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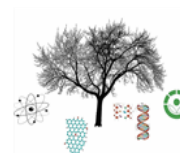
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ABSTRACT BOOK



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Invited Speaker

New Concept in Isolation and Determination of Biologically Active Compounds from Polish Plants

Bogusław Buszewski^{1,2}, Katarzyna Rafińska^{1,2}, Magdalena Ligor¹, Hossam Al-Suod^{1,2}, Olga Wrona³,
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Abstract

A rich source of biologically active compounds in human nutrition is alfalfa or lucerne (*Medicago sativa* L.). During various investigations it has been indicated that besides protein lucerne contains many secondary metabolites – compounds that are not directly involved in the normal plant growth and development. Among secondary metabolites identified in extracts obtained from alfalfa, the saponins and flavonoids are the most interesting and well characterized. The goldenrod (*Solidago* L.) is an herbaceous plant in the family *Asteraceae*, coming from North America, but well grows in Poland. Honey is obtained from this plant. It serves as a benefit for bees in late summer, when there is a lack of flowers. Goldenrod herb contains essential oils, triterpenoid saponins, diterpenoid acids, phenyl acids (phenolic diglycoside-leucocozoid), flavonoids (quercetin, kaempferol and isorhamnetin and their derivatives), and derivatives of caffeic acid, polysaccharides, carotenoids and other. Infusions of the goldenrod are primarily used in treatments as a diuretic. Moreover, phenyl acids and tannins from goldenrod, makeup with harmful metabolic products easily water-soluble complexes. Antiseptic and anti-inflammatory properties of this herb are caused by the presence of salicylates and phenyl acids.

The main aim of investigations was to develop methodologies for the extraction and determination of biologically active compounds from mentioned plants. Classical solvent extraction and modern methods such as supercritical fluid extraction (SFE), accelerated solvent extraction (ASE) and other allow obtaining extracts rich in biologically active compounds and characterized by a high antioxidant activity. The method of rapid assessment of the qualitative analysis of extracts by means of TLC, HPLC-MS were proposed. Moreover, the application of MALDI-TOF-MS for the screening analysis of main sugars in plant extracts has been discussed.

Keywords: *Medicago sativa* L., *Solidago* L., extraction methods, chromatography, mass spectrometry

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Invited Speaker

Chemistry, Culture-Forming Science and Technology: Are Ethical Guidelines Needed?

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Abstract

The creative power of chemistry, as a science and as a technology, has strongly contributed in enabling the lifestyle most enjoy today, not only those in the “developed” world. Impressive improvements in men’s living conditions have been achieved, such as greatly increased agricultural yields (e.g. through fixation of atmospheric nitrogen as fertilizers), safe food supplies for a continuously growing world’s population (e.g. through invention and synthesis of plant and crop protection agents), rising life expectancy worldwide (e.g. through chemotherapeutics), and more freedom in mobility (light materials for construction and energy storage) and information (e.g. liquid crystals for electronic displays). All this takes place at rates over-proportionally to global population growth, and in concert with the other scientific-technological and engineering disciplines.

Chemists know that, according to the second law of thermodynamics, every useful, beneficial effect is inextricably associated with entropic, dis-ordering effects. The enthalpic fraction of invested energy can only be maximized (and the entropic fraction in the form of accidents and pollution kept as low as possible) through optimization of each step in the flow of energy; this is similar for socio-economic systems. In this sense, ethical guidelines should be viewed as methodology for optimizing the overall benefit gleaned from the global investments in human energy.

Such optimization can be achieved by recognizing the needs and limitations of socio-technologic-economically interacting groups and their shareholders. The latter often have different priorities; if not considered and balanced, societal gains derived from scientific-technological advances become marginal or even negative. Exchange of opinions and perceived needs - among scientists/engineers, and between scientists/engineers and the public - is key transforming scientific knowledge into technological advances and ultimately into societal wealth. So, truthfulness in communication of research outcomes (in journals, as patents, or by other media) is of major importance for the well-being of a socio-political system. The interplay to find the best balance can, however, only function well under participatory-democratic conditions; ideological or group-egotistic constraints make modern scientific-technological systems inefficient and waste-producing.

Therefore, chemists – as other scientists - must understand their responsibility for the well-being of the socio-economic systems they live in. To be competent, besides proper scientific-technical training, education in applied ethics is a core need, especially in intellectual capabilities, for practicing fair cooperative relations. This requires a new concept of academic education in chemistry and science within an ethical-empathic, perhaps even spiritual, paradigm. In principle, modern information-technological possibilities (in particular the Internet) offer the practical means to do so.

Invited Speaker**Mass Spectrometric Analysis of Invasive Clinical Samples**

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Abstract

Jonas Bergquist, MD-PhD, is Full Chair Professor in Analytical Chemistry and Neurochemistry at the Department of Chemistry at Uppsala University, Sweden, Adjunct Professor in Pathology at the Department of Pathology, School of Medicine, University of Utah, USA, and Distinguished Professor in Precision Medicine, Binzhou Medical University, Yantai, China. Professor Bergquist's group is continuously developing general analytical tools for screening and discovery of biomarkers of pathological states. Technologies include all important links: identifying relevant clinical applications, invasive in-situ sampling of complex samples, advanced sample pretreatment, multidimensional liquid based separation, high resolution mass spectrometry, and multivariate data analysis. Professor Bergquist among other things focus to explore the neuroimmunological involvement by using proteomics and metabolomics in various disorders with a special interest in cerebrospinal fluid and hard-to-reach tissue studies. In this presentation the invasive sampling strategies will be exemplified.

Invited Speaker**Seeing the Unseen-Paving the Way for Understanding Separations**

Staffan Nilsson,

*Lund University***Abstract**

- [1]*Real-Time Fluorescence Imaging of Capillary Electrophoresis*. S. Nilsson, J. Johansson, M. Mecklenburg, S. Birnbaum, S. Svanberg, K.-G. Wahlund, K. Mosbach, A. Miyabayashi and P.-O. Larsson. *J. Capillary Electrophoresis*. 2. no. 1, 46 (1995)
- [2]*Real-Time Fluorescence Imaging of Isotachophoretic Preconcentration for Capillary Electrophoresis*. J. Johansson, D. T. Witte, M. Larsson and S. Nilsson. *Anal. Chem.* 68, 2766 (1996)
- [3]*Deactivation of Frits for use in Capillary High-performance Liquid Chromatography and Capillary Electrochromatography with characterization by Imaging with Laser-induced Fluorescence*. B. Behnke, J. Johansson, S. Zhang, E. Bayer and S. Nilsson. *J. Chromatogr. A*, 818, 257, 1998
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- [5]*Fluorescence Imaging of frit effects in Capillary Separations*. B. Behnke, J. Johansson, E. Bayer and S. Nilsson, *Electrophoresis* 21, 3102, (2000)

Invited Speaker

Application of Surface Analysis Techniques for Biomaterials

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Abstract

The importance of surface analysis is constantly increasing due to the enhancement of research topics dedicated to the nanotechnology and nanostructured materials for the use in the fields of microelectronics, anti-corrosive and anti-wear coatings, biomedicine, biotechnology, gas sensing, etc. Constantly shrinking dimensions of the devices and increasing role of the processes, taking place on the surface of the material, are defining the increasing demand for surface characterization of new materials. The present lecture is dedicated to the principles and applications of the mostly diffused techniques of surface analysis: electron spectroscopies XPS and AES.

X-ray Photoelectron Spectroscopy (XPS), also known as Electron Spectroscopy for Chemical Analysis (ESCA), is used to determine quantitative atomic composition and chemistry. Its sampling volume extends from the surface to a depth of approximately 5 -10 nm. Alternatively, XPS can be utilized for sputter depth profiling to characterize thin films and coatings by quantifying chemical species as a function of depth. XPS as an analytical technique is unique in providing chemical state information of the detected elements.

Auger Electron Spectroscopy (AES) is a surface-specific technique that utilizes a high-energy electron beam as an excitation source. Atoms excited by the electron beam can relax through the emission of Auger electrons with kinetic energies, which are characteristic of elements present at the sample surface. AES can be successfully employed for sputter depth profiling and for high-resolution chemical imaging of conductive materials.

Numerous examples of practical application of XPS and AES for the characterization of different materials (organic supramolecular assemblies, composite materials, biocompatible ceramic coatings, 2D carbon, enzymes, etc.) will be disclosed in this lecture.

Keywords: XPS, AES, surface analysis, biomaterials

Invited Speaker

Development of Antimicrobial Properties Nutraceuticals - Gummy Candies with Addition of Bovine Colostrum, Essential Oils and Probiotics

Elena Bartkiene¹, Modestas Ruzauskas¹, Vita Lele¹, Paulina Zavistanaviciute¹, Jurga Bernatoniene¹, Valdas Jakstas¹, Liudas Ivanauskas¹, Daiva Zadeike², Dovile Klupsaite², Pranas Viskelis³, Joana Bendoraitiene², Vesta Navikaite-Snipaitiene², Grazina Juodeikiene²

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Abstract

The aim of this study was to develop antimicrobial properties gummy candies based on bovine colostrum (BC), essential oils (EOs), lactic acid bacteria (LAB) strains, and their combinations. In addition, the heteropolysaccharide (agar), as a multifunctional polymer, was used for the antimicrobial candies preparation. The antimicrobial activities of BC, EOs (*C. reticulata* L., *Eugenia caryophyllata*, *C. paradisi* L., *Thymus vulgaris*), and LAB strains (*Lactobacillus plantarum* LUHS135 and *Lactobacillus paracasei* LUHS244), and their combinations against pathogenic bacteria (*Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus mutans*) were investigated. The highest antimicrobial activities were demonstrated by *Thymus vulgaris* and *Eugenia caryophyllata* EOs, and their emulsions (12%), and the best formulation of components for antimicrobial gummy candies production would incorporate the BC fermented with *L. paracasei* LUHS244 in combination with *Thymus vulgaris* or *Eugenia caryophyllata* EOs, which inhibited growth of all the tested pathogenic microorganisms (except *Pseudomonas aeruginosa*). Gummy candies formula consisting of the fermented BC (up to 3%) and thyme EO (up to 0.2%) with mandarin or grapefruit EOs (up to 0.2%) for taste-masking, allowed obtaining good texture and high overall acceptability products containing desirable antimicrobials, thus, antimicrobial gummy candies could be consumer preferred form of nutraceuticals.

Keywords: bovine colostrum, essential oil, probiotic, antimicrobial activity, gummy candy.

Acknowledgements: The authors are thankful to Lithuanian University of Health Sciences and Kaunas Technology University for providing the financial support to carry out this research work (LSMU-KTU joint project).

Invited Speaker

The Influence of Ultrasound Treatment, Fermentation with *Lactobacillus* Strains, and Different Methods of Dehydration on Bovine Colostrum Chemical Composition and Microbial Contamination

Elena Bartkiene¹, Vadims Bartkevics^{2,3}, Paulina Zavistanaviciute¹, Vita Lele¹, Modestas Ružauskas¹, Jurga Bernatoniene¹, Valdas Jakstas¹, Daiva Zadeike⁴, Pranas Viskelis⁵, Grazina Juodeikiene⁴

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Abstract

The aim of this study was to evaluate the influence of fermentation with *Lactobacillus plantarum* LUHS135 and *Lactobacillus paracasei* LUHS244 strains, different methods of dehydration, and ultrasound treatment on bovine colostrum (BC) chemical composition, including fatty (FA) and free amino acids (FAA) profile, and micro and macro elements. In addition, lactic acid bacteria (LAB) count, microbial contamination, and biogenic amines (BAs) formation in BC samples were analyzed. All the investigated treatments of BC reduced myristoleic (C14:1) (on average 25.5 %), alpha-linolenic (C18:3 n3) (on average 20.0 %), butyric (C4:0) (on average 17.9 %), caproic (C6:0) (on average 18.0 %), caprylic (C8:0) (on average 22.2 %), capric (C10:0) (on average 17.1 %), lauric (C12:0) (on average 18.0 %), myristic (C14:0) (on average 14.0 %), pentadecanoic (C15:0) (on average 11.1 %), and stearic (C18:0) acid content (on average 10.1 %), total saturated fats (on average 5.1 %), *trans* fats (on average 43.3 %), and omega-3 (on average 31.7 %). Results of the ANOVA test indicated, that the treatment method has significant influence ($p < 0.0001$) on the concentrations of FAA and most of the macro- and micro- elements in BC samples. The highest content of cadaverine, histamine, and tyramine in nontreated BC samples (28.62 ± 3.1 mg/kg, 15.75 ± 2.1 mg/kg, and 662.68 ± 12.4 mg/kg, respectively) was established. In all of the treated BC samples spore-forming aerobic mesophilic bacteria, enterobacteria, *Escherichia coli*, and fungi/yeast were not found, however, LAB count was ranging from 8.36 log₁₀ CFU/ml to 6.00 log₁₀ CFU/g. Thus, the ultrasound treatment, fermentation, and dehydration are perspective methods for BC treatment, in the case to reduce microbial contamination, however, the detailed chemical composition should be evaluated, especially BAs formation.

Keywords: bovine colostrum, chemical composition, microbial contamination, ultrasound treatment, fermentation, dehydration.

Acknowledgements. The authors are thankful to Lithuanian University of Health Sciences and Kaunas Technology University for providing the financial support to carry out this research work (LSMU-KTU joint project).

Invited Speaker**Cellular Hypoxia: Information for Revelation**

Arvydas Kanopka

*Vilnius University, Institute of Biotechnology**Corresponding author: arvydas.kanopka@bti.vu.lt***Abstract**

Cancer cells are often confronted with a significant reduction in oxygen availability, which is a major reason for a changeover of major cellular processes.

Hypoxic regions have been identified within all solid tumors and their presence has been linked to malignant progression, metastasis, resistance to therapy, and poor clinical outcomes following treatment. Cellular responses to hypoxia are mediated by hypoxia-inducible transcription factors (HIFs). This presentation will focus on currently available data on cell survival under reduced oxygen tension and speculation on biological relevance of cellular adaptation to hypoxic conditions.

Keywords: Cancer, Hypoxia, HIF.

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Invited Speaker

Cell Lines Possess Differential Splicing Factor Expression and Tumor-Associated mRNA Isoform Formation Profiles

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Abstract

Cell lines derived from human tumors have been extensively used as experimental models of neoplastic disease. Although such cell lines differ from both normal and cancerous tissue. The data obtained used DNA and RNA microarray systems do not give full information about protein expression levels in cells and tissues. We present experimental evidence that splicing factor SRSF1, SRSF2, U2AF35, U2AF65 and KHSRP expression levels in gastrointestinal tract (colon, gastric and pancreatic) tumors differ compare to healthy tissues and in cell lines, derived from corresponding organs.

Obtained results provided a novel molecular characterization of this important group of human cell lines and their relationships to tumors *in vivo*. Expression levels of individual splicing factors in tumors might serve as tumor markers. Not all experimental results obtained from cell lines reflect changes that occur in tumors. Also, Fas and Rac, cancer-associated genes, tumor-associated sFas and Rac1b mRNA isoform profiles in cell lines do not correspond to profiles that are observed in tumors.

In conclusion, our results showed that not all experimental results obtained in cell lines reflect changes that occur in real tumors.

Keywords: Tumor, splicing factor, Fas, Rac, mRNA, cell lines, SR proteins.

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Oral presentation

Lactic acid bacteria as natural neutralization agents of mycotoxins

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Abstract

The *Fusarium* family is an important cereal pathogen, because of its ability to produce toxic secondary metabolites (mycotoxins) such as zearalenone. After infection, mycotoxins can accumulate into cereal plant, resulting in contamination of animal feed and human cereal food. Zearalenone is mostly present in corn, but it can be also found in other important crops such as wheat, barley, sorghum, and rye. Chemically, ZEA is a resorcylic acid lactone and has structural similarity to the natural estrogens – it can mimic endogenous estrogens, change their mechanism of synthesis and metabolism, which contributes to change and neoplastic i.e. breast cancer. There are a few methods which have been developed to control the occurrence of mycotoxins such as physical and chemical approaches, but they are non-efficient and contribute to changes in the value of food products and the occurrence of toxic substances. Recently, microbiological methods have received much attention; they have been found to be safer and more effective. One of the most promising organisms able to ZEA neutralization seem to be lactic acid bacteria (LAB), which are widely used for the production of fermented foods, are part of intestinal microflora and have beneficial health effects in humans. In our study, a novel approach of ZEA neutralization by *Lactococcus lactis* and *Bifidobacterium* sp. is investigated. The process was confirmed by identification of binding kinetics and spectroscopic studies such as FTIR spectroscopy and MALDI-TOF-MS spectrometry. According to the obtained results, bacterial strains isolated from milk products have the ability to adsorb and neutralize the zearalenone, but biosorption processes for them are not the same. The kinetic process of zearalenone binding to *L. lactis* in comparison with *Bifidobacterium* sp. cells is not homogeneous but expressed with two main stages. The first one is quite rapid and consists of most of the zearalenone biosorption (about 90%). The second stage is much slower and corresponds to the diffusion of ZEA into bacterial cells. FTIR analysis showed that in immobilization of ZEA by LAB deprotonated carboxyl groups of bacterial proteins and peptidoglycan are mainly involved. Moreover, the assessment of dead and live lactic acid bacteria cells after zearalenone treatment was performed using fluorescence microscopy.

Keywords: zearalenone, toxicity, neutralization, lactic acid bacteria.

Acknowledgements: This work was supported by Maestro-6, No. 2014/14/A/ST4/00641 (2015-2017) and Opus 11 No. 2016/21/B/ST4/02130 (2017- 2020) from the National Science Centre, Poland.

Oral presentation

Chemical and Biological Insights on *Lepidium sativum* Extracts Obtained by Modern and Conventional Extraction Techniques

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Abstract

Lepidium sativum (garden cress) is an annual, herbaceous edible plant belonging to the cruciferous family. Many sources indicate that *L. sativum* exhibits hypoglycemic, anti-inflammatory and antidiarrheal activities. Despite the many valuable properties, both chemical composition and biological activity of *Lepidium sativum* extracts are so far poorly known.

Isolation of bioactive compounds from the plant is usually performed by using toxic organic solvents such as methanol, hexane or acetone. This approach has numerous drawbacks which are primarily related to the negative impact on the environment. Nowadays, the efforts have been directed to the development of environmentally friendly technologies and thus many studies focus on the development and application of green technologies. In our studies, we used three different solvents considered as non-toxic or at a low level of toxicity such as water, carbon dioxide and ethanol.

Biologically active compounds were isolated from garden cress seeds and sprouts using conventional as well as non-conventional techniques such as maceration (M), ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE). For biological activities, antioxidant and antimicrobial properties were evaluated. Moreover, enzyme inhibitory effects of obtained extracts were studied. Antimicrobial activity was measured by using well-diffusion method and determination of minimal inhibitory concentration (MIC) against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Escherichia coli*. For chemical characterization, the total content of phenolic and flavonoids was analyzed by spectrophotometric methods. Antioxidant capacity was measured by using DPPH and ABTS as a free-radicals, while for evaluation of reducing potential ferric reducing antioxidant power (FRAP) methodology was applied.

Comparison of used extraction techniques and solvents showed that ethanol was generally more effective solvent than carbon dioxide and water. On the other hand maceration with water was an especially favorable technique for extraction of phenolic compounds, while extracts from SFE were especially rich in flavonoids. Despite the low efficiency of SFE, extracts obtained with this technique exhibited significant antimicrobial effect against tested strains of bacteria. For *P. aeruginosa* and *P. mirabilis*, minimal inhibitory concentration was 0.5 mg/mL.

Keywords: *Lepidium sativum*, extraction, antioxidant potential, antibacterial activity, enzyme inhibition.

Acknowledgements: This study was supported by PLANTARUM project No. BIOSTRATEG2/298205/9/NCBR/2016 from National Centre for Research and Development, Poland.

Oral Presentation

Development of experimental set up for characterization of plasmonic structures

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Abstract

Plasmonic structures are commonly used in areas such as nanophotolithography, biosensing, nanophotonic devices and electronic circuits. These structures, used as sensors, allow measuring of changes in geometrical parameters such as angles and distances. Such structures are widely used in biotechnology for measurement of the concentration of reaction products, reaction speeds, etc. Plasmonic structures greatly improve the effectiveness of semiconductor light emitting devices. Although at the same time plasmonic sensors are a new technology in some areas like radiotherapy and medical diagnostics. Same can be said about specific applications in biotechnology.

Plasmonic sensors, in general, are considered small in dimensions (surface area smaller than 1mm^2) compared to conventional biotech measurement systems. Such sensors are stable, sensitive to minute changes in the measured quantity. One of the methods of devising plasmonic sensors is the use of metal diffraction gratings. By adding a layer of the sensing material, a whole sensor structure is formed. Because of the economic reasons these types of sensors are attractive for routine measurements where stability is paramount as well as research where high resolution is key. Although to “read-out” such detectors a special measurement system is always required, which not only adds to the cost of the whole tandem but also is a source of additional errors of measurements.

All of the plasmonic sensor readout systems must have high spectral and angular resolutions for an accurate and precise read-out. In this r a surface plasmon-based sensor read-out system was developed with the previously mentioned characteristics. To read-out the CCD matrix at different light incident angles a mechanical modulator capable of rotating the plasmonic structure with an angular resolution of 0.1° was developed. Since the light source for inducing surface plasmon resonance have to be polarized, a HeNe laser was used as a source of light for the experiments.

In this research, a sensor with gold diffraction grating was chosen to test a newly devised surface plasmon resonance observation system. Results obtained using a clean gold diffraction grating showed good agreement with recently published works of other researchers.

Keywords: Surface plasmon resonance, optical measurements, biosensors, measurement systems

Oral Presentation

How to Get Perspective Hypotheses in Multidisciplinary Research Work: Phytochemicals, Antivirals and Data Mining

Tomas Drevinskas¹, Rūta Mickienė¹, Audrius Maruška¹, Mantas Stankevičius¹, Nicola Tiso¹, Algirdas Šalomska², Raimundas Lelešius², Agneta Karpovaitė², Ona Ragažinskienė³

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Abstract

Highly complex research work requires high level expertise, which can lead to the development of perspective hypotheses that can be investigated. In this work methodology of determining perspective compound classes in different medicinal plant extracts is described. The methodology covers virology, phyto-chemistry, chemical analysis and data mining. Issues, findings and perspectives will be presented during the conference.

Keywords: Phenolic compounds, Capillary electrophoresis, Contactless conductivity detection, Gas chromatography, Infectious bronchitis virus, Radical scavenging activity, Medicinal Plants, Methodology.

Acknowledgements. The research was granted by Research Council of Lithuania, project No. MIP-065/2015.

Oral presentation

Tools and methods for the investigation on filamentous fungi interactions

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Abstract

Interactions between species shapes the ecosystems. In their natural environment, fungi closely interact with other organisms. Despite their importance, fungus-fungus interactions are still scarcely investigated.

Intra- as well as inter-specific fungal interactions may be mediated upon contact or even at a distance. The functions and specificity of these interactions are not well understood and their mechanisms are largely unknown. Understanding these basic mechanisms is essential to improve the applicability of fungi for further usage.

Simple efficient methods for the investigation on filamentous fungal interaction are proposed. They include the macroscopic and microscopic observation of the morphological changes induced by the interactions, the biomass development evaluation and the instrumental analysis of the extracellular enzymes, volatile and diffusible secondary metabolites produced during the interactions.

A particular focus is given to filamentous fungi and the screening and selection of consortia composed by two or more interacting strains, usable for enhancing specific efficiency for practical applications.

Keywords: Fungal interaction, filamentous fungi, fungal consortia, volatile organic compounds, basidiomycetes.

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Invited Speaker

Cyclitols-biologically important components

Ryszard Gorecki

The University of Warmia and Mazury in Olsztyn

Oral presentation

Development and application of instrumental analysis: current projects

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Abstract

In this report recent and present research projects carried out in the Instrumental Analysis Open Access Centre of Vytautas Magnus University will be presented. Presently 8 doctors of sciences, two of them holding habilitated doctor degree, numerous PhD, master and bachelor students are involved in the activities of the center. The research personnel is not limited with the researchers from one university and one country.

All the research activities at the Open Access Centre can be classified as fundamental and applied research. Fundamental research covers development of novel analytical and separation methods, synthesis of selective and efficient materials, design and testing of new apparatus. Trends in instrumental analysis, development of miniaturized, integrated analytical methods and wirelessly operated tools will be reviewed. Applied research includes bioremediation of pollutants, downstream biotechnology and analytical methods application for revealing of quantitative and quantitative composition of the biological samples, industrial raw materials and products, toxic chemicals etc. Projects related to the increase of food safety using natural additives and biotechnologies or increase of quality of medicinal plant raw material, searching of anticancer and antiviral phytochemicals will be reported.

The importance of the interdisciplinary and multidisciplinary cooperation will be pointed out based on the research experience, completed and ongoing projects.

Keywords: instrumental analysis, miniaturization, integration of methods

POSTER PRESENTATIONS

Poster presentation

The Impact of Extraction Method on Bee Pollen Antioxidant Activity

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Abstract

Pollen is one of the most appreciated natural products collected by bees. In our days it is increasingly thought that in the near future bee pollen could not only be used just as a food additive but as one of the most important components of medications. Well known medical and nutritional properties of bee pollen are determined by the considerable content of phenolic compounds, mainly flavonoids [1]. The presence of these compounds is considered as the main reason for bee pollen antioxidant properties. The aim of this research was to determine the effect of extraction method of bee pollen on its antioxidant activity. Lithuanian pollen was used for the extraction of flavonoids with three different solvents: methanol, water and methanol:acetonitrile mixture (50:50 (v/v)), using ultrasound-assisted or only solvent extraction techniques at room and 40°C temperatures. Also, macromolecular compounds, such as macrogol, were used for extraction using the same conditions. Extracts made with macrogol may be used for the preparation of eyes drugs and would be safe for children [2]. Total phenolic compounds content, total flavonoid content and antiradical activity of the extracts was evaluated by spectrophotometric methods [3]. The total content of phenolic compounds was measured using Folin–Ciocalteu reagent. The flavonoid content analysis was carried out by colorimetric reaction with aluminum chloride. Antiradical activity was characterized by the total radical scavenging activity which was measured using 2,2–diphenyl–1–picrylhydrazyl (DPPH) free radical. The results will be presented during the conference.

Keywords: bee pollen, extracts, antioxidant activity, phenolic compounds, flavonoids.

Acknowledgements: Financial support from Research Council of Lithuania project 09.3.3-LMT-K-712-03-0127 is acknowledged.

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Poster presentation

Identification and Evaluation of Antibacterial Agents Produced by Bacteria in Dairy Products

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Abstract

Many bacteria with a wide range of antibacterial activity are promising microorganisms that can be used extensively, not just in the food industry. Some lactic acid bacteria release bacteriocin(s) - proteinaceous Substances that may inhibit the growth of similar or closely related bacteria. Bacteriocins are widely investigated to evaluate their use as narrow-spectrum antibiotics. Bacteriocins are of interest in medicine, food industry and marine environment because they are made by non-pathogenic bacteria that normally colonize the human body.

The aim of this work is to find bacteria in dairy products, in which they release different bacteriocins during their growth, to identify them, to isolate and evaluate the resulting antibacterial activity. Plates with MRS medium and inoculant were incubated at 37 °C for 48-72 h and all isolates were further purified by streak planting and preliminarily identified based on their staining by Gram [1]. The antibacterial activity of bacteria isolates was evaluated, in order to select bacteriocin(s) producing isolates. Detailed results of the work will be presented during the conference.

Keywords: lactic acid bacteria, bacteriocin, antibacterial activity, dairy products.

Acknowledgements: Financial support from Research Council of Lithuania project 09.3.3-LMT-K-712-03-0131 is acknowledged.

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Poster presentation

Hemagglutinin from *Echinacea purpurea* L. root separation and identification

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Abstract

Lectins or hemagglutinins are non-immune origin glycoproteins, which can bind carbohydrate structures in specific and reversible manner. They have big potential for their therapeutical applications such as immunomodulatory, anticancer antibacterial and other activities. Hemagglutinins from *Echinacea purpurea* L. (Moench) roots haven't been investigated.

The aim of the experiment: To purify and identify hemagglutinins from *Echinacea purpurea* L. (Moench) roots.

Experiment tasks: 1. Purify hemagglutinins from purple coneflower roots; 2. Identify hemagglutinating glycoproteins.

Materials and methods: 1. Affinity column with immobilized D-(+)-mannose ligands was equilibrated and unbound proteins were washed out with phosphate buffer saline (PBS) pH 7.4. Hemagglutinins were eluted out of the column with 0.2 M lactose solution in PBS. Following hemagglutinin fraction was collected and checked for hemagglutinating activity. 2. Hemagglutinating active fraction was separated by polyacrylamide gel electrophoresis and immunoblotted for glycosylated proteins. 3. Glycosylated protein bands were identified by liquid chromatography tandem mass spectrometry by sunflower genome database search.

Results: 1. Hemagglutinating active proteins from purple coneflower root were separated from non-active proteins and collected into one fraction. 2. Lysin motive (LysM) peptidoglycan binding domain was identified after database search with identity score equal 182.0.

Conclusions: 1. Affinity chromatography method was suitable for separation of hemagglutinating active proteins from purple coneflower root crude protein extract. 2. Identified glycoprotein – LysM domain was responsible for the hemagglutinating activity.

Keywords: *Echinacea purpurea* L., purple coneflower, hemagglutinins, lectins, LysM domain.

Poster presentation

The Association between Rs1800625 and Age-Related Macular Degeneration

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Abstract

Introduction: Age-related macular degeneration (AMD) is the leading cause of blindness in people aged 65 and older in developed countries [1]. The pathogenesis of AMD has been linked to mechanisms involving inflammation, oxidative stress and basal laminar deposit formation between retinal pigment epithelium (RPE) cells and basal membrane caused by advanced glycation end (AGE) products. The AGEs are implicated in the pathogenesis of AMD through the AGE and receptor for AGE (RAGE) interaction, which can be altered by polymorphisms of RAGE gene [2]. The aim of this work was to examine rs1800625 variant in *RAGE* gene contributing to AMD development.

Methods: The study enrolled 300 patients with early AMD, 300 patients with exudative AMD and 800 healthy control subjects. DNA was extracted from white blood cells using the silica-based membrane technology utilizing a genomic DNA extraction kit (GeneJET Genomic DNA Purification Kit, Thermo Scientific), according to the manufacturer's recommendations. The genotyping was carried out using the RT-PCR method. Statistical analysis was performed using the SPSS / W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA).

Results: The statistical analysis revealed that rs1800625 allele G at rs1800625 is associated with 1.5-fold increased risk for exudative AMD after adjustment for age (OR=1.545; 95% CI: 1.003-2.379; p=0.048). Results suggest that allele G at rs1800625 is a risk-allele for exudative AMD development.

Conclusion: We revealed a significant association between rs1800625 polymorphism and AMD risk. We considered allele G at rs1800625 to be as a marker of poor prognosis in AMD development.

Keywords: rs1800625, age-related macular degeneration, polymorphism.

Acknowledgements: This work was funded by a grant (No. SEN-11/2015) from the Research Council of Lithuania.

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Poster presentation

Studies in Development of Formulations Containing Coniferous Greenery Products and Aloe Extract

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Abstract

Pine needles thick extract and spruce needles sodium chlorophyllin – products from coniferous greenery – promote gastrointestinal mucosal damage restoration processes, stimulate the body's defenses, and possess regenerative, antioxidant, antimutagenic properties. Aloe leaf mesophyll dry extract (aloe extract) can be combined with coniferous greenery products to enhance their effects.

The aim was to investigate the possibility of producing formulations containing pine needles thick extract or spruce needles sodium chlorophyllin, and aloe extract.

Six compositions were prepared from pine needles thick extract (moisture content about 40%) or spruce needles sodium chlorophyllin (moisture content about 60%) and aloe extract, with the addition of emulsifier (glycerol monooleate), water and ethanol 50% (V/V). These compositions intended for filling in aluminum tubes as an oral dosage form. The mixture of pine needles thick extract, aloe extract and glycerol monooleate produces uniform mass. Preconditioning of the aloe extract using water or ethanol gives no significant differences in homogeneity.

Possibility to get dry spruce needles sodium chlorophyllin and its mixtures with aloe extract and excipients have been studied.

Spruce needles sodium chlorophyllin was dried at 30°C, 60°C and 90°C temperature to a moisture content of not more than 10%. It was found that the drying regime and addition of aloe extract does not affect the chlorophyll content in the preparation. But the addition of excipients: lactose, calcium carbonate, potato starch or calcium lactate significantly decreases the chlorophyll content. Dried spruce needles sodium chlorophyllin and aloe extract mixture 4:1 was prepared and filled into hard gelatin capsules.

Conclusions. Formulations containing coniferous greenery products and aloe extract can be prepared by mixing with an emulsifying agent and filled in aluminum tubes. The mixture of dried spruce needles sodium chlorophyllin and aloe extract can be filled in hard gelatin capsules.

Keywords: pine needles thick extract, spruce needles chlorophyllin, aloe leaf mesophyll extract, formulations, dosage forms.

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Poster presentation

Exudative Age-related Macular Degeneration Association with MMP-2 (-1306 C/T) Rs243865 Gene Polymorphism

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Abstract

Introduction: Age-related macular degeneration (AMD) is a disease of the macula, which significantly affects the eyesight and leads to irreversible central vision loss [1]. Recent studies have demonstrated that angiogenesis is the most important mechanism of AMD development and is associated with important extracellular remodeling involving different proteolytic systems, among which matrix metalloproteinases play an essential role the etiopathogenesis of AMD still remains unclear [2]. The main objective of the present study was to determine the relation between the exudative AMD and MMP-2 (-1306 C/T) rs243865 polymorphism.

Methods: The study included 267 patients with exudative age-related macular degeneration and 318 healthy control subjects. DNA was obtained from peripheral venous blood leukocytes using commercial kits. Genotyping of MMP-2 (-1306 C/T) rs243865 was executed using real-time polymerase chain reaction method.

Results: The analysis of MMP-2 (-1306 C/T) polymorphism did not reveal any differences in the distribution of CC, CT, and TT genotypes between the exudative AMD and control groups: 58.8%, 31.5 % and 9.7 % vs. 59.75%, 33.96% and 6.29%, respectively, $p=0.287$). MMP-2 (-1306 C/T) rs243865 CT genotype showed 5.7-fold increased the risk of exudative AMD development compared to CC and TT genotypes together in younger (<65 years) males group ($p=0.05$).

Conclusion: MMP-2 (-1306 C/T) polymorphism is associated with exudative AMD development in younger males.

Keywords: exudative age-related macular degeneration, matrix metalloproteinases, gene polymorphism.

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Poster presentation

Free radical reactions mechanism and kinetics by use of synthetic DPPH•

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Abstract

In the recent time, the influence of free radicals on human organism and their significant role in the development of civilization diseases has been considered. In limitation of the free radical activity, some antioxidants are very helpful, which providing free electrons for free radicals and deprive them of their activity. Antioxidants are found in fruits, vegetables, herbs, tea, coffee and others. The most common are vitamin C, E and A.

The ability to suppressing of free radicals is important to exam the antioxidants properties. A method commonly used for this purpose the well-known reagent DPPH• (2,2-diphenyl-1-picrylhydrazyl) is used. It has unpaired electron on the nitrogen. DPPH• belongs to the stable free radical group. It creates a stable cation radical, whose solution is violet color, and the maximum of absorbance. During the reaction, a hydrogen atom that can be devoted to DPPH• creates a form of reduced. His violet colored then fades and the decrease in absorbance is proportional to the remaining in the solution a number of oxidized forms of DPPH•. Free radicals in terms of properties and causes have been described. In particular, properties of synthetic free radical DPPH• have been specified in detail.

The main aim of investigations was to determine the antioxidant capacity of vitamin C and vitamin E derivative - Trolox and tocopherol. Measurements have been done to check the kinetics of the reaction between free radical DPPH• and used antioxidants. The antioxidant activity of the aforementioned substances was determined by means of spectrophotometry method with a free DPPH• radical by measuring absorbance at wavelength $\lambda = 517$ nm. As results of performed method, it was confirmed that the free radical DPPH• reacted most intensively with the antioxidant tested up at time period 3 - 15 minutes and after that, the reaction was often reversible. It is also significant that in this method the concentration of the test substance was important for the course of the reaction velocity. Tested antioxidants generally showed good antioxidant properties, the most effective was tocopherol.

Keywords: antioxidants, synthetic free radical DPPH•, spectrophotometry

Acknowledgements: This work was financed in the framework of grant entitled: Cultivated plants and natural products as a source of biologically active substances assign to the production of cosmetic and pharmaceutical products as well as diet supplements" (nr BIOSTRATEG2/298205/9/NCBR/2016) attributed by the National Center for Research and Development (Warsaw, Poland).

Poster presentation

Development and Integration of Miniaturized Capillary Electrophoresis Equipment into Unmanned Aerial Vehicle for Analysis of Complex Mixtures (DRONOMOUS)

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Abstract

In recent years, autonomous chemical analysis devices for environmental research have become increasingly popular. Integration of capillary zone electrophoresis (CZE) equipment into unmanned aerial vehicle have never been done. Successful integration of CZE will open up new opportunities for ecology and chemical analysis research. Analysis system consists of several modules. One of the modules is extraction system of chemical compounds from air samples. The extraction system is based on gas – liquid extraction method. Another module is miniaturized capillary zone electrophoresis system with programmable sample and electrolyte tray. Detector type – contactless conductivity detector. LaRa – R2 LTE 3G module (or similar) can be used for long range (up to 300 meters) wireless data transmission system. All non – electronic plastic parts are designed using 3D printer. Miniaturized capillary electrophoresis equipment allows to conduct not only simple air composition analysis special air quality monitoring but also can be used in more advance air pollution research. The aim of this project is to design, manufacture and optimize capillary zone electrophoresis equipment for airborne chemical analysis. Integration of miniaturized capillary electrophoresis equipment into Unmanned Aerial Vehicle allows more flexibility in conducting airborne analysis, especially where environmental conditions could be dangerous for human health.

Keywords: Capillary zone electrophoresis, Air analysis, Unmanned aerial vehicle, Contactless conductivity detection

Acknowledgements: The research was granted by Research Council of Lithuania, project No. 09.3.3-LMT-K-712-03-0130.

Poster presentation

LIPC rs10468017 polymorphism association with age-related macular degeneration and macular pigment optical density

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Abstract

Introduction: Age-related macular degeneration (AMD) is a degenerative disease affecting central part of the retina, usually occurring among persons older than 50 years of age. It is characterized by the retinal pigment epithelial changes as early features. In drusen lipids represent at least 40% of the volume because of that attention is dedicated to the genes responsible for lipid metabolism in AMD pathogenetic mechanisms.

Purpose: To determine the association of LIPC rs10468017 involved in lipid metabolism and MPOD with early AMD.

Materials and Methods: The study included 279 patients with early (mild and moderate) AMD and 829 healthy persons and macular pigment optical density (MPOD) testing was performed for 46 of patients, and for 101 of healthy persons. DNA isolated from venous blood leukocytes. The genotyping was carried out using the real-time polymerase chain reaction (RT-PCR) method. MPOD obtained in the 30° fundus images, by MPOD mode. Used Visucam 500 module (Carl Zeiss Meditec). Statistical analysis was performed using the computer program "IBM SPSS Statistics 20.0"

Results and discussion: Statistical analysis revealed that LIPC rs10468017 CT genotype is associated with 1.6-fold decreased risk of early AMD development compared with CC genotype (OR=0.624; 95 % CI: 0.462-0.842; p=0.002) and 1.5-fold decreased risk compared to CC and TT genotypes (OR=0.649; 95 % CI: 0.484-0.870; p=0.004). Also, CC and TT genotypes are associated with 1.5-fold decreased risk of early AMD (OR=0.651; 95 % CI: 0.494-0.859; p=0.002) and each copy of allele T is associated with 1.3-fold decreased risk of early AMD (OR=0.764; 95 % CI: 0.617-0.947; p=0.014) as well. Also, we found that patients with early AMD had statistically significantly lower MPOD levels in the right eye than healthy people (p<0.001). The same results were found in the left eye. Also, a woman with early AMD had statistically significantly lower MPOD levels in both right and left eyes than healthy women (p<0.001) while a man with early AMD had statistically significantly lower MPOD levels than healthy men only in left eye (p<0.001). Statistically significantly lower MPOD levels were observed in patients with early AMD in LIPC rs10468017 CC and CT genotype groups than in healthy persons with LIPC rs10468017 CC and CT genotypes, respectively. Summarizing research done by scientists, there is observed a close link between the genes that are involved in lipid metabolism and their polymorphisms, such as LIPC rs10468017, and the occurrence of AMD.

Conclusions: We found that LIPC gene rs10468017 polymorphism is associated with decreased risk of early AMD possibility and patients with early AMD had statistically significantly lower MPOD levels than the control group.

Keywords: Age-related macular degeneration, macular pigment optical density.

Poster presentation

Association of CYP4F2 rs1558139 gene polymorphism with age-related macular degeneration

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Abstract

Background: Age-related macular degeneration (AMD) is multifactorial neurodegenerative disease characterized by progressive degeneration of photoreceptors in the macular region of the retina and resulting in irreversible central vision loss [1].

The aim of the study was to investigate the association of CYP4F2 rs1558139 with the development of early and exudative AMD.

Materials and methods: A total of 204 patients with early AMD, 204 with exudative AMD and 294 healthy controls. The genotyping of rs1558139 was carried out using the real-time polymerase chain reaction method.

Results: The A/A genotype of CYP4F2 rs1558139 was more frequently observed in women with exudative AMD than men (31.9% vs. 17.3%, $P < 0.001$), and the A/A genotype was more frequently found in women with exudative AMD aged ≥ 65 years than in men with early AMD aged < 65 years (31.2% vs. 8%, $P = 0.0228$, respectively). The G/A genotype under the overdominant model showed a 2.8-fold greater risk of early AMD in the male group aged < 65 years (OR = 2.807, 95% CI = 1.034–7.619; $P = 0.043$).

Conclusions: The CYP4F2 rs1558139 polymorphism was not found to be associated with early and exudative AMD in the overall study population.

Keywords: Age-related macular degeneration, rs1558139, gene polymorphism.

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Poster presentation

Development of lipid nano carriers containing soybean oil for dihydroquercetin delivery into human skin

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Abstract

Dihydroquercetin is a perspective flavonoid compound for treating environment-associated skin conditions. However, dihydroquercetin is poorly soluble in water (< 0.1 %) which brings a lot of challenges in the formulation of topically applied products. Incorporating dihydroquercetin in nano carriers is one of the strategies to improve the solubility. Moreover, our previous study demonstrated that soybean oil can enhance the skin penetration of dihydroquercetin [1]. In the current study, experimental design approach was used to formulate nano carriers containing 1 % of dihydroquercetin and soybean oil as skin penetration enhancer. The ranges of selected compounds were: purified phosphatidyl choline (Lipoid S100, Lipoid GmbH) 500 – 2000 mg, Tween 80 15 – 200 mg, soybean oil 25 – 300 mg. All compounds were mixed with 100 mg dihydroquercetin, hydrated with 10 ml of 3-10 % ethanol and stirred overnight, followed by 20 sonication cycles for 30 s. The optimal formulation was obtained based on particle size, polydispersity index and encapsulation efficiency with desirability index of 0.8. Final formulation contained 13.26 % phospholipids, 1.71 % Tween 80, 0.60 % soybean oil, 1 % dihydroquercetin and hydrated with 4.38 % ethanol. Particle size was 60.738 nm, polydispersity index 0.21 and 80.7 % encapsulation efficiency of dihydroquercetin. These results were in accordance with desirability coefficient and calculated values for optimal formulation. Final formulation was tested for human skin penetration *ex vivo* using flow-through diffusion cells. Skin penetration of dihydroquercetin in the formulation was compared to the control solution of dihydroquercetin in PEG400. Results showed, that there was no significant difference between control solution and formulation after 6 hours. After 24 hours dihydroquercetin content was significantly higher in the skin samples where formulated nano carriers were applied. These results demonstrate that experimental design approach is useful for designing an optimal composition of nano carriers which can improve the solubility and skin penetration of poorly water-soluble compounds, such as dihydroquercetin.

Keywords: dihydroquercetin, skin penetration, nano carriers, soybean oil.

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Poster presentation

L-Lactic acid production from wheat straw using combined lactic acid bacteria strains belonging to *Lactobacillus* genera

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Abstract

Recently, considerable interest has arisen to bio-recycle of the agro by-products such as straw into the valuable chemicals such as lactic acid. Cheap cellulosic materials are regarded as economically attractive feedstocks for lactic acid fermentation, which allow the utilization of agro waste as a source of carbohydrate [1]. Lactic acid is a platform chemical, and its salts have a long history of commercial uses and applications [2]. The Higher economic effect has been found by using biological conversion of wheat biomass to lactic acid vs chemical synthesis by increasing the energy efficiency by 47% and decreasing the total costs by 17% [3]. The chemical route produces a racemic mixture of DL-lactic acid, while optically pure L(+)- or D(–)-lactic acid can be obtained by microbial fermentation. Since elevated levels of the D-isomer are harmful to humans, L-(+)-lactic acid is the preferred isomer in food and pharmaceutical industries [4], therefore the search of microorganisms producing a high content of L-lactic acid from a lignocellulosic material such as wheat straw is outstanding importance.

The aim of the research was to investigate the usability of wheat straw in the production of L-lactic acid via fermentation applying by newly isolated lactic acid bacteria (LAB) strains belonging to *Lactobacillus* genera and its combinations.

Agro-industrial by-products were fermented with thermophilic and mesophilic LAB belonging to *Lactobacillus sanfranciscensis*, *Lactobacillus delbrueckii*, *Lactobacillus rossiae*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus* and other species. Before fermentation enzymatic treatment of by-products was carried out using carbohydrases. An enzymatic test K-DLATE 08/11 (Megazyme Int. Ireland, Wicklow, Ireland) was used for lactic acid and D/L-lactates determination.

The results show that the proposed mixed LAB starter cultures of mesophilic *L. sanfranciscensis* MW15, *L. crustorum* W19 and *L. sanfranciscensis* MR29 strains and combination of thermophilic *L. delbrueckii* subsp. *bulgaricus* DSM 20081 and *L. delbrueckii* subsp. *bulgaricus* MI strains can be successfully used to enhance lactic acid production from bio-treated wheat straw. Moreover, *L. crustorum* W19 and *L. sanfranciscensis* MR29 strains, as well as a mixture of those strains, could be used for pure L-lactic acid isomer from wheat straw medium production by revealing the possible synergistic effect of the combined LAB on L-lactic acid production.

Keywords: Cellulase, *Lactobacillus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, lactic acid, lactic acid bacteria, wheat straw.

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Poster presentation

Comparative Analysis of Biologically Active Compounds From Cow Colostrum And Milk Fats

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Abstract

Colostrum is a mammary secretion which has unique benefits for health [1]. The cow colostrum contains a wide range of growth factors, fatty acids, immunoglobulins and antimicrobial peptides [2]. First milking colostrum is an especially valuable source of fats, making up about 20-25% of total solids. Fat plays an important role in energy supply, improve metabolism, protects against microbial infections. The important fat-soluble vitamins, as well as some of the immunoglobulins/antibodies stick to the surface of fat particles [3]. Recently, scientists have been interested in the appliance of colostrum fat for cosmetic purpose and cell regeneration. So, the aim of research work was to compare the fatty acid content of cow colostrum and milk fats. Fatty acids were determined by gas chromatography with flame ionization detector. According to obtained chromatograms, 24 out of a possible 37 fatty acids were identified in colostrum fat. The amount of saturated fatty acids (C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C15:0, C18:0, C21:0, C23:0) were observed less in colostrum in comparison with milk. Meanwhile, the content of palmitic acid (42.94%) was observed significantly higher in colostrum in comparison with milk (29.83%). Among monounsaturated fatty acids the oleic acid was dominant in both fats: milk and colostrum (approx. 20.83%). In colostrum was found polyunsaturated eicosapentaenoic acid and docosahexaenoic acid, whereas in milk these acids were not detected. Due to the high content of biologically active compounds such as palmitic acid, and polyunsaturated fatty acids the colostrum fat could be applied in cosmetic production for rebuilding and repairing cellular tissues as well as skin elasticity increasing.

Keywords: cow colostrum fat, milk, fatty acids

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Poster presentation

The Combined Impact of Heat Wave and Drought on Oxidative Stress and Antioxidant Enzymes Activity of Barley

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Abstract

Heat waves in Europe, associated with lack of precipitation, are increasing in frequency, intensity and duration as a consequence of rapid climate warming. Yet there is still lack of evidence as to what degree the interactive impact of a heat wave and drought would affect plants growth and how they would recover from this combined stress. One of the main plant stress response is an intensification of oxidative stress and the shift in antioxidative system activity. Growth, changes in two antioxidant enzymes (superoxide dismutase (SOD) and catalase (CAT)) activities and lipid peroxidation (malondialdehyde (MDA) content) under the short-term (3-day long) impact of heat wave (31/21 °C vs. 21/14 °C day/night) single and in combination with drought (i.e. fully watered and not watered during the heat wave period) as well as the recovery following stress were investigated in barley (*Hordeum vulgare* L., var. Aura) plants grown in growth chambers at control environment at their leaf development stage. Under the single heat wave treatment, the activities of SOD and CAT were increased by 23% ($p < 0.05$) and 17% ($p > 0.05$), respectively, and MDA content by 42% ($p < 0.05$), but it has had no significant effect on above-ground dry weight. After one-day regeneration period, full recovery of MDA content was observed, while SOD and CAT activities were significantly reduced, compared to the control ones. Whereas under the combined impact of heat wave and drought, the activities of SOD and CAT were increased by 94 and 83% ($p < 0.05$), respectively, MDA content was more than two times ($p < 0.05$) higher, and the above-ground dry weight was reduced by 20% ($p < 0.05$). After one-day regeneration period CAT activity, as well as MDA content in drought-stressed plants, were still higher by 42 and 54% ($p < 0.05$), respectively, while above-ground dry weight was significantly lower by 14% ($p < 0.05$), meaning that drought-stressed barley plants suffered to a significantly larger extent from oxidative stress caused by combined impact of heat wave and drought than that from single heat wave treatment.

Keywords: Heatwave, drought, oxidative stress, antioxidant enzymes, barley

Poster presentation

Biologically Active Compounds of Hop (*Humulus lupulus* L.) Plant and Cones

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Abstract

Hop (*Humulus lupulus* L.) is a plant which is probably the best known for being used in the brewery as its cones are responsible for the characteristic bitter taste of beer. Bitter α - and β -acids found in hops cones have antibacterial properties, which in part provides resistance against beer-spoiling microorganisms. Also, other studies show that there are more biologically active compounds (i.e. xanthohumol, 8-prenylnaringenin and others) found in hops that have anti-cancer, radical scavenging, peroxide reducing and antioxidant properties [1-3]. It is very likely that biologically active compounds are accumulated not only in the cones of hops but also in other parts of the plant. There are little data about biologically active compounds in other parts of the hop plant. Identification and optimization of purification processes of biologically active compounds in other parts of the hop plant would probably allow finding more diverse applications of hop harvest. The distribution of biologically active compounds depends on the part of the plant, variety, phase of vegetation and geographical location of the plant. The purpose of this study was to evaluate and compare the phytochemical composition of biologically active compounds in different parts of *H. lupulus* L. plant growing in Kaunas Botanical Garden of Vytautas Magnus University, Lithuania. To our knowledge, it is the first study about antioxidant activity of different parts of hops collected in Lithuania.

The phytochemical composition was analyzed using spectrophotometric methods. The total amount of phenolic compounds, the total content of flavonoids and antiradical activity (using 1,1-diphenyl-2-picrylhydrazyl (DPPH)) were determined [4]. Results were expressed as rutin equivalents mg/g of the plant. The results of the assays will be presented during the conference.

Keywords: *Humulus lupulus*, biologically active compounds, phytochemical composition, spectrophotometry.

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Poster presentation

Essential Oil Composition and Antimicrobial Activity of *Agastache foeniculum* (Pursh) Kuntze

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Abstract

The present work focuses on to evaluate antimicrobial activity of essential oils of diploidal (unmodified) and polyploidal (biotechnologically modified) *Agastache foeniculum* (Pursh) Kuntze forms. *Agastache foeniculum* (Pursh) Kuntze is an aromatic perennial plant belonging to the Lamiaceae family and widely use: in perfume industry, in medicine, as herbal remedy, as decorative plant or in anise-flavored spices. It is a natural product to lower the blood pressure, improve metabolism or alleviate cough [2].

The polyploidal form of *Agastache foeniculum* (Pursh) Kuntze were cultivated in the Laboratory of Cell Engineering at the Institute of Botany (Lithuania) and at the Botanical Garden of Vytautas Magnus University (Lithuania), during the project, that was supported by Science and Studies Foundation of Lithuania (Grant No. N-14/2009). The essential oil of dry material (flowers, leaves and stems) was extracted by hydro-distillation method, for 2 h, using a Clevenger-type apparatus. The essential oils were analyzed by gas chromatography–mass spectrometry (GC-MS) [1]. The major constituents of the essential oils were estragole (87.5%), limonene (2.4%), 1,8-cineole (2.0%) and globulol (1.4%) [2]. The herb was collected during full-blooming period in 2016. The antimicrobial activity of secondary metabolites in *Agastache foeniculum* (Pursh) Kuntze originated from the sector of medicinal plants botanical garden of Vytautas Magnus University Lithuania, were tested by the agar well diffusion method, disk diffusion susceptibility method against different species of microorganisms. Agar well diffusion method and disk diffusion susceptibility method is official methods used in microbiology laboratories for routine antimicrobial susceptibility testing evaluate the antimicrobial activity of plants extracts. Antimicrobial susceptibility testing methods are published by the Clinical and Laboratory Standards Institute (CLSI) [3]. Essential oils of *Agastache foeniculum* (Pursh) Kuntze were tested against *Fusarium solani* and *Aspergillus* sp. microorganisms and results showed that antimicrobial activity of essential oils were detected only at the highest concentrations of estragole [4].

Keywords: essential oils, microorganisms, *Agastache foeniculum* (Pursh) Kuntze.

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Poster presentation

Optimisation of the Extraction of Anthocyanin from American cranberry (*Vaccinium macrocarpon* Aiton) using Response Surface Methodology

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Abstract

The object of interest for this research were fruits of American cranberry (*Vaccinium macrocarpon* Aiton). Preparations made from cranberry extracts are widely used for treatment of urinary tract inflammations. Cranberry fruits also have potent antioxidant, anti-inflammatory properties, a positive impact on the cardiovascular system, vision and prevention for obesity. Cranberry contains a lot of different chemical substances: phenolic compounds, organic acids, vitamins, micro- and macro elements and etc. There has been found the presence of flavonoids, such as anthocyanins, which were the main interest in this study.

The extraction procedure is of great importance for the extraction of natural colorants. In the present study, the main focus was on optimization of anthocyanin extraction from the cranberries.

The optimization was based on three factors: concentration of ethanol, extraction time and ultrasound power. In the experiments used ethanol concentration varied from 16.36% to 83.64% (v/v), the time varied from 6.36 minutes to 73.64 minutes and ultrasound power was from 339 W to 1130 W. pH adjustment of ethanol was made by addition of 0.1% of hydrochloric acid. The total amount of extracted anthocyanin was evaluated by UV-VIS spectrophotometry. The results showed that the relationship between the three variables and the total anthocyanin content followed a quadratic model. The best conditions to extract anthocyanins seemed to be when the concentration of ethanol was 70 % (v/v), time – 20 minutes and ultrasound power was 452 W. Total anthocyanin content extracted using this extraction conditions was 2.48 mg/g.

Optimized cranberry fruits extraction conditions were applied to 8 different cultivars that were grown in Lithuanian climate conditions. The tested cultivars was 'Baiwjay', 'Holliston', 'Searles', 'Drever', 'Bergman', 'Woolman', 'Brin', 'Pilgrim'. The highest total anthocyanin content (7.90 ± 0.004 mg/g) was determined in 'Baiwjay' cranberry samples and the lowest content (2.21 ± 0.01 mg/g) - in 'Holliston' samples.

In the future, the research of cranberry fruits individual anthocyanin composition will be performed by using HPLC.

Keywords: American cranberry, anthocyanin, extraction optimization, response surface methodology, UV-VIS spectrophotometry.

Poster presentation

The improvement of fatty phase quality indexes in dry-fermented sausages

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Abstract

The study was carried out with dry-fermented sausages and selected antioxidants (vitamin C 1.0%, rosemary extract (*Rosmarinus officinalis*) 0.05% and L-carnosine 0.5%), in order to effectively reduce the number of acids and peroxides, to evaluate the effect of the chosen antioxidant additive on the fat hydrolysis and oxidation. Experimental dry-fermented sausages were produced in the company “X”. Samples 1, 3 5, 7 th of experimental dry-fermented sausages were with lactic acid additive used for the acceleration of the fermentation process and the ninth sample was the control (without additives). Determination of antioxidative activity, evaluation of total phenolic compounds, tests of the number of acids and the number of peroxides were carried out on the first day after the production of dry-fermented sausages and 120 days after production. Active acidity pH and color coordinates were determined additionally 30 days after the production of sausages. The study confirmed the positive effect of antioxidants on the reduction of the number of acids and peroxides during the storage period of dry-fermented sausages. 120 days after the production of dry-fermented sausages, the statistically reliable ($p < 0.01$) minimum acid number 1.75 ± 0.01 mg KOH / g was determined in the eighth sample (with the additive of 0.05% of rosemary extract, 1.0% of vitamin C and L-carnosine), and statistically reliable ($p < 0.01$) minimum acidity according to the oleic acid $0.87 \pm 0.01\%$. The additive of rosemary extract 0.05% and vitamin C 1.0% reduced the oxidation of fatty phase in the dry-fermented sausages. After 120 days, a statistically significantly lower ($p < 0.001$) number of peroxides 3.40 ± 0.10 , in comparison with other samples, was determined. The processes of hydrolysis and oxidation of fat depended on similar factors, as a strong positive linear relationship was determined between the number of acids and peroxides with a correlation coefficient $r = 0.8647$. The lactic acid and rosemary extract positively affected the sensory properties of the sausages, the acceptance of the control sample was significantly lower.

Keywords: dry-fermented sausage, antioxidant activity, peroxides, acidity.

Poster presentation

Fusarium Pathogens Associated with Spring Wheat Black-Point and Their Responsibility for Deoxynivalenol Concentration in Grain

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Abstract

Cereals are a primary source of a human diet, wheat being the third most produced grain worldwide. The cereal-infecting species *Fusarium graminearum* (teleomorph *Gibberella zeae*), *F. culmorum* and *F. pseudograminearum* produce a range of type B trichothecenes, including deoxynivalenol (DON) and its acetylated derivatives, which inhibit protein translation in eukaryotes that are known virulence factors on wheat. In recent years, many *Fusarium* species have emerged which now threaten the productivity and safety of small grain cereal crops worldwide. During floral infection and post-harvest on stored grains *Fusarium* spp. produce various types of harmful mycotoxins which subsequently contaminate food and feed products. *Fusarium graminearum* teleomorph *Gibberella zeae* has not been spotted on cereal grain in Lithuania before and has not posed any problems for grain producers and purchasers so far. Visually black-point on grain and under grain hulls was identified as the fruit bodies of ascospores producing perithecia. Black-pointed wheat grains have caused a lot of discussion among grain growers, grain purchasers, manufacturers, scientists and consumers.

According to the quality requirements for a marketable grain of wheat (LST 1580), when estimated visually, *Fusarium*-damaged grain must not exceed 1 %. To estimate DON contamination in black-point infested wheat grain, 44-grain samples were divided into 4 groups: visually healthy grain, *Fusarium*-damaged grain, black-pointed grain, and a composite sample. All grain samples were subjected to DON analyses using commercial mycotoxin detection kits. The analysis of the visually healthy grain showed that DON-contaminated samples accounted for 27 %, and the average concentration was below the level of detection. In the group of *Fusarium*-damaged grain, DON was detected in all samples tested and its concentration averaged $9198 \pm 741 \mu\text{g kg}^{-1}$. In our study, DON was detected in 45 % of the black-pointed grain samples and its concentrations were low and averaged $346 \pm 36 \mu\text{g kg}^{-1}$.

In summary, we would like to emphasize that even though the black-pointed grain had low concentrations of DON, such grains are unquestionably the source of contamination and pathogen transmitters that need to be treated with a high degree of responsibility and thoughtfulness. Storage mistakes and use of untreated seed can have unpredictable consequences.

Keywords: Black-pointed grain, deoxynivalenol (DON), spring wheat, *Fusarium*-damaged grain

Acknowledgements: This study was supported by The Ministry of Agriculture of the Republic of Lithuania, and by the long-term research program 'Harmful Organisms in Agro and Forest Ecosystems implemented by LAMMC.

Poster presentation

Phytochemical Analysis of Biologically Active Compounds of *Artemisia dubia* Wall. Using Spectrophotometric Methods

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Abstract

Energy crops are becoming more popular in today's world. Many of these plants are not examined well (because their only purpose is to obtain energy), but they can also accumulate many other valuable substances that can be used to improve the quality of life, for example, in the field of health improvement. One such plant is from the *Artemisia* tribe - *Artemisia dubia* Wall. [1].

The aim of this investigation was to identify biologically active compounds, such as phenolic acids and flavonoids of *Artemisia*. *A. dubia* was collected from three different experimental areas with fertilization type: Non-fertilized; fertilized with N₉₀ fertilizer and N₁₇₀ fertilizer during different period: July 8, August 18 and September 7 in year 2016. Collected raw material was air-dried in Lithuanian Research Centre for Agriculture and Forestry and ground to ca. 3-5 mm size fraction. The extracts were prepared using 0.5 g of dried raw material and 20 mL 75% methanol. Extracts were left in a shaker for 24 h, and filtered afterward using filtering paper. Modified spectrophotometric analysis methods were used: Folin-Ciocalteu reagent was used to determine the total amount of phenolic compounds and AlCl₃ method was used to determine flavonoids [2].

The results showed that the biggest content of phenolic compounds in rutin equivalents (RE) 81.52 mg/g including flavonoids 22.86 mg/g were determined in extracts of the plant collected in July in an experimental area with no fertilizers. The lowest content of bioactive compounds was determined in the extract of the plant, collected in September in an experimental area with N₉₀ fertilizer.

Spectrophotometric analysis showed that total amount of phenolic compounds and the total amount of flavonoids were not affected by fertilization of the plant but showed considerable differences between periods of a collection of the plant material.

Keywords: *Artemisia dubia*, phenolic compounds, flavonoids, spectrophotometry.

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Poster presentation

Lettuce Leaf Optical Properties: Linking Spectral Characteristics to Photosynthetic Productivity

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Abstract

Green leafy vegetables are the main crops in the novel type of controlled environment horticulture – plant factories. This type of cultivation system enables full control of environmental parameters, therefore allow to predict the timing and quality of vegetable production. However, to control these processes properly, efficient, non-destructive analysis method to evaluate plant wellness is necessary. In this study, we review how the light emitting diode (LED) lighting spectrum affect growth and photosynthetic parameters of lettuces and analyze the relations between these parameters and leaf optical indices, measured using the non-destructive spectrometric method. Green baby leaf lettuces (*Lactuca sativa* ‘Lobjoits green cos’) were cultivated in closed environment growth chambers (21/17°C day/night temperature, ~55% relative humidity). Lettuces were cultivated under different LED lighting spectra: combinations of red (660, 638 nm), blue (445 nm) and far-red (735 nm) with supplemental green (530 nm), orange (620 nm), yellow (595 nm) and UV-A (385 nm) wavelengths, total photosynthetic photon flux density 300 $\mu\text{mol m}^{-2}\text{s}^{-1}$. At the maturity stage, leaf reflection and absorption spectra were registered, stomatal parameters and photosynthetic parameters evaluated. The obtained results show, that leaf optical properties depend on LED lighting spectrum, as well as on other cultivation environment parameters. The main correlations and interfaces will be discussed.

Keywords: light emitting diodes, stomata, chlorophyll/flavonoid index, leaf reflection/absorption spectra.

Acknowledgements: This research was funded by a grant (No. 09.3.3.-LMT-K-712-03-0009) from the Research Council of Lithuania.

Poster presentation

The Association between TBX15 rs984222 and Age-Related Macular Degeneration

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Abstract

Introduction: Age-related macular degeneration (AMD) is progressive eye condition leading people in developed countries to blindness at the age of 65 or later [1]. AMD causes defects in the central part of a retina (macula). Although etiology and pathogenesis of the disease is not clear [1], some studies showed that the main pathological processes in AMD development are due to drusen formation caused by changes in lipid metabolism, inflammation and pathological angiogenesis [2]. The aim of this study was to determine the association between TBX15 rs984222 and age-related macular degeneration.

Methods: Study enrolled 160 patients with early AMD, 121 patients with exudative AMD and 283 healthy controls. DNA was extracted from peripheral blood leukocytes using DNA salting-out method. Genotyping was carried out using real-time polymerase chain reaction (RT-PCR) method. Statistical analysis was performed with „SPSS version 20.0“.

Results: Genotype (GG, GC, CC) distribution between early AMD and control groups did not reveal statistically significant differences (48.8%, 38.1%, 13.1% vs. 50.5%, 38.9%, 10.6%, respectively, $p=0.724$). Exudative AMD analysis showed statistically significant differences comparing genotype (GG, GC, CC) distribution between exudative AMD and control groups (38.8%, 43.8%, 17.4% vs. 50.5%, 38.9%, 10.6%, respectively, $p=0.049$). Logistic regression was performed after controlling for age, but it did not reveal any significant associations.

Conclusion: We found associations between *TBX15* rs984222 and exudative AMD development but further analysis with bigger sample size is needed to confirm our results.

Keywords: TBX15; rs984222; age-related macular degeneration; polymorphism.

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Poster presentation

Comparative Characteristic of Experimental Models of Immunosuppression for Cellular Therapy of Myocardium

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Abstract

Objective: to evaluate the effectiveness of various experimental immunosuppression techniques for cardiomyoplasty with human stem cells (hCS).

Materials and methods. Female Wistar rats ($n = 24$) were used in the experiment. The immunosuppression model was formed by intraperitoneal administration of tacrolimus ($n = 9$) at a dose of 1 mg / kg of weight for initiation and 0.2 mg / kg per day of a maintenance dose (group 1- G1) or prednisolone ($n = 9$) at a dose of 80 mg / kg of weight for initiation and 16 mg / kg per day of maintenance dose (G2). Control (CG) were animals ($n = 6$), which was administered an equivalent dose of saline. We studied rats with transthoracic electrocardiography (ECG) and echocardiography (Echo), blood test, morphometric and histopathologic analyses.

Results and discussion. The blood test showed that the administration of tacrolimus or prednisolone led to a decrease in monocyte content and an increase in granulocytes on the 14th day of the experiment. The total content of erythrocytes and leukocytes did not differ from the data of CG. There were no ECG or Echo signs of myocardial damage. However myocardial hypertrophy (LVH) of the LV without an expansion of the LV cavity was observed in OG1 on day 14. The gravimetric analysis confirmed the development of LVH in OG: relative heart weight in OG1 was 3.43 ± 0.13 g / kg, in OG2 3.70 ± 0.20 g / kg versus 2.86 ± 0.15 g / kg in CG.

Histological examination of the heart with hematoxylin and eosin staining showed no difference between CG and experimental groups. At the same time, the study of hearts in the MSB staining revealed interstitial perivascular and subendocardial proliferation of connective tissue in 100% of rat hearts in a group with the administration of tacrolimus and in 50% of ones with the prednisolone administration. Small single foci of the disappearance of the myocardiocyte transverse striation and cytoplasm clumping without signs of cytolysis were observed in all examples in both experimental groups. Consequently, the morphological study indicated the induction of collagen formation by studied immunosuppressants but the absence of cardiotoxic action.

Conclusions. The administration of tacrolimus or prednisolone does not have a myelosuppressive and cardiotoxic effect, which makes it possible to use these drugs for immunosuppression in carrying out experimental studies on cell cardiomyoplasty in xenotransplantation.

Keywords: prednisolone, tacrolimus, experiment.

Changes of Biologically Active Compounds in the Forest Litter during the Vegetation of Understory Plants Before and After Clear Cutting of Forest

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Abstract

The impact of forest clear cutting to the understory plants and insects populations, to the soil or forest litter is not clear and is discussible between scientists nowadays [1-3]. Therefore, the aim of this study was to evaluate changes of the total phenolic compounds content and antiradical activity in the forest litter at different collection times before and after clear-cutting of the forest. The samples of forest litter were collected considering the vegetation phases of understory plants of *Ericaceae* family, such as *Vaccinium myrtillus*, *V. vitis-idaea*, and *Caluna vulgaris*, in the tested forests. In the previous study, it was determined, that highest contents of phenolic acids are in aqueous extracts of *C. vulgaris* from one-year-old clear cuttings, which demonstrated a strong phytotoxicity on Scots pine seed germination [4]. Forest litter from Lithuanian *Pinetum vaccinosum* and *Pinetum vaccinio-myrtillosum* forest types were analyzed in the present project. Samples were collected in April (the beginning of vegetation of understory plants), in May (blooming of *Vaccinium myrtillus* and *V. vitis-idaea*), in July (development of *V. myrtillus* and *V. vitis-idaea* berries, and blooming of *C. vulgaris*), and in November (the end of the vegetation of the understory plants). Total phenolic compounds content and antiradical activity varied in the samples depending both on the forest type and collection period. The reduction of total phenolic compound content and antiradical activity was observed after clearcutting in the samples collected in *Pinetum vaccinosum* forest type; while the content of total phenolic compounds in the samples from *Pinetum vaccinio-myrtillosum* remains constant during three periods out of four, and the reduction was noticed in the samples collected in November. Antiradical activity of two samples from this forest remarkably increased, and slightly was reduced in one sample.

Keywords: Forest litter, clear-cutting, total phenolic compounds, antiradical activity.

Acknowledgement: This research was funded by a grant (No. SIT-1/2015, MEKODINA) from the Research Council of Lithuania.

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Poster presentation

Supercritical Fluid Extraction of *Medicago sativa* Leaves: Phenolic Profile and Antioxidant Activity

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Abstract

Medicago sativa L. (lucerne or alfalfa) is one of the most cultivated forage legumes in the world. Besides its high nutritional value as a fodder for livestock, this plant was used from ancient times in folk medicine. As well as it is a rich source of many valuable secondary metabolites as biologically active compounds from the groups of phenols and flavonoids.

The conventional methods of extraction of plant material with organic solvents have many drawbacks, such as low selectivity or consumption of large volume of solvent. From the point of view of green chemistry, supercritical fluid extraction (SFE) with carbon dioxide (SC-CO₂) has gained much popularity. Nowadays, this is the most promising method which is able to provide extracts of high activity while precluding any toxicity associated with the used solvent. Due to the fact that carbon dioxide is non-polar, modification of SC-CO₂ through the adding of polar co-solvent or modifiers is needed to extract bioactive compounds.

In the frame of this work, SFE extraction was utilized for extraction of phenolic compounds from leaves of *M. sativa*. Taking into account that operational conditions may influence the chemical composition optimization of supercritical extraction was performed. Effect of three different extraction parameters (pressure, temperature and ethanol concentration) was explored while optimization. The total of flavonoids and phenolic content and antioxidant activity of extract from *M. sativa* leaves were investigated by spectrophotometric methods. Selected phenolic compounds were determined by HPLC-MS/MS. Antioxidant properties were evaluated using two methods, including free radical scavenging (DPPH, ABTS). Obtained results showed that the highest content of flavonoids and phenolic as well as the highest antioxidant activity increase with temperature and pressure up to 70°C and 300 bar, respectively.

Keywords: *Medicago sativa* L., supercritical fluid extraction, phenols, antioxidant activity.

Acknowledgements: This study was supported by PLANTARUM project No. BIOSTRATEG 2/298205/9/NCBR/2016 from National Centre for Research and Development, Poland.

Poster presentation

Association of variants at *FGFR2* and *CYP4F2* locus with risk of atrophic age-related macular degeneration

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Abstract

Introduction: Age-related macular degeneration (AMD) is a common cause of blindness in developed countries and can progress from an early to an intermediate and, finally, to a late form, which can be atrophic or neovascular, and until this moment there is no effective treatment for geographic atrophy [1]. Until now AMD etiology remains poorly understood, especially the geographic atrophy form. It is known that AMD development is determined by many factors but genetic factors have a significant propensity to AMD development [2]. So the aim of our work is to determine the frequency of *FGFR2* (2981582) and *CYP4F2* (rs1558139) gene polymorphisms in patients with atrophic AMD.

Methods: A total of 852 subjects were evaluated, including 52 patients with atrophic AMD and 800 healthy controls. The genotyping was carried out using the RT-PCR. DNA was extracted from white blood cells using the silica-based membrane technology utilizing a genomic DNA extraction kit (GeneJET Genomic DNA Purification Kit, Thermo Scientific), according to the manufacturer's recommendations. The genotyping was carried out using the RT-PCR method. Statistical analysis was performed using the SPSS / W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA).

Results: The variant in *FGFR2* gene was found to be associated with the 2.1-fold decreased risk of atrophic AMD only under the over-dominant model ($p=0.027$). No association with *CYP4F2* and atrophic AMD was detected.

Conclusion: The study showed that *FGFR2* gene polymorphism may have a protective role in atrophic AMD development.

Keywords: *FGFR2* (2981582) and *CYP4F2* (rs1558139), atrophic age-related macular degeneration, polymorphism.

Acknowledgements: This work was funded by a grant (No. SEN-11/2015) from the Research Council of Lithuania.

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Poster presentation

***LIPC* rs493258 Role in Females With Early Age-Related Macular Degeneration**

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Abstract

Introduction: Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly individuals in developed countries [1]. The etiology and pathophysiology of AMD are not fully understood. Formation of drusen is the main pathological change in AMD. Lipids make up about 40% of drusen volume, thus possible relation between AMD and genes controlling lipid metabolism could provide novel insights into AMD [2]. Women carry higher AMD risk than men [3]. Our purpose was to determine the genotype frequencies of *LIPC* rs493258 in women with early AMD.

Methods: Study enrolled 192 women with early AMD and 530 healthy control women. DNA was extracted from peripheral blood using commercial kits (Gene JET Genomic DNA Purification Kit, Thermo Scientific), according to the manufacturer's recommendations. The genotyping was carried out using the RT-PCR method. Statistical analysis was performed using the SPSS/W20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA).

Results: *LIPC* rs493258 genotype (CC, CT, TT) distribution analysis showed statistically significant differences comparing women with early AMD and healthy control women (43.2 %, 46.4 %, 10.4 % vs. 35.1 %, 47.0 %, 17.9 %, $p=0.023$, respectively). Binomial logistic regression after adjustment for age did not reveal significant results.

Conclusion: *LIPC* rs493258 polymorphism is not associated with early AMD development for women.

Keywords: *LIPC* rs493258, age-related macular degeneration, women.

Acknowledgements: This work was funded by a grant (No. SEN-11/2015) from the Research Council of Lithuania.

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Poster presentation

HS-SPME-GC/MS method as an analytical technique for tracking of changes in volatile patterns of salivary bacteria affected by silver nanoparticles

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Abstract

Silver nanoparticles (AgNPs) are well-known antimicrobial agents and their antimicrobial activity against bacteria is attributed to their high reactivity with proteins and initiation of structural changes in the cell wall and the membrane. Silver nanoparticles can interact with SH-groups of amino acids and therefore inhibit protein synthesis and function. Also, uptake of free silver ions followed by disruption of ATP production and DNA replication is one of the possible mechanisms of toxicity. In consequence, it leads to inhibition and death of cell [1]. Volatile organic compounds (VOCs) are naturally occurring products or by-products of metabolic pathways found in bacterial headspace and their profiles can be affected by stressing agents, including AgNPs [2].

The main goal of our study is to register the changes in profiles of VOCs from three salivary bacteria after addition of silver nanoparticles at a concentration of 12.5 µg mL⁻¹. Profiles of volatile organic compounds emanated from these bacteria after treatment with AgNPs were analyzed using headspace-solid phase microextraction-gas chromatography/mass spectrometry (HS-SPME-GC/MS). We have investigated three bacteria related to oral cavity: *Escherichia coli*, *Klebsiella oxytoca* and *Staphylococcus saccharolyticus*. The last purpose is to evaluate the changes in functional group distribution of obtained compounds and to point out which volatiles can serve as biomarkers of particular bacteria.

Keywords: saliva, bacteria, volatile organic compounds, solid-phase microextraction, gas chromatography-mass spectrometry.

Acknowledgements: This study was supported by Preludium grant No. 2014/15/N/ST4/03702 from the National Science Centre, Poland.

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Poster presentation

Determination of Volatile Organic Compounds as Products of Glucosinolates Decomposition in Garden Cress (*Lepidium Sativum*) by SPME-GC/MS

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Abstract

Glucosinolates are organic compounds having a molecule β -D-glucose, sulfonated oxime and a side chain derived from protein amino acids. The basic products of the degradation of glucosinolates are organic isothiocyanates, thiocyanates or nitriles. They occur mainly in plants of the order *Capparales* and *Brassicales* crops. They have economical and medical significance. Glucosinolate degradation products play important roles in plant interaction with herbivores and pathogens. In addition, they are important for human life. For example, some degradation products exhibit anticarcinogenic activity e.g. they can reduce the risk of development of breast, prostate, lung and gastric cancer [1-2].

The aim of the study was to determine volatile different organic product of glucosinolates degradation present in garden cress extract. In the plant extracts could be found alkaloids, flavonoids, glycosides, polyketides, vitamins, minerals, proteins, fats, carbohydrates which give antimicrobial, anti-inflammation, antioxidant, chemoprotective properties. Garden cress is a valuable nutritional supplement [3].

For determination of volatile organic compound (VOCs) in garden cress extracts solid phase microextraction (SPME) and gas chromatography-mass spectrometry (GC/MS) were applied. These techniques were used for the extraction, separation and determination of analytes present in trace amounts. The SPME-GC/MS allowed identifying benzyl cyanide, benzyl isothiocyanate and benzyl thiocyanate as the mainly volatile secondary plant metabolites in the plant extracts.

Keywords: glucosinolates, garden cress (*Lepidium sativum*), solid phase microextraction, gas chromatography-mass spectrometry

Acknowledgements: This study was supported by PLANTARUM project No. BIOSTRATEG2/298205/9/NCBR/2016 from National Centre for Research and Development, Poland.

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Poster presentation

The Association Between SIRT1 Rs3740051 And Age-Related Macular Degeneration

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Abstract

Introduction: Age-related macular degeneration (AMD) is a neurodegenerative disease that causes loss of vision mostly in people over 60 years in developed countries [1]. The precise pathogenesis of AMD not yet clarified, although it has been associated with genes regulating complement, lipid, angiogenic, and extracellular matrix pathways [2]. The SIRT1's dysregulation leads to the disappearance of a protective effect against retinal degeneration [3]. The aim of this work was to examine rs3740051 variant in SIRT1 gene promoting to AMD development.

Methods: The study enrolled 181 patients with early AMD, 140 patients with exudative AMD and 189 healthy control subjects. DNA extracted from blood using the silica-based membrane technology utilizing a genomic DNA extraction kit (QIAamp DNA Mini Kit, Genomic DNA Purification Kit, QIAGEN), according to the manufacturer's recommendations. The genotyping performed using the RT-PCR method. Statistical analysis was performed using the SPSS / W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA).

Results: Genotype (AA, AG, GG) distributions were determined in early AMD, exudative AMD and control groups: 84.2 %, 15.3%, 0.6%; vs. 86.1%, 13.1%, 0.7% and vs. 85.6%, 12.8%, 1.6%, respectively. Statistical analysis did not reveal significant differences between patients with early AMD and healthy controls ($p=0.519$). Also, genotype distribution did not differ comparing exudative AMD and control groups ($p=0.781$).

Conclusion: There was not found any associations of SIRT1 rs3740051 with early and exudative AMD development.

Keywords: age-related macular degeneration, SIRT1, rs3740051, polymorphism.

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Poster presentation

Association of *SIRT1* rs3740051 Gene Polymorphism with Pituitary Adenoma

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Abstract

Pituitary adenomas (PAs) are the most common central nervous system (CNS) tumors. They generate a large group of neoplasms which can secrete hormones that depends on their cell of origin. The incidence of pituitary adenomas within the general population to be as high as 16.7% [1]. The present study was to determine the association between sirtuin 1 (*SIRT1*) gene polymorphism and PA development and how they associated with gender.

Methods: The study included 64 patients with a diagnosis of PA. The reference group involved 173 healthy subjects. The genotyping of *SIRT1* rs3740051 was performed using the quantitative polymerase chain reaction method. The potential association with single nucleotide polymorphism (rs3740051) was evaluated for all patients.

Results: Statistical analysis did not reveal significant genotype (AA, AG, GG) distribution differences between the PA and control groups: 76.6%, 28.3%, 3.1% vs. 86.1%, 12.7%, 1.2%, respectively (P=0.182). Analysis of *SIRT1* rs3740051 polymorphism genotype distribution by gender did not reveal any statistically significant differences as well. Genotype (AA, AG, GG) distribution in PA and control females (77.1%, 20.0%, 2.9% vs. 89.5%, 8.4%, 2.1%, respectively, p=0.175) and males (75.9%, 20.7%, 3.4% vs. 82.1%, 17.9%, 0%, respectively, p=0.237).

Conclusion: The *SIRT1* rs3740051 polymorphism was not associated with PA and gender.

Keywords: pituitary adenoma, gene polymorphism, sirtuin 1 rs3740051.

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Poster presentation

Determination of FGFR2 rs2981582 single gene polymorphism in patients with oral cancer

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Abstract

Introduction. Fibroblast growth factor receptor 2 (FGFR2) is a member of the FGFR family of tyrosine kinase receptors, which via cell growth, invasiveness, motility and angiogenesis contributes to the process of tumorigenesis [1,2]. We performed a case-control study to determine possible involvement of *FGFR2* gene polymorphism rs2981582 on oral cancer in Lithuanian subjects.

Methods. The study included 35 patients with a diagnosis of oral cancer and 100 healthy subjects as a reference group. DNA samples of the represented patient population were extracted from peripheral venous blood. Genotyping of FGFR2 rs2981582 was performed using the real-time polymerase chain reaction method. Statistical analysis was performed using „IBM SPSS Statistics 20.0“.

Results. It was determined that *FGFR2* rs2981582 polymorphism has no effect on a development of oral cancer. The analysis of *FGFR2* gene polymorphisms did not reveal any differences in the distribution of GG, GA, and AA genotypes between the oral cancer group, and the control group (42.9%, 48.6%, and 8.6% vs. 46%, 37% and 17%, respectively).

Conclusion. Our study showed that there is no association between *FGFR2* rs2981582 and oral cancer. Moreover, a further study with a larger sample sizes are required.

Keywords: Fibroblast growth factor receptor 2, gene polymorphism, oral cancer.

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Poster presentation

Volatile Compounds Composition of *Elsholtzia ciliata* Fresh, Frozen and Dried Herbal Materials

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Abstract

Elsholtzia ciliata (Thunb.) Hyl. belonging to Lamiaceae family commonly known as crested late-summer mint is very interesting herb for its chemical composition and pharmacological activities. The aim of this study was obtaining a chemical composition of the volatile compounds from fresh, frozen and dried *E. ciliata* herbal materials. The samples were prepared by the dynamic headspace solid-phase microextraction (SPME) and analyzed using gas chromatography-mass spectrometry method (GC-MS). This is the first study of volatile compounds determined from fresh, frozen and dried herbal samples of *E. ciliata*. The plant growing in Lithuania does not exhibit a lot of volatile compounds. Sixteen different compounds have been obtained from all SPME samples. Dehydroelsholtzia ketone, elsholtzia ketone, beta-bourbonene, caryophyllene, alpha-caryophyllene, germacrene D and alpha-farnesene were identified and found to be predominant compounds in fresh, frozen and dried herbal samples. The major amounts of ketones were found in dried herbal samples and made up 21.94% (dehydroelsholtzia ketone) and 71.34% (elsholtzia ketone) of headspace SPME composition. Sesquiterpenes were the second major group of identified compounds and obtained from dried (3.3%), frozen (1.73%) and fresh (1.95%) *E. ciliata* samples. Predominant sesquiterpenes were α -caryophyllene and β -bourbonene in fresh (1.04% and 0.53%), frozen (0.84% and 0.49%) and dried (1.6% and 0.97%) herbal materials.

Keywords: *Elsholtzia ciliata*, volatile compounds, SPME, ketones, sesquiterpenes.

Poster presentation

***RAD51B* RS8017304 Gene Polymorphism Association with Exudative Age-related Macular Degeneration**

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Abstract

Introduction: Age-related macular degeneration (AMD) is currently the leading cause of irreversible central vision loss in the elderly [1]. AMD is a complex and multifactorial disease that involves the significant interplay between genetic and environmental factors. *RAD51B* is a known member of the *RAD51* paralogs and is involved in homologous recombinational repair of DNA double-strand breaks by promoting the activity of the central recombinase [2]. The absence of the *RAD51B* protein is thought to disrupt the formation of the *RAD51* nucleoprotein filament, the initial stage of homologous recombinational repair [3]. Considering the demonstrated link between DNA damage and AMD, there have been attempts to find a relation with AMD and *RAD51B* polymorphism rs8017304. Our aim was to determine the frequency of the genotype of rs8017304 in patients with exudative AMD and control group.

Methods: The study enrolled n = 100 patients with exudative AMD and a random sample of the population n = 100. The genotyping of rs8017304 was carried out using the real-time polymerase chain reaction (RT-PCR) method. Exudative age-related macular degeneration was confirmed after optical coherent tomography examination.

Results: The analysis of rs8017304 gene polymorphism in exudative AMD and the control group did not reveal any differences in the distribution of AA, GA, and GG genotypes (50.0%, 43.0%, and 7.0% in AMD group and 60.0%, 28.0% and 12.0%, in control group, p=0.066). Further statistical analysis revealed that AG genotype comparing with AA increase possibility of manifestation of exudative AMD 1.8 times (OR=1.843; 95 % CI:1.005-3.379; p=0.048). Moreover, comparing with AA and GG together, this possibility increases 1.94 times (OR=1.940; 95% CI:1.076-3.497; p=0.028).

Conclusions: *RAD51B* rs8017304 gene polymorphism is associated with increased risk of exudative AMD development.

Keywords: Age-related macular degeneration, polymorphism.

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Poster presentation

Alternative Technologies for Plant Proteins, in Natural Matrices and Isolates, Nutritional Value and Functionality Increasing

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Abstract

The aim of this study was to evaluate the influence of submerged (SMF) and solid state (SSF) fermentation with *Pediococcus pentosaceus* KTU05-8, KTU05-9, KTU05-10 strains on the parameters of *Lupinus luteus* and *Lupinus angustifolius* wholemeal protein and protein isolates. In addition, the formation of biogenic amines (BAs) in lupine wholemeal protein and protein isolates were evaluated. *Pediococcus pentosaceus* KTU05-8, KTU05-9, KTU05-10 strains are suitable starters for *Lupinus luteus* and *Lupinus angustifolius* wholemeal and protein isolates fermentation, in order to improve nutritional value. Both, fermentation and protein isolation, processes increases protein digestibility *in vitro*, however, higher total phenolic compounds content in fermented lupine wholemeal could be obtained. There is a significant effect of protein isolation on BAs content in lupine products, and in most of the cases, isolates are safer from this point of view. SMF and SSF with selected *pediococcus* strains is the useful technology for lupine protein value increasing.

Keywords: Lupine protein isolates, *pediococcus*, digestibility, phenolic compounds, biogenic amines.

Poster presentation

Screening of Microorganisms for Production of Cyclodextrins from Starch

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Abstract

Raw starch degradation pathway via cyclodextrins is usually carried out by alkalophilic bacteria by producing cyclodextrin glucanotransferase (CGTase) enzyme. Alkalophilic bacteria most commonly found in starch sources or alkaline saline environment (i.e. corn soil, soda lakes, rotten potatoes and etc.). The latter bacteria require an alkaline pH and have an optimal growth at ca. pH 10. Nevertheless, other studies show that alkaliphiles can be isolated from neutral and acidic soil samples. CGTases are bacterial enzymes that convert starch and other 1,4-linked α -glucans to cyclodextrins (CDs), which are closed ring structures composed mainly of 6, 7 and 8 glucosyl units, named α , β and γ -cyclodextrins and are featured by hydrophilic outside and hydrophobic central cavity. The quantity ratio of CDs production depends on individual bacteria strain and specific chemical properties of bacterial CGTase. [1] Because of the ability to form inclusion complexes with various organic and inorganic compounds (i.e. SBE- β -CD, α -CD-cinnamic acid), CDs can be used to modify the physicochemical characteristics of low soluble drugs and natural preservatives. [2,3]

The objective of our study was to find and isolate cyclodextrin glucanotransferase producing bacteria for CDs synthesis from different raw starch sources. However, no case of the similar studies are known in Lithuania up-to-date. The discovery of CGTase producing bacteria and optimization of qualitative and quantitative CDs synthesis from raw sources would allow to reduce production costs and extend the uses of these cyclic polysaccharides in pharmaceutical and food industries. All the samples were chosen according to the published data and were collected from different locations of Lithuania.

During the study, we detected and isolated CGTase producing bacteria and evaluated enzymatic activity. Isolation and identification of CGTase production was completed by using Horikoshi – phenolphthalein agar method by fixing yellow halo zones around the bacteria colonies. Enzymatic activity was evaluated spectrophotometrically by phenolphthalein assay method by measuring the quantitative ability to produce CDs. All the data with detailed explanation will be presented during the conference.

Keywords: alkalophilic bacteria, alkaliphiles, cyclodextrin glucanotransferase, cyclodextrins, starch, spectrophotometry

Acknowledgements: Financial support from Research Council of Lithuania project 09.3.3-LMT-K-712-03-0128 is acknowledged.

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Poster presentation

The Effects of Light Intensity on Nitrate and Ascorbic Acid Contents in Green Vegetables, Cultivated in Plant Factories

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Abstract

Novel, progressive type of controlled environment horticulture – plant factories enables the precise control of cultivation environment parameters, however, it is not yet well known, how these environmental parameters (lighting, temperature, nutrition etc.) should be balanced seeking for maximal vegetable productivity, optimal internal and external quality. In this study, we present how light emitting diode (LED) light intensity affect nitrate and ascorbic acid contents in different green vegetables, cultivated in the closed environment phytotron. Green and red leaf lettuces (*Lactuca sativa* ‘Lobjoits green cos’ and ‘Red cos’) and tatsoi (*Brassica rapa* var. *rosularis* ‘Rosetto’ F1) were cultivated under combinations of red (660, 638 nm), blue (445 nm) and far-red (735 nm) LED lighting under PPFD of 100-500 $\mu\text{mol m}^{-2}\text{s}^{-1}$. At the stage of technical maturity, nitrate, nitrite contents and ascorbic acid contents were evaluated. The results propose the metabolic interface between nitrates and ascorbic acid in green vegetables, sensitive for lighting intensity. According to WHO, ascorbic acid in green vegetables acts as an antioxidant and neutralize the possible negative effect of nitrate metabolites (nitrites and nitrosamines) for the human organism. Lighting intensities, resulting in low nitrate and high ascorbic acid contents in green vegetables will be elaborated.

Keywords: light emitting diodes, ascorbic acid, nitrates nitrites.

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Poster presentation

Silica Anionite for Solid Phase Extraction: Synthesis and Optimisation

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Abstract

Solid-phase extraction is used in laboratories in order to concentrate and purify samples for the further use for analysis. For that, a particulate stationary phase or monolith with corresponding functional groups is used together with a mobile phase which contains desired analyte.

For this work silica gel was used modified with 3-glycidyloxypropyltrimethoxysilane (GLYMO) and diethylamine (DEA) in order to attach amino groups for positive charge of stationary phase. The modification was carried out using a different ratio of GLYMO and DEA, different temperatures, different solvent as reaction medium and different modification time for the optimisation of the adsorptive properties of the monolith. The amount of nitrogen fixed on the monolith was determined using Kjeldahl method for nitrogen determination. Results have shown that the highest amount of nitrogen (0.617%) is obtained at the ratio of GLYMO and DEA of 1:1.2 in alkaline solution using dimethyl sulfoxide as a solvent and proceeding the modification for 24 hours in ca. 85°C temperature, meanwhile the lowest amount (0.08%) of nitrogen is obtained at the ratio of GLYMO and DEA of 1:1.2 in alkaline solution using dimethyl sulfoxide as a solvent, proceeding the modification for 7.5 hours at ca. 68°C temperature. The optimized monolith will be used in further experiments for solid-phase extraction of phytochemicals.

Keywords: solid-phase extraction, silica, anionite, amine.

Poster presentation

Protein extraction from diatom for MALDI analysis

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Abstract

This study aim was to investigate protein extracted from natural silica collected from diatoms using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) method, alongside with the investigation on which extraction reagent was the most suitable for this analysis. Diatoms are one of the main group of algae that can produce natural silica particles from the cell wall, which can be used as a natural sorbent. Biosilica is obtained by removing the organic part from diatom cells. The biosilica particle is strictly defined (e.g. by the structure of pores) and possess active functional groups such as carboxyl, amine. For further analysis of organic deposit of biosilica, a MALDI analysis was carried out. This method is widely used for a fast peptides/protein analysis as well as microorganism identification.

For protein extraction, five different combination of solutions were used: 0,1% ACN, 0,1% TFA, TA30, 5% TFA and 0,1% TFA/ACN solutions were used. For MALDI analysis silica extractions were spotted on MALDI target and analyzed with Bruker Ultraflex II TOF/TOF MALDI mass. The analysis of peptides from biosilica showed, that almost all peaks contained modified peptides residue or additions to matrix molecules.

Keywords: diatoms, MALDI, silica, protein.

Poster presentation

Normal-Phase Separation of Racemic Phenylpiracetam on Immobilized Amylose-Based Chiral Stationary Phases

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Phenylpiracetam (2-[2-oxo-4-phenylpyrrolidin-1-yl] acetamide), a member of the racetam group of nootropic drugs, has been introduced into clinical practice in its racemic form. Recent studies in our Institute were aimed at the resolution of racemic phenylpiracetam into individual *S* and *R* enantiomers and their subsequent pharmaceutical analysis [1]. It was found that chlorinated polysaccharide-based chiral stationary phases (CSP), and especially amylose-based coated CSP Lux Amylose-2, have shown good enantioseparation ability for racemic phenylpiracetam under normal-phase (NP) liquid chromatography conditions [2].

During the past decade, different immobilized CSPs have become one of the most powerful tools to obtain pure enantiomers. It is known that immobilized columns exhibit greatly enhanced robustness, efficiency and remarkable stability. Moreover, immobilized CSPs instead of coated CSPs have the advantage of allowing applying a much broader range of solvents as the mobile phase. In continuation of our racemic phenylpiracetam resolution research under NP mode chromatographic properties of coated (Lux Amylose-2) and four recently commercialized immobilized amylose-based chiral stationary phases (CHIRALPAK ID, IE, IF and IG) have been examined. Alcohol/*n*-hexane mixtures were studied as mobile phases in present work. Application of non-standard eluents, such as dioxane or tetrahydrofuran mixtures with *n*-hexane, 100% ethyl acetate and methyl *tert*-butyl ether, chloroform or dichloromethane mixtures with alcohols, has been investigated on immobilized CSPs. It was established that immobilized CSP CHIRALPAK IG shows good chiral recognition ability with non-standard mobile phases.

Keywords: Column liquid chromatography, Enantioseparation, Amylose-based chiral stationary phases, Normal-phase mode.

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Poster presentation

***Lactobacillus crustorum* W19 and *Lactobacillus sanfranciscensis* MR29 Strains Application for L-lactic Acid Production from Wheat Bran**

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Abstract

Lactic acid or 2-hydroxy propionic acid has a market with great growth potential. It can be produced by fermentation or chemical synthesis and can employ a large variety of different waste materials as substrates. The economics of lactic acid production by fermentation is dependent on many factors, of which the cost of the raw materials is very significant. It is very expensive when sugars, e.g., glucose, sucrose, starch and other etc., are used as the feedstock for lactic acid production [1]. Therefore, industrial by-products such as wheat bran is a promising feedstock for lactic acid production considering its great sustainability, availability, and low cost compared to refined sugars. From the last several years, in serious consideration of the worldwide economic and environmental pollution issues, there has been increasing research interest in the value of bio-sourced lignocellulosic biomass such as wheat bran and its recycling into the various valuable chemicals such as lactic acid [1-3]. The search for low-cost raw materials and L-lactic acid isomer producing bacteria strains to be used in the production of lactic acid by fermentation has been promoting the development of competitive processes. The search for low-cost raw materials and lactic acid, especially L-lactic acid isomer producing strains to be used in the production of lactic acid by fermentation has been promoting the development of competitive processes. L-(+)-lactic acid is the preferred isomer in food and pharmaceutical industries [4], whereas elevated levels of the D-isomer are harmful to humans.

The aim of the research was to investigate the usability of wheat bran in the production of L-lactic acid via fermentation applying by newly isolated lactic acid bacteria (LAB) strains belonging to *Lactobacillus* genera and its combinations. Wheat bran previously pre-treated with carbohydrases were fermented with thermophilic and mesophilic LAB belonging to *Lactobacillus sanfranciscensis*, *Lactobacillus delbrueckii*, *Lactobacillus rossiae*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus* and other species. An enzymatic test K-DLATE 08/11 (Megazyme Int. Ireland, Wicklow, Ireland) was used for lactic acid and D/L-lactates determination.

The experiment was carried out using 19 different strains of LAB in wheat bran medium. The results show that the highest lactic acid content is produced by *Lactobacillus sanfranciscensis* MW15 strain (108.6 g/kg), whereas *Lactobacillus crustorum* W19 and *Lactobacillus sanfranciscensis* MR29 strains produce pure L-lactic acid isomer (respectively 84 and 77.8 g/kg).

The application of LAB strains combinations for lactic acid production via fermentation increased total lactic acid production by 14.6 % by revealing the possible synergistic effect of combined LAB strains fermentation. Moreover, the fermentation using combined *L. crustorum* W19 and *L. sanfranciscensis* MR29 strains increased pure L-lactic acid production from wheat bran medium by 29.4 %.

The results confirm that wheat bran could be used for L-lactic acid production using selected enzymes and lactic acid bacteria strains. The results show that the application of combined LAB strains as starter cultures can be successfully used to enhance lactic acid production from bio-treated wheat brans. Moreover, *Lactobacillus crustorum* W19 and *Lactobacillus sanfranciscensis* MR29 strains produce pure L-lactic acid isomer in wheat bran medium.

Keywords: *Lactobacillus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus crustorum*, lactic acid, lactic acid bacteria, wheat bran.

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Poster presentation

Evaluation of Chemical Enhancers for Transungual Delivery

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Abstract

Introduction. Topical therapy of nail diseases is considered highly challenging due to poor permeability of the nail plate for the active substances. Chemical penetration enhancers may be used to improve the drug delivery across the nail plate. In the present study, several chemicals were evaluated as transungual delivery enhancers.

The aim of the study. To determine the efficacy of various chemicals in improving amorolfine HCl and naftifine HCl transungual delivery.

Materials and methods. Freshly slaughtered bovine hooves were used for nail model membrane preparation. The fully hydrated hooves were cut into 60 µm sections by cryotome according to the method of Mertin & Lippold, then prepared bovine hooves membranes were cut to produce membranes of 5 mm x 5 mm. For “enhancer only” formulations the predefined concentrations of chemicals in acidified water (pH 3) were produced. Membranes were placed into 2 ml of above formulations and kept at 35°C for 24 hours. Then the hooves’ membranes were washed with acidified water (pH 3) and dried for 4 hours at 40°C. Hooves’ membranes were weighed, placed into 2 ml of active substance solutions (500 µg/ml) and incubated for 24 hours at 35°C. Membranes were washed and active substances were extracted and analyzed. The controls were carried out using “drug only” formulation. Three replicates were used for each enhancer.

Results. The keratolytic agent – salicylic acid failed to enhance uptake of naftifine HCl, but increased amorolfine HCl uptake two-fold if compared to control. It was determined, that nonionic surfactants Tween 60 or Tween 40 decrease the uptake of tested drugs when compared to control. 5%, 10% thioglycolic acid and 10% urea increased both amorolfine HCl and naftifine HCl uptake two-fold compared to control. Also, it was observed, that 10% thioglycolic acid aggressively etches the surface of membranes, and such concentration should not be recommended for human nails treatment.

Conclusions. 5% thioglycolic acid and 5% urea were identified as potential enhancers for incorporation into amorolfine HCl and naftifine HCl formulations for transungual delivery.

Keywords: naftifine hydrochloride, amorolfine hydrochloride, transungual delivery.

Poster presentation
Capillary Gelelectrochromatography

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Abstract

Capillary electrochromatography is an analytical technique combined with high-performance liquid chromatography and capillary electrophoresis. This analytical technique is based on separation of molecules according to mass and charge ratio and the affinity to the stationary phase. An important feature in capillary electrochromatography is the electroosmotic propulsion of the mobile phase. In our previous research, it was demonstrated, that entirely homogeneous agarose thermoreversible gel with covalently attached ionic groups and alkyl ligands can be used for reversed phase electroseparation of hydrophobic neutral molecules according to reversed phase mechanism. To our knowledge, there is no report of capillary electrochromatography for size exclusion separation of the macromolecules. Agarose is a linear polysaccharide polymer that can be used in analytical methods in order to separate molecules. The purpose of this research was to optimize the preparation and modification of agarose gel and use it for capillary gelelectrochromatography. Agarose gel was modified using epoxyamine in 2% of NaOH medium with NaBH₄ additive. The reaction was performed for 24 hours. Capillary was filled with molten modified agarose gel using pressure. 75 µm I.D. fused silica capillary was filled with prepared modified agarose gel for the analysis of neutral macromolecules using electrical field. The results obtained will be reported in the presentation.

Keywords: capillary electrochromatography, agarose gel, neutral molecules.

Acknowledgements: Financial support from Research Council of Lithuania project 09.3.3-LMT-K-712-03-0128 is acknowledged.

Poster presentation

Acrylic Continuous Beds for Bioseparation: Synthesis and Characterization

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Abstract

Continuous beds, also described as monoliths, are the fourth generation of stationary phases. S. Hjertén was the first who published about continuous beds fabrication and application of high-performance liquid chromatography in 1989 [1]. Since then continuous beds have gained a lot of interest, because of its easy preparation and rapid high-efficiency separation. Over the years, there is a trend of miniaturization and for this reason, capillary monolith columns are a great solution.

In this work a reviewed how capillary for liquid chromatography is modified using Bind Silane, how during *in situ* polymerization continuous bed columns are prepared using a solution of acrylic monomers and other additional compounds will be described [2]. Additionally, an evaluation of porosity, hydrodynamic properties and other characteristics of continuous beds will be reviewed [3].

Keywords: continuous beds, monoliths, capillary liquid chromatography.

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Poster presentation**Assessment of Concentrations of Antibiotic Accumulated into *Escherichia coli* using Mathematical Modeling**Violeta Vaitkevičienė, Lina Ragelienė*Vytautas Magnus University, Faculty of natural sciences, Department of Biochemistry, Vileikos st.8, Kaunas**Corresponding author: violeta.vaitkeviciene@vdu.lt***Abstract**

Nowadays bacteria can be resistant to multiple antibiotics. This study is aimed to assess the antimicrobial activity of tetracycline (TC)—against *Escherichia coli* during mathematical modeling. Some aspects of the tetracycline mode of action, including pH, temperature, EDTA, some heavy metals and sorption on bacteria surface were investigated.

The model was then used to prove the possibility of approximating tetracycline's bioaccumulation using only a single species of the antibiotics, specifically neutral, zwitterionic species. Uptake of antibiotics by bacteria was described and computed as adsorption of neutral species of antibiotics with their $\log K$ values 2.36 for tetracycline. In addition, the tetracycline was combined with an extracellular solution in order to assess any synergistic effect of elements existing. The created model also made it possible to assess the speciation of tetracycline in aqueous phase under various conditions. This method could prove as a potential alternative to laboratory routine.

Keywords: antimicrobial activity, mathematical modeling, *E. coli*, tetracycline.

Poster presentation

Associations of *SIRT1* rs12778366, *FGFR2* rs2981582 and *STAT3* rs744166 Polymorphisms With Early Age-Related Macular Degeneration Development

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Abstract

Introduction. Age-related macular degeneration (AMD) is progressive eye condition that affects central part of a retina (macula). Etiology and pathogenesis of the disease are not clear [1]. On the other hand, drusen formation caused by changes in lipid metabolism, inflammation and pathological angiogenesis are considered as main pathological processes in AMD development [2]. The aim of the study was to determine the association of polymorