins, compounds used in direct and indirect plant protection against pathogens. Comparing with the control, increased phenolic concentrations in the young plants were observed in all TRS25 treated material regardless of the application method. At the early flowering stage the heightened levels of phenolics persisted in the plants derived from the seeds treated directly with this strain.

The present results suggest that TRS25 can be used to stimulate cucumber seeds germination, plant growth promotion as well as protection of plants against downy mildew. The application of *Trichoderma* spores with organic carrier into the soil before cucumber seed sowing seems to be less successful in the enhancement of phenolic synthesis.

**Key words:** *Trichoderma*, cucumber, defence response, downy mildew

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## **The role of reactive oxygen and nitrogen species and enzymes involved in their metabolism in response of *Phaseolus vulgaris* to pathogen attack**

Agata NOWOGÓRSKA, Justyna NAWROCKA, Adrian WITCZAK, Monika SKWAREK

*Supervisor:* Assoc. prof. Jacek Patykowski, PhD.

Reactive oxygen and nitrogen species (ROS and RNS, respectively) are the first molecules of defense mechanisms produced in response to pathogen attack. They are involved in reactions directly related to the creation of a toxic environment for pathogens, as well as other reactions including cell signaling or modifications of structural compounds triggering resistance to stress factors. *S*-nitrosylation is one of the mechanisms modifying protein activity under various stresses. This process is mostly regulated by the activity of specific enzyme *S*-nitrosogluthathione reductase (GSNOR). On the other hand metabolism of ROS is strictly related to enzyme activity maintaining redox state such as peroxidase (POX), superoxide dismutase (SOD) as well as glutathione reductase (GR) and monodehydroascorbate reductase (MDAR) functioning in the ascorbate-gluthathione cycle.

The aim of this study was to determine the changes in a generation of selected reactive oxygen and nitrogen species and activity of enzymes involved in their metabolism under selected biotic stress. Biochemical reactions in common bean leaves after sequential infection with first biotrophic bacteria *Pseudomonas syringae* pv. *phaseolicola* and subsequently necrotrophic fungus *Botrytis cinerea* were analyzed. Moreover histochemical detection of NO and H<sub>2</sub>O<sub>2</sub> was performed.

The results showed that the infection of common bean with selected pathogens caused significant changes in the generation of ROS and RNS, as well as in the activity of enzymes comparing to the control. The results indicated that plant inoculation with a specific pathogen significantly affected the final response to the sequential infection and was mainly determined by the mechanisms induced after the bacterial infection.

**Key words:** *common bean, infection, Pseudomonas syringae, Botrytis cinerea, antioxidant enzymes*

**Acknowledgement:** Work was supported by faculty grant for young scientists and PhD students entitled: "Elements of redox regulation in response of common bean (*Phaseolus vulgaris*) to sequential infection of *Pseudomonas syringae* pv. *phaseolicola* and *Botrytis cinerea* "

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## **Defensive enzyme activities in cotton lines and varieties with different insect-resistance**

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*Supervisor:* Olga N. Veshkurova, Shavkat I. Salikhov

Cotton is one of major crop plants grown in Uzbekistan, the world's sixth largest cotton fiber producer and fourth largest exporter, accounting for 5 percent of global production of cotton fiber. Biting and sucking pests as well as pathogens cause the crop losses of cotton plant. Insect pests can cause significant yield losses from the time of plant emergence through boll maturity. Estimates indicate that cotton fiber losses due to insect infestations can amount up-to 15% of world production. In this manner, host plant resistance plays an important role in pest management strategy resulting in reduced losses due to the herbivores, less insecticide use, more crop yields, and safer environment, in addition being cost effective. Among the destructive cotton plant pests, cotton bollworm *Helicoverpa armigera* is one of the economically important and commonly found in some cotton growing countries, the damage of which was estimated up-to 30%.

In plants, resistance against insect-pests is supplied by a number of defensive compounds such as phytoalexins, defensins, thionins, pathogenesis-related (PR) proteins obtaining enzymic activities. Among these defense-related chemicals PR proteins are certainly of major importance to be protected from insect-pests. Oxidoreductase enzymes are of the great importance among defensive compounds of a protein nature. Enzymes, inducing in damaged tissues, such as peroxidases (POX) and Polyphenoloxidases (PPO) are considered as involved in resistance to pathogenic microorganisms and insects. Besides the role of urease in plant defense against a number of insects has been reported. In this regard we investigated the activities of POX, PPO and urease in 24 different varieties and lines of cotton plant differing by the percentage of damage by cotton bollworm. The aim of this research was to study the relationship between these enzymes activities in cotton leaves of different cotton lines and varieties which were estimated as high, moderately and low resistant to cotton bollworm. Results showed that the POX and PPO activities were significantly higher in the high resistance varieties and lines compared to the moderately and low resistant ones. POX and PPO activities were significantly lower in low resistance varieties and lines. The higher POX and PPO activities corresponded with higher insect resistance, and their activities positively correlate with the various levels of resistance of cotton plant.

However, we determined negative correlation between ureolytic activity and resistance against cotton bollworm. Higher urease activities were defined in low resistant lines and significantly lower urease activity values were found in high resistant plants.

Obtained results support that POX and PPO activities can be used as indicator for resistance of cotton plant against cotton bollworm during breeding.

**Keywords:** *Cotton plant, peroxidase, polyphenoloxidase, urease, insect resistance*

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## **The antimicrobial activity of cationic peptides from the seeds of milk thistle *Silybum maricinum* (L.).**

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Olga VESHKUROVA, Shavkat SALIKHOV

*Supervisor:* Veshkurova Olga Nikolaevna

The intensive use of antibiotics leads to the rapid development of pathogenic bacteria resistant to classical antimicrobial drugs. In this connection, in recent years the finding of most effective antibacterial substances is performed worldwide. Therefore, very relevant it is the question of the creation of alternative treatments, when bacterial resistance to them is limited or absent. The antimicrobial peptides may be such means.

Cationic peptides capable of forming an ion-permeable pores in liposomes differing in the size and lifetime. After penetration through the cell wall, the peptide binds to the negatively charged surface of the cytoplasmic membrane and across it by the formation of micelle-like complex from the group of these peptides, or passes through the cytoplasmic membrane under the action of the transmembrane electric potential.

In this study the peptides from milk thistle seeds (*Silybum marianum* L.) are considered as the research object. The peptides were isolated from the milled and defatted meal of milk thistle using of acid extraction (10% acetic acid), precipitation with cold acetone and followed ion-exchange chromatography.

The precipitate was dissolved in 0.05 M acetate buffer (pH 9.0) and eluted from DEAE column previously equilibrated with 0.05 M ammonium acetate (pH 9.0) at a flow rate of 0.5 ml / min. The eluate was adjusted to pH 6.0 with HCl and analyzed on of CM-TSK column, equilibrated with 0.05 M ammonium acetate (pH 6.0). Adsorbed proteins fractions were eluted with stepwise gradient concentrations in 300 ml of NaCl ranged from 0.0 to 1 M prepared in the same 0.05 M ammonium acetate (pH 6.0) at a flow rate of 0.5 ml/min and 6 different fractions were collected. Each of obtained fractions was desalted on G-10 column and tested for antimicrobial activity against gram-negative and gram-positive bacteria.

Antimicrobial activity was determined by radial diffusion on Petri dishes with previously inoculated pathogenic bacteria using 50 µl of each of obtained peptide fractions in various concentrations. Antimicrobial activity was evaluated on nine strains of human pathogenic bacteria. As a standard was used gramicidin (an antimicrobial peptide) at the concentration of 50 µg/ml to refer to activity of obtained peptides.

As the result, the most active against *Staphylococcus saprophyticus* and *Proteus vulgaris* were fractions №1 and №2. Fraction №6 has shown high antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aerogenosa*, *Actinomyces*. The activity of these three factions exceeded gramicidin activity 20-25 times at the same concentration.

**Keywords:** *peptides, ion-exchange chromatography, antimicrobial activity*

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## **The antioxidant enzymes function in the protection of *Quercus robur* leaves against oak powdery mildew**

Monika SKWAREK, Adrian WITCZAK, Justyna NAWROCKA, Agata NOWOGÓRSKA

*Supervisor:* prof. Jacek Patykowski

In recent years the increase in infestation of trees by pathogens has been reported. In Polish forests oak powdery mildew is most commonly caused by a species of fungus *Erysiphe alphitoides*. Fungi which cause powdery mildew belong to the subdivision *Ascomycetes* (*Ascomycotina*). Oak powdery mildew is responsible for significant damage in nurseries. The biggest disease symptoms are observed on young leaves, growing in early spring. The susceptibility of mature oak leaves to pathogen infection is much lower.

Each of stress, biotic and abiotic which initiates changes in plant cells lead to distortions of their metabolism. Plant cells as a result of a series of follow-up reactions attempt to restore normal metabolism. The attack of the pathogen induces a burst of reactive oxygen species (ROS), that can act as a signal activating of enzymatic pathways defense mechanisms.

The aim of the study was to determine the concentration of hydrogen peroxide ( $H_2O_2$ ) and superoxide anion ( $O_2^{\cdot-}$ ), selected elements of ascorbate-glutathione cycle such as ascorbate peroxidase activity (APX), dehydroascorbate reductase activity (DHAR), monodehydroascorbate reductase activity (MDAR) and the concentrations of ascorbate (AA) and dehydroascorbate (DHA) in the *Quercus robur* leaves infected with powdery mildew.

The study material was constitute one-year old seedlings of oak uninfected and infected with *Erysiphe alphitoides* from the container nursery, Forest District Gidle, Poland. For analysis we used leaves with different size of infection area (<5%, 12-15%, 25%).

The results showed the changes in the  $H_2O_2$  and  $O_2^{\cdot-}$  concentrations, antioxidant enzyme activities and in AA and DHA concentrations that may play an important role in defensive system of powdery mildew-infected leaves.

**Key words:** *oak powdery mildew, antioxidant enzymes, biotrophic pathogen*

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## **Investigation effects of inhibitors of enzymatic hydrolysis of holocellulose from poplar wood**

Dominika SZADKOWSKA, Jan SZADKOWSKI, Anna LEWANDOWSKA

*Supervisor:* professor Janusz Zawadzki, dr inż. Andrzej Radomski

Bioethanol production from biomass is a technology of growing importance. The second-generation biofuels, based on conversion of lignocellulosic substrate, especially wood of fast-growing tree species, are considered as a desired product of “green” technology. Hydrolysis of polysaccharides is conducted using enzyme complexes, commonly obtained from fungi. Enzyme molecules are extremely sensitive to process conditions, thus detailed optimisation is necessary to give the highest yield of glucose and xylose. Various organic and inorganic substances are known to be enzyme inhibitors. Many of them occur in raw wood or are formed during wood pre-treatment. The aim of this study was to find the influence of potential enzyme inhibitors on the yield of enzymatic hydrolysis. Holocellulose obtained from white poplar wood according to chlorite method was used as a substrate. Prior to holocellulose isolation, wood was extracted with azeotropic mixture of chloroform and ethanol, containing 7 % of ethanol (by weight). Hydrolysis experiments were conducted using commercial mixtures of enzymes from Dyadic: Cellulase CP CONC and Xylanase 2 XP CONC. The effectiveness of hydrolysis reactions was determined as simple sugars yield, defined as the ratio of the resulting glucose or xylose amount to theoretical value, based on the content of cellulose or xylan, respectively. High-performance liquid chromatography (HPLC) using “amino” stationary phase was applied to sugar analysis, along with size exclusion chromatography (SEC) for enzyme and sugars separation. Gas chromatography coupled with mass spectrometry (GC-MS) was applied for analysis and identification of compounds in wood extractives and recognising them as potential inhibitors. The influence of iron and manganese cations, which are present in water used for processing, was tested. Dependence of hydrolysis rate on selected organics was determined as well. Vanillin, which corresponds to lignin structure, was tested, along with water and alcohol extract from white poplar wood. Another compound tested was furfural, which is formed in wood during its thermal or hydrothermal pre-treatment. It has been shown that the content of manganese and iron in tap water does not affect the efficiency of enzymatic hydrolysis. On the other hand furfural was found to show strong impact, observed as decrease in the yield of the enzymatic hydrolysis. In the case of extractives obtained from the white poplar wood, there is no significant inhibition observed, as the differences did not exceed those registered in parallel hydrolysis experiments, conducted without additives.

**Key words:** *hydrolysis, furfural, extractives, poplar wood*

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**Activity of selected antioxidant enzymes in Scots pine (*Pinus sylvestris*) needles infected by *Lophodermium seeditiosum***

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Anna LEWANDOWSKA, Jan SZADKOWSKI

*Supervisor:* prof. Małgorzata Sławska, PhD.

Plants in response to stress conditions, including contact with a pathogen, have developed defense mechanisms that are intended to inhibit infection. The first defense reaction is to increase the level of reactive oxygen species (ROS) in a plant cell, which can lead to increased activity of antioxidant enzymes.

The aim of the study was to determine the changes in activity of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPOD) and syringaldazine peroxidase (SPOD) in Scots pine (*Pinus sylvestris*) needles infected with a fungal pathogen that causes *Lophodermium* needle cast.

The plant material was collected from forest plantations in Spała Forest District from different types of forest habitats: fresh coniferous forest, fresh mixed forest, moist coniferous forest. Infected needles were collected 14 days after the occurrence of the first symptoms of fungal pathogen infection. The needles used in the analysis had visible symptoms of the infection that encompassed 25% of needle area.

The results demonstrated that the activity of SOD increased in the infected needles in the early phases of infection. There were no changes in CAT activity. We also observed a significant increase in GPOD and SPOD activity in the infected pine needles.

The increase in enzyme activities may indicate initiation of defensive processes in the infected cells.

**Key words:** *Lophodermium seeditiosum*, infection, antioxidant enzymes, reactive oxygen species

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SECTION  
*Plant Production*

## **Mineral composition and dry mass production of safflower (*Carthamus tinctorius* L.) grown under low salt concentrations**

Milena DANIČIĆ, Ivana MAKSIMOVIĆ, Marina PUTNIK-DELIĆ

*Supervisor:* dr. Marina Putnik Delić

Combination of highly mineralized irrigation waters and native substrate may lead to accumulation of excess salts in the soils. This can cause damage to crops in sense of their nutritive and market value, and also jeopardize agricultural production. Therefore, significance of growing more salt-tolerant crops in the future is going to be more considered. Safflower (*Carthamus tinctorius* L.) is drought tolerant species which is mostly grown in the arid and semiarid areas of the world, where the process of evapotranspiration overcomes the rainfall. In this paper, the influence of relatively low concentrations of NaCl (0, 0.2, 0.6, 1.2 g NaCl·L<sup>-1</sup>), on dry mass of root, stems and leaves and mineral nutrition of safflower, has been examined. The plants were grown under semi-controlled conditions in the greenhouse. After germination in sterilized sand in thermostat, plants were moved to pots containing ½ Hoagland solution. Afterwards, different NaCl concentrations were added to growth solution. After the end of the experiment, plant parts were harvested, dried and weighed afterwards. The concentrations of Na, K and Ca were determined by flame photometry. Concentration of P was established by the ammonium vanadate-molybdate method whereas the concentrations of Mg, Fe, Mn, Cu and Zn were obtained using atomic absorption spectrophotometer. Inhibitory effect of salt on leaf production of treated plants was proven (dry mass of leaves of the treated plants was reduced by 19.5-13.3%). Also, the dry mass of stems and roots decreased in the presence of 0.6 and 1.2 g NaCl·L<sup>-1</sup>. It was found that the presence of salt changed the accumulation and distribution of mineral elements. Concentration of K, Ca, P and Mg decreased following higher NaCl concentration in nutrient solution actually up to 26.6% comparing to control. The concentration of Na increased in root (up to 21%) and shoot (up to 67.5%) of all treated plants with respect to control and the concentration of Fe, Zn, Mn and Cu showed no consistency. Even though concentrations of NaCl examined in this experiment were relatively low (up to 1.2 g NaCl·L<sup>-1</sup>) and safflower is relatively salt-tolerant species, significant changes in growth and chemical composition of plant tissues occurred.

**Key words:** *dry mass, growth, mineral nutrition, safflower, salinity, stress*

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## Comparison of lawn varieties of turfgrass kentucky bluegrass (*Poa pratensis* L.) to use 'park'

Barbara JANUS, Beata GRYGIERZEC

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The study was realized in 2011-2012 in Gnatowice (50° 11'51,36" N and 20° 10'36,44" E, 220 m asl) next to Krakow (220 m asl). It included six varieties of kentucky bluegrass lawn, namely Alice, Ani, Bile, Conni, Evora as well as Limousine. The experience was operated by the 'park' system extensively. A randomized block methods with four replications, with each plot area of 1 m<sup>2</sup>, was assumed in September 2010. Plots were separated from each other by wooden strips embeded in the soil to a depth of 5-10 cm, creating a cassette system. Pas plots began and ended with zero plots, sown kentucky bluegrass (a variant of Alice). The nitrogen fertilizer was applied in the amount of 60 kg · ha<sup>-1</sup> N, phosphorus-potassium fertilizer being applied once in late September and October. Phosphorus is used in an amount of 20 kg · ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and potassium 40 kg · ha<sup>-1</sup> K<sub>2</sub>O.

The goal was to evaluate the characteristics of selected varieties of kentucky bluegrass lawn in 'park' extensive system. The varieties were analyzed for the following features lawn: the state of the plants in spring and autumn, the overall aesthetic aspect (OA), compactness (C), the intensity of regrowth (IR) and diseases (*Microdochium nivale*, *Drechslera poae*, *Puccinia striiformis*, *Entyloma dactylidis*).

There are 23 varieties of kentucky bluegrass lawns, which account for 78% of all the varieties of this species, are currently registered in the COBORU National Register. There exists justified, and the continuing need to research new varieties of kentucky bluegrass in many respects, with particularly important knowledge of resistance emerging during the lifetime of the disease. Rational use of Vanna [2013] should be based on proper selection of varieties, consistent with fertilization and chemical protection applied. In this study were used Polish and foreign varieties. Short description of the results shown in the next paragraph.

During the study period the best state in the spring was characterized by a Limousine, while the worst being Evora. Winter survival was significantly different in the studies in each variety. The overall aesthetic aspect and compactness was dependent not only on the variety, season and the year of the study. The most attractive plants look like in summer while the worst in autumn. In general, the greater the intensity of regrowth was characterized by variations in the second year of the studies with the exceptions of Conni and Evora in this respect. The greatest concentration of two diseases *Microdochium nivale* (observed in two varieties: Ani and Conni) and *Entyloma dactylidis* (varieties Alice, Bila and Conni) observed in spring. The most affected *Puccinia striiformis* was variety Ani in autumn. On the other hand, in the case of *Drechslera poae* the lowest symptoms of the disease were recorded in summer in all varieties of kentucky bluegrass.

**Key words:** kentucky bluegrass, use 'park', varieties

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**Impact of substrate supplemented with CaCO<sub>3</sub> on mycelial growth, yield, morphological features and storability of fruiting bodies of Black poplar mushroom *Agrocybe cylindracea* (DC.) Marie.**

Agnieszka JASIŃSKA, Luiza DAWIDOWICZ

*Supervisor:* prof. dr. hab. Marek Siwulski

Black poplar mushroom deserves special attention, above all due to its medicinal properties. Water and alcohol extracts from fruiting bodies of the fungus have an anti-oxidant, anti-cancer, anti-fungal, cholesterol triglycerides blood level abilities. *A. cylindracea* is moreover rich in protein, low in fat, having a mild flavor and unique taste. This mushroom is also rich in vitamins and mineral elements. The aim of experiments was to determine the effect of a substrate supplementation with calcium carbonate on mycelial growth, yield, morphological features and storability of fruiting bodies of *Agrocybe cylindracea* (DC.) Marie. The study was conducted in the laboratory of the Department of Vegetable Crops of Poznań University of Life Sciences and air-conditioned chambers of mushroom farm – Wytwórnia Grzybów Uprawnych in Łobez near Jarocin. The object of the study was 4 strains of *A. cylindracea*: AE02, AE05, AE06, AE11. The effect of addition calcium carbonate in an amount of 2, 4 and 8 g per 100 g of substrate on mycelium growth, yield and fruiting and morphological characteristics of storability was examined. Total yield, which accounted whole fruiting bodies, marketable yield accounted for hats of fruiting bodies without stems, cap diameter, length and diameter of stem, average weight of fresh carpophore and carpophores dry mass were determined. The results of mycelial growth and yield were established in fully randomized design in 4 replications and 2 series. On biological tubes, thickness of substrate mass occupied by mycelium was measured after 18 days from inoculation. Mycelial growth rate is dependent on the growing substrate. The amount of additive to sawdust substrate affects the rate of mycelial growth and yield of investigated strains. *A. cylindracea* mycelial growth was not affected by addition of calcium carbonate to substrate, however a significant effect of this additive was found on the yield, which was the highest with addition of calcium carbonate in an amount of 8.0g per 100g<sup>-1</sup>. Carpophores characterized with the largest hats diameter, and the largest individual mass obtained of substrate enriched with addition of calcium carbonate in an amount of 8.0g per 100g<sup>-1</sup>. In addition, it was found that addition of calcium carbonate to substrate affect storability of *A. cylindracea*. The lowest weight loss of fruiting bodies after 3 and 7 days of storage was found with addition of calcium carbonate to substrate in an amount of 4.0g per 100g<sup>-1</sup>.

**Key words:** *additives, calcium carbonate, carpophores, cultivation, fungi, harvest, shelf life*

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## **Hormonal and pro / antioxidant balance in cotton under the effect of chloride salinity and exogenous phytohormones**

Karomat M. KULDOSHOVA, Sanjar B. NAVRUZOV

*Supervisor:* prof. Ali A. Akhunov, PhD. Zamira Golubenko, PhD. Nigora A. Abdurashidova

Investigation of mechanisms of plant development stress-reaction and the formation of adaptation to changing environmental conditions acquire special relevance. In plants there is a complex multi-component signaling network, carrying out the reception of external factors and the activation of the system. Phytohormonal and superoxide-producing signal systems are among the most important ones.

One of the problems of studying the hormonal system in the adaptation process is investigating the possible correlations of hormonal balance in order to enhance the resistance of cotton.

We analyzed the relationship between changes in the quantity and proportion of phytohormones; studied physiological parameters under the effect of salinity and water deficiency on the background of abscisic acid (ABA) and indoleacetic acid (IAA). Studies have shown that the hormonal balance in the initial stages of the impact of stress on cotton seedlings undergoing significant changes reduces IAA content and increases the level of ABA, as a result the ratio of IAA/ABA falls. These changes in hormonal balance are not only the result of the stress response, but also are adaptive value. Exogenous phytohormones action improves the morphological indicators with abiotic stresses. It was revealed that the nature of the physiological response of cotton depends on the level of stress, changes in the content of endogenous hormones mediated by their direction and their ratio. We have defined that phytohormones processing steps under stress factors have protective effect.

Displacement of pro/antioxidant balance is observed in response to various natural factors and considered as one of most important links during the development of the stress response. Formation of the protective reaction occurs with the participation of phytohormones since they are important regulators of plant life and therefore changes in phytohormonal balance is observed under various external influences.

In our work, the effects of exogenous IAA and ABA on the level of lipid peroxidation and activity of antioxidant enzymes were studied in cotton seedlings for the first time. It has been shown that IAA has prooxidant effect and ABA was revealed as an antioxidant.

The investigations demonstrated that in cotton varieties Gulistan, resistant to salinity, quantity of water-soluble proteins is higher than variety C-4727, non-resistant to stresses.

**Keywords:** *abscisic acid, indoleacetic acid, salinity, phytohormone*

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## Evaluation of total phenolics and carotenoids of selected varieties of non-traditional cereals

Ľudmila LEVÁKOVÁ, Magdaléna LACKO-BARTOŠOVÁ

*Supervisor:* prof. Ing. Magdaléna Lacko-Bartošová, CSc.

Wheat is a good source of polyphenols, secondary metabolites of plants with beneficial effects on human health. However, little information is available on phenolic composition and carotenoids concentrations in different *Triticum* species. Phytochemicals in whole grain cereals have not received as much attention as the phytochemicals in fruits and vegetables, although the increased consumption of whole grain cereals and whole grain-based products has been associated with reduced risk of chronic diseases such as cardiovascular diseases, type II diabetes, obesity, cancer and some other chronic diseases. Recent research has shown that the total phytochemical content and antioxidant activity of whole grain cereals have been commonly underestimated in the literature and they contain more phytochemicals than was previously reported. The phenolic compounds are one of the most widely occurring groups of phytochemicals, synthesized during plant development and in response to stress conditions. These compounds, most of which are present in wheat bran, play an important role in plant growth and reproduction, providing protection against pathogens and predators and also contributing to the colour and sensory characteristics of plants. In addition to their role in plants, phenolic compounds in human diet provide many health benefits associated with reduced risk of chronic diseases in which reactive oxygen species are involved. The objective of this study was to evaluate total phenolic content (free and bound phenolics) and carotenoids content (lutein and  $\beta$ -carotene) in selected varieties of non-traditional cereals produced in organic farming system. Two varieties of *Triticum spelta* (Rubiota, Franckenkorn) and *Triticum dicoccon* (Farvento, Molise sel Colli) and *Triticum monococcum* (Einkorn) were analysed. A field stationary experiment was established at the Research Experimental Station of the Slovak University of Agriculture in Nitra, at Dolná Malanta during the years 2013 – 2014. The average free phenolic content of selected varieties was  $157.10 \pm 53.20 \mu\text{g FEA.g}^{-1}$ . There were significant differences between varieties. *Triticum spelta* varieties had the significantly lowest concentrations of free phenolics. The average content of bound phenolics was  $741.48 \pm 122.39 \mu\text{g FEA.g}^{-1}$ . In general, carotenoids are very minor constituents in cereal grains except for einkorn that contains relatively high levels of carotenoids. The lutein content in einkorn was  $2.51 \mu\text{g.g}^{-1}$  and the concentrations of  $\beta$ -carotene reached the value of  $0.57 \mu\text{g.g}^{-1}$ .

**Key words:** *carotenoids, non-traditional cereals, organic farming system, phenolic compounds*

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## **Influence of nitrogen fertilizer with inhibiting effect on the content of available nitrogen forms in the soil under winter barley (*Hordeum vulgare* L.) growing**

Zuzana PANÁKOVÁ

*Supervisor:* doc. Ing. Pavol SLAMKA, PhD.

The objective of this research was to investigate the influence of liquid nitrogen fertilizer with inhibiting effect on the content of nitrate and ammonium nitrogen in the soil and the effectiveness of nitrogen-sulphur nutrition of winter barley. The research was conducted in field small-plot experiment with winter barley (variety "Wintmalt") on heavy brown soil in vegetation period 2015/2016. The dose of nitrogen in all experimental treatments was  $140 \text{ kg} \cdot \text{ha}^{-1}$  and was applied at one shot or split in three partial doses. The following fertilizers were applied in the experiment: solid nitrogen-sulphur fertilizer Ammonium nitrate + Ammonium sulphate DASA 26/13 (26% N, 13% S) and liquid nitrogen-sulphur fertilizer Ammonium thiosulfate ATS (12% N, 26% S) with inhibiting effect. Ammonium thiosulfate is used to enhance N utilization efficiency of fertilizers such as ammonium nitrate. Fertilizers DASA 26/13, ATS concentrated and ATS watered down (1:1) were applied three times by split application during regenerative fertilization, production fertilization and qualitative fertilization, respectively. Fertilizers DASA 26/13, ATS concentrated and ATS watered down (1:1) were applied by one shot application during regeneration fertilization. Soil samples from all examined treatments were taken from two soil depths (0.0-0.3m and 0.3-0.6m, respectively) by probe rod in 3-4 week intervals. Inhibitory effect of fertilizer ATS was evaluated as a portion of  $\text{N-NO}_3^-$  on  $\text{N}_{\text{an}}$  in respective soil profiles. Achieved results indicate that during vegetation period of winter barley and on the average of two depths of the soil profile, application of nitrogen-sulphur fertilizer Ammonium thiosulfate considerably reduced portion of nitrate nitrogen from the content of mineral nitrogen in the soil by 4-22%. The application of fertilizer Ammonium thiosulfate considerably increased content of ammonium nitrogen in the soil by 4-22%. A favourable effect on increase of ammonium nitrogen content and reduction of nitrate nitrogen content was found out in spite of the fact that in this treatment the total dose of fertilizer was applied at one shot.

**Keywords:** *ammonium cation, fertilization, inhibitory effect, nitrates, nitrification*

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## **Application of inversed size exclusion chromatography for the determination of pore size distribution in *Populus* sp. wood after explosion of supercritical carbon dioxide**

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*Supervisor:* dr. hab. inż. Tomasz Zielenkiewicz, dr. inż. Andrzej Radomski

The aim of this paper is to apply the innovative technique, which is inversed size exclusion chromatography, to study the differences of porous structure of *Poplar* sp. wood after explosion of supercritical carbon dioxide. Inversed size exclusion chromatography consist on the application of analyzed material as the stationary phase in the chromatographic column with the solution of the set of polymers of known molecular mass as the mobile phase during chromatographic experiment. The volume of pores with specified radius may be determined on the basis of retention times of different polymers. Developed porous structure is an advantage in case of the material application as a substrate for the enzymatic hydrolysis. The information about pore size distribution may be helpful for the proper choice of material for further processing.

Three kinds of poplar chips after supercritical carbon dioxide explosion were the analyzed material in this paper. The first sample was poplar chips of humidity in ambient conditions (8.5%), second was dried poplar chips (0%), and the last was poplar chips of saturated basis (moisture content above 30%). Results of changes in porosity were related to the material of 8.5% humidity which weren't subjected to the supercritical carbon dioxide explosion. Presented method of inversed size exclusion chromatography is relatively fast.

**Key words:** *ISEC's- inversed size exclusion chromatography, steam explosion, pulps, Poplar sp., supercritical carbon dioxide explosion*

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SECTION

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

## **Bioaccumulation of cadmium and its effect on total polyphenols content and antioxidant activity in selected variety of potatoes**

Miriama KOPERNICKÁ

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Cadmium is mainly accumulated in the environment by industrial activities. Soil factors such as content of organic matter, phosphate and zinc as well as pH affect Cd accumulation and its bioavailability. Its higher doses can lead to toxic effects and accumulates predominantly in kidneys, liver, bones, respiratory and digestive tracts. According to a WHO recommendation, the daily intake of cadmium should not exceed 1 µg per kg of body weight. The aim of this study was to determine total cadmium content and its accumulation in roots, aboveground biomass and potato tubers depending on different application of CdCl<sub>2</sub>·2.5 H<sub>2</sub>O and comparison of Cd effect on total polyphenol content and antioxidant activity of blue potato tubers Bergerac variety. The cadmium content in roots of blue potatoes of control treatment was 1.76 ± 0.68 mg.kg<sup>-1</sup>. In the second variant, after the application of 1 mg.kg<sup>-1</sup> CdCl<sub>2</sub>·2.5 H<sub>2</sub>O, cadmium content was 161.05 ± 26.47 mg.kg<sup>-1</sup>, that was about 91.51 times higher compared to the control treatment. The third variant after application of 5 mg.kg<sup>-1</sup> CdCl<sub>2</sub>·2.5 H<sub>2</sub>O, cadmium content was 675.76 ± 111.59 mg.kg<sup>-1</sup>, that was about 383.95 times higher compared to the control treatment and in the last fourth variant, after application of 25 mg.kg<sup>-1</sup> CdCl<sub>2</sub>·2.5 H<sub>2</sub>O, was 1775.11 ± 227.38 mg.kg<sup>-1</sup> of cadmium content, and it was up to 1,008.59 times higher compared to the control treatment. The cadmium content in the aboveground biomass of blue potatoes control treatment ranged from 1.16 ± 1.00 mg.kg<sup>-1</sup>. In a second variant was 11.01 ± 1.20 mg.kg<sup>-1</sup>, that as about 9.49 times higher compared to the control treatment. The third variant has cadmium content of 26.97 ± 5.14 mg.kg<sup>-1</sup>, that was about 23.25 times higher compared to the control treatment and in the last fourth variant was 50.20 ± 8.15 mg.kg<sup>-1</sup> of cadmium content, and it was about 43.27 times higher compared to the control treatment. The cadmium content in potato tubers of blue potatoes control treatment ranged from 3.68 ± 1.38 mg.kg<sup>-1</sup>. In a second variant was 6.83 ± 2.28 mg.kg<sup>-1</sup>, that was 1.86 times higher compared to the control treatment. The third variant has cadmium content of 29.90 ± 4.05 mg.kg<sup>-1</sup>, that was about 8.13 times higher compared to the control treatment and in the last fourth variant was 104.99 ± 21.53 mg.kg<sup>-1</sup> of cadmium content that was about 28.53 times higher compared to the control treatment. Statistically, we confirmed the effect of application in different doses of CdCl<sub>2</sub>·2.5 H<sub>2</sub>O to total polyphenols content and antioxidant activity too. The lowest determined content of total polyphenol (mg.kg<sup>-1</sup> dry matter) in potato tubers was measured in control treatment (417.63 ± 38.58 mg.kg<sup>-1</sup> dry matter) and the highest content of total polyphenols in was measured in a second variant (566.03 ± 182.23 mg.kg<sup>-1</sup> dry matter). In a third variant (519.78 ± 40.91 mg.kg<sup>-1</sup> dry matter) and in the last fourth variant (469.99 ± 89.39 mg.kg<sup>-1</sup> dry matter) the content of total polyphenols decreased. The values of antioxidant activity were in interval from 8.05 % to 9.42 %. The relationship between total polyphenols content and antioxidant activity in different variations was not confirmed. Environmental contamination by Cd and its impact to harvested agricultural production is very important regards to the protection of consumer's health.

**Key words:** *potatoes, cadmium, total polyphenols content, antioxidant activity*

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## **Antioxidant properties of cereal snacks with apple pomace**

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Apple is one of the most favored fruit of millions of people. Poland is a significant producer of apple, and is range on the 4th place in world production. During apple processing for consumption, by-products are also produced, the largest portion of which is pomace. The pomace is characterized by a high content of pro-health compounds like: vitamins, minerals, crude fibre or polyphenols. Polyphenols are the bioactive components in apple pomace with a high importance of health. They have many activities, like: antitumor activity, antithrombotic, anti-inflammatory, or antiviral activity. They can also reduce the risk of chronic diseases. Therefore the food enrichment by pro-health raw material full of polyphenols is very desirable. Cereal snacks could be an example of such incorporation. The aim of this research work was to study the influence of the amount of additive apple pomace on the antioxidant properties of cereal snacks. Five kinds of cereal snacks have been used for this experiment (with different addition). Two extractions types were used: 80% ethanol (EtOH) and double: 80% methanol (MetOH) with 70% acetone. The extraction was performed for 120 minutes. The following analyzes were performed:

- the content of phenolic acids, flavonols, anthocyanins using spectrophotometrical method, according to Mazza et al. (1999);
- total polyphenols by spectrophotometric method, according to Singleton et al. (1999);
- the content of flavonoids using spectrophotometrical method, according to El Hariri et al. (1991);
- the antioxidant activity with ABTS, according to Re et al. (1999).

It was found that with the addition of apple pomace to cereal snacks, the content of all measured bioactive compounds and antioxidant activity were increased. The highest increase was observed in phenolic acids, and the lowest for anthocyanins. Antioxidant activity was almost four times higher compare to control. It shows that apple pomace is good source of bioactive polyphenols, particularly in cereal snacks. But still needed more research.

**Key words:** *cereal snacks, apple pomace, antioxidant properties*

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## Comparison of fermentation processes in the production of ethanol from corn and wheat

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The basic raw materials for ethanol production are agricultural plant with a high content starch or other total sugars, which are processed by fermentation. The fermentation is the conversion of organic substances, mainly total sugars to less complex substance in the presence of enzymes as catalysts. The spectrum of components for the production of ethanol is of course largely depends on quantities of agricultural cultivation in a given geographical area. From the crops grown in our latitudes are the most commonly used of these cereals (wheat, triticale, corn); and potatoes. Wheat grains contain 58 to 70% of starch (depending on the variety) stored in the inner part of the endosperm, in the form of starch grains. Corn contains more than 60% starch which predetermines the use of fermentation.

The aim of our study was to observe the process of conversion of starch into total sugars in the fermentation mash. In the grain of corn and wheat we set the content of starch. Fermentation mash was important for make out the number of total sugars and yield. The samples were collected from the factory, which produces ethanol from agricultural raw materials, wheat and corn. The average starch content in corn was 63.7% and 61.5% from wheat. The determination of total sugars in the mash was carried out at intervals of 12, 24, 36, 48 hours from the beginning of fermentation and before distillation. The total time of fermentation of a corn and wheat was 70 hours. In both fermentation cases the yeast *Saccharomyces cerevisiae* was used. A sample of the mash was filtered through a fabric filter and the filtrate of a volume 0.0003 litre with distilled water was diluted to 0.1 litre. To the sample, the 5 % solution of phenol and sulfuric acid were added. The absorbance was measured spectrophotometrically at a wavelength of 500 nm against a blank.

The average value of the total sugars in samples of corn mash after 12 hour fermentation was 255.0 g.L<sup>-1</sup>, after 24 hour 162.1 g.L<sup>-1</sup>, after 36 hour 86.9 g.L<sup>-1</sup>, after 48 hour 35.7 g.L<sup>-1</sup>. After 70 hour of fermentation, the value of total sugars was 1.6 g.L<sup>-1</sup>. In wheat was a decrease from the value 177.4 g.L<sup>-1</sup> total sugars after 12 hour to 120.3 g.L<sup>-1</sup>, then after 36 hour from 86.9 g.L<sup>-1</sup> to 48.0 g.L<sup>-1</sup>. And the 28.7 g.L<sup>-1</sup> was total sugars in wheat before distillation. The yields of these two commodities were also different. For the production of 1 litre of absolute alcohol 2.56 kg of corn, but 2.66 kg of wheat were spent. From these results follow that the corn is more suitable raw material for ethanol production, because of its less consumption to achieve the same volume of ethanol. According to Gay-Lussac equation the calculate of theoretical yield of individual carbohydrates is possible. In practice, however, the theoretical yields aren't achieved, because during the production of ethanol another by-products are formed. Creation of these products is affected by many factors such as the type of applied yeast strain, technological parameters of the process and the raw material used as the main constituent of the fermentation medium.

Higher content of carbohydrates in the maize meal resulted in a better yield of corn, and therefore corn is more suitable raw material for producing ethanol, than wheat. In addition, corn is more economically preferable such as wheat. Both tests used same enzymes, fermentation condition, especially temperature and pH.

**Key words:** *fermentation, corn, wheat, ethanol, yield*

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## Utilisation of ozone to decrease microbiological load in walnuts and hazelnuts

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Walnuts and peanuts are very attractive substrates using in food processing industry. They are the ingredients of many final products, such as chocolates or subproducts like confectionery mass. They are also valuable in nutritional product for direct human consumption. In its composition there are a number of desirable fatty acids, proteins, dietary fiber, vitamin E, folic acid and magnesium. Qualities of nuts cause that the food producers and individual consumers are seeking for high-quality raw material. Very important parameter of raw material quality is a level of molds and the total number of microorganisms. These parameters are normalized and after exceeding the maximum level, the material is eliminated from the market. These excesses are observed very often so producers are searching for non-invasive methods which allow elimination of this problem. One of the simplest solution is thermal process of microorganisms inactivation. However, this method results in a loss of bioactive compounds which in elevated temperature are usually labile. The method that could be utilized to solve this problem is ozonation. Ozone is an allotropic form of oxygen consisting of triple atom molecules. It is a strong oxidant and has antiseptic properties. It is one of the most effective disinfectants. Its bactericide action is observed at a concentration as low as several  $\mu\text{g}/\text{dm}^3$ . It is successfully used for water disinfection, fumigation of production facilities and the removal of odorants. Furthermore, it is used in crop production and contributes to increase the shelf life of fruits such as highbush blueberry or raspberry (Balawejder et al., 2015). Ozone in gaseous form can penetrate only the surface of raw material therefore it has impact only on the microbial load present on the surface. It is killing microorganisms by disintegration of cell membrane. This is usually done by peroxidation of lipids constituting the membrane. After the penetration inside the cells, ozone can inhibit the action of cellular enzymes responsible for cellular respiration which can lead to its halting. Preliminary studies showed that the ozonation process can be successfully used to reduce the microbial load in walnuts and hazelnuts. Reduction of the registered molds and total number of microorganisms was confirmed using microbiological test. However, there is an issue concerning the susceptibility of fatty acids to oxidation. After the ozonation process a modification the smell of nuts was observed furthermore some of carbonyl compounds were identified. Those products are produced during the ozonolysis process from unsaturated fatty acids which are plenty of in nuts.

**Key words:** *ozone, walnuts, hazelnuts, microbiological load, decontamination*

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## The content of elements in *Melilotus albus* during plant development

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Supervisor: Małgorzata Dżugan<sup>1</sup>

White sweet clover (*Melilotus albus*) known as honey clover, belongs to *Fabaceae* family. It is an annual or biennial plant. In its natural environment grows in a habitat of roadsides and waste places. White sweet clover is adapted to a wide range soil but not to acidic soil. This plant is a major source of nectar, thus it is used to produce honey. In addition, due to the high protein content *Melilotus* is used as an animal feed. Unfortunately, honey clover because of the content of coumarin compounds must be used carefully, it can cause internal bleeding. There is little literature data about chemical composition and content of biologically active compounds of this plant.

The aim of this study was to determine the content of elements in white sweet clover plant during vegetation. The research material was the harvest and dried *Melilotus albus* plants, collected in three stages: (I) during budding, (II) at the beginning of flowering and (III) in the middle of flowering from experimental plots, where additionally different seed density was used (3 g/m<sup>2</sup>, 6 g/m<sup>2</sup>, 9 g/m<sup>2</sup>) during sowing. The content of heavy metals, micro- and macroelements using ICP-OES method with prior wet microwave was measured.

In all tested samples the content of heavy metals, such as lead, cadmium, chromium and arsenic was not detected. Element, which was present in the largest amount, especially in I and II stages was potassium (about 40.03 mg/g), its level was almost twice lower in last analysed stage. The decrease in the content of elements during plant development was observed in the case of calcium (from 13.92 to 10.28 mg/g), magnesium (3.21-2.16 mg/g), sulfur (3.41-2.25 mg/g) and phosphorus (3-2 mg/g). Nickel was determined only in III stages of vegetation in low concentration (0.001 mg/g), inversely as zinc which has been marked in the I and II stages only. Aluminum was measured in all samples, the value ranging between 0.031-0.045 mg/g. Other microelements elements (molybdenum, manganese, iron, copper) were detected in trace amounts. Additionally, differences in content of the elements, based on seed density used during sowing was observed.

In conclusion white sweet clover is the rich source of many elements, especially potassium and calcium. The content of individual elements changes during plant development, in most cases during vegetative cycle the decrease was observed, but the mechanism of these changes needs explanation.

**Key words:** white sweet clover, *Melilotus albus*, elements, ICP-OES

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## Bioactive compounds of buckwheat honey

Sylwia SWACHA

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Honey is a natural food product that is available across the world. Among natural products it is extensively used for various applications. The chemical composition and physical properties of honeys fluctuate based on the plants from which the raw materials were collected by bees. Moreover, differences in the type of flora, climatic conditions and geographical region also influence on mineral compounds and physicochemical properties. Buckwheat (genus *Fagopyrum*) is a significant minor crop (around 1.5 million tons production globally per year) with long-established uses in eastern Europe, China and Japan. Buckwheat honey is a dark, with strong aroma of buckwheat flowers and low viscosity. Compared with other honeys, dark honeys has higher antioxidant capacity, as well as flavonoid and phenolic content. There are over 150 polyphenolic compounds that have been identified in the honeys. These include phenolic acids, flavonoids (catechins) and flavonols. The important medicinal properties of buckwheat honey are connected with the presence of rutin – a unique component characteristic for this type of honey. Among the many advantages of rutin most frequently mentioned are purifying and strengthening the circulatory system.

The aim of this study was to investigate the content of macro- and microelements as well as antioxidant properties of buckwheat honeys. Material for this study were buckwheat honey samples (n=8) collected from beekeepers localized in Poland (Podkarpacie region) produced in the 2014. For the content of the elemental composition the ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) method using Thermo iCAP 6500 spectrophotometer (Thermo Fisher Scientific) was performed. The ferric reducing antioxidant power (FRAP) and Folin-Ciocalteu tests were applied to measure the antioxidant activity and total phenolic content, respectively.

Buckwheat honeys are a source of essential macronutrients, in average: potassium ( $662.84 \text{ mg.kg}^{-1}$ ), calcium ( $39.01 \text{ mg.kg}^{-1}$ ), magnesium ( $25.72 \text{ mg.kg}^{-1}$ ) as well as micronutrients: manganese ( $7.44 \text{ mg.g}^{-1}$ ), copper ( $0.75 \text{ mg.kg}^{-1}$ ), iron ( $0.26 \text{ mg.kg}^{-1}$ ), nickel ( $0.06 \text{ mg.kg}^{-1}$ ) and selenium ( $0.11 \text{ mg.kg}^{-1}$ ). Studies of antioxidant activity using FRAP test have shown that tested honeys has high antioxidant activity  $1715.47 \text{ mmol Trolox/100g}$ . Total phenolic content ranged of  $259.62$  to  $485.14 \text{ mg gallic acid equivalent per 100 g}$ . In conclusion, buckwheat honey from Podkarpacie region are rich source of antioxidants and high mineral content. All tested honeys are characterized by high quality according to low limits.

**Key words:** *honey, antioxidant activity, total phenolic content, ICP-OES*

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## Honey powder production by spray drying method

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From all honey produced in Poland, only 1% is used in the industry. Limited use of honey is related to its viscous consistency which hinders washing equipment as well as crystallization forcing its re-liquefaction. A perfect solution to this problem seems to be honey powder. Unfortunately, drying of honey is not an easy task. Honey, due to the high content of monosaccharides – at about 80%, exhibits a low glass transition temperature, which leads to the formation of the gummy structure, impeding drying. The use of carrier materials allows increase the glass transition temperature and then dry honey. Therefore, the production of honey powder having good functional properties, with simultaneous small addition of carriers and a small loss of biologically active compounds is still technical and technological challenge. The aim of this study was to obtain powders based on honey bees honeys by spray drying method and to examine physical properties and antioxidant activity of the resulting product. Material for the study consisted of different origin lime honey samples (n=3) and three different types of carriers: maltodextrin, whey protein concentrate (10 % protein content) and rennet whey. As a solvent, water and herbal extracts (nettle and mint) were used. A 30 % w/v honey solutions with addition of various carriers in different proportions were subjected to drying process using a laboratory spray-drying chamber DC1500 (China). The resulting powders were subjected to microscopic observation and biochemical testing: analysis of antioxidant activity and total content of phenolic compounds using FRAP and Follin-Ciocalteu tests, respectively. Powders with addition of maltodextrin as a carrier took the form of small crystals while powders with addition of whey protein concentrate and rennet whey were in the form of dust. The highest antioxidant activity measured by FRAP test showed powders with addition of nettle and mint extracts, 1983.69 and 2621.74 mmol Trolox.100g<sup>-1</sup> respectively. The rest of the tested samples had similar antioxidant activity (about 150 mmol Trolox.100g<sup>-1</sup>). The most rich in phenolic compounds turned out to be also powders with herb extracts addition 385.46 and 495.46 mg gallic acid equivalents.100g<sup>-1</sup> respectively. The level of phenolic compounds in the rest powder samples ranged from 43.05 to 100.46 mg gallic acid equivalents.100g<sup>-1</sup>. Physical properties of the obtained powders showed different crystal size depending on the carrier. Antioxidant activity of honey powders can be multiplied by addition of some herbs.

**Key words:** *honey, spray drying, antioxidant activity*

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## The risk of contamination of raspberries by cadmium in locations with environmental load

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The old mines, scrap-heaps, landfills, industrial and municipal wastes are important sources of the environment contamination by heavy metals. Metals entering the soil can be received by plants and entered to the food chain with the consequence for the human health. The aim of this study was to evaluate the risk of consumption of wild raspberries from Slovak regions (Upper Orava, Donovaly and Banská Štiavnica) with environmental loads, from the aspect of cadmium content. Samples of soil, fruits and leaves of raspberries were taken from individual sampling points (chosen by random search). In soil samples were determined agrochemical parameters such as active soil reaction pH/ H<sub>2</sub>O, content of oxidizable carbon (C<sub>OX</sub>, %), and percentage content of humus (Hum, %) calculated from C<sub>OX</sub> content. Pseudototal content of cadmium was determined in soil extract by *aqua regia* and content of mobile forms in soil extract by NH<sub>4</sub>NO<sub>3</sub> by flame AAS. The determined values were compared with limits given by European and National legislation. In fruit and leaves samples were determined total Cd content by flame AAS. The obtained results were expressed as mg/kg FM (fresh matter) and compared with hygienic limit given by the Food Codex of the Slovak Republic. Each analysis was done in 4 repetitions. Statistical processing of the results was carried out using software Statgraphics Centurion XVII. In soil samples the active soil reaction (pH/H<sub>2</sub>O) ranged from 3.04 (extremely strong acidity) to 4.85 (strong acidity). The % of humus in soil samples was in range 2.03 (middle humid soil) to 7.93 (very high humid soil). In all soil samples the pseudototal content of cadmium exceeded the limit value 0.70 mg.kg<sup>-1</sup> DM (dry matter) given by European and National legislation (2.55-3.85 mg.kg<sup>-1</sup> DM). Values of content of mobile cadmium forms determined in soil extracts by NH<sub>4</sub>NO<sub>3</sub> were in the range 0.09-0.42 mg.kg<sup>-1</sup> DM. In all samples from Upper Orava (0.16-0.30 mg.kg<sup>-1</sup> DM) and Banská Štiavnica (0.11-0.42 mg.kg<sup>-1</sup> DM) regions the determined content of cadmium mobile forms exceeded the limit (0.1 mg.kg<sup>-1</sup> DM). In all fruit samples the total content of cadmium exceeded the limit value 0.05 mg.kg<sup>-1</sup> FM given by Food Codex of the Slovak Republic. The samples were in range 0.11-0.19 mg.kg<sup>-1</sup> FM. In leaves samples the range for total Cd content was 0.92-2.35 mg.kg<sup>-1</sup> DM. The limit 1.00 mg.kg<sup>-1</sup> DM for Cd content in tea mixtures given by the Food Codex of the Slovak Republic was exceeded in all samples from Upper Orava (1.25-1.39 mg.kg<sup>-1</sup> DM) and all samples from Banská Štiavnica region (1.36-2.35 mg.kg<sup>-1</sup> DM). Eating wild raspberries from the burden regions of Slovakia may present a potential risk for the human health. It is necessary to monitor the soil content of hazardous elements because of food safety.

**Key words:** *berries, environment, pollution, cadmium, heavy metals*

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SECTION

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

## Using Chipron Meat 5.0 LCD Kit for identification meat species

Zuzana DRDOLOVÁ, Jozef GOLIAN

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

The detection of animal species in meat products is very important for consumer protection purposes and for detecting cases of counterfeiting. Counterfeiting meat products have broad societal impact in the economic sphere, in the exercise of religious practices in general and in influencing the health aspects of the consumer. Based on this fact, issues related to safety and quality in the meat products have brought up in that manner as well. Hygiene and right labelling notified on the label of any food stuff are very important criterias especially for public health. Food safety covers all the preventive measures for the delivery of food in healthy and hygienic conditions to the consumer. Inadequate management of food safety causes serious health problems. In meat and meat products the variety of animal species on the label should be inspected regularly. The regulations to protect the public health against adulteration and zoonoses strictly prohibit the inedible and lower quality meat either to be directly launched or to be processed in the food chain. In this study, we compared the results of the identification cooked meat products of the two brands. Selected brands come from two different countries. All the samples were examined for notification on the label and assessment of adulteration by DNA Microarray method at 4 C for sample preparation and DNA isolation. The pisces taken by means of lancet and spatula. Chipron LCD Array System can detect cattle, buffalo, pig, sheep, goat, horse, donkey, rabbit, hare, chicken, turkey, goose, and two duck varieties in food sample. The detection in this system is based on specific sites within 16S rRNA mitochondrial locus of all meat species in the analyzed food sample. A dark precipitate is formed by the enzyme substrate provided in the test kit, and it indicates a positive hybridization reaction. After staining procedure completed the chip was read with the scanner, and analysis was done by the software from the “Analysis-Package” provided by Chipron. Three different spots on the chip are called the control points (C) to detect a positive reaction which are located in upper-left, upper right and lower right corners, respectively. If no darker visualization occurs, the test should be repeated. When analyzing we kept the recommendations and the report drawn up by the producer. Eleven percent samples were not in accordance with the composition of the product by the manufacturer on the product packaging in the first set examined samples. Contamination in this set of samples accounted identified DNA *Gallus gallus* and *Sus scrofa*. In the second set examined samples 41% of products were not in accordance with the composition of the product mentioned on the product packaging. Contamination in this set of samples was identified DNA *Gallus gallus*, *Sus scrofa*, *Bos taurus*, *Anas platyrhynchos*, *Meleagris gallopavo*, *Bos bison*.

**Key words:** *identification, microarray, meat, falsification*

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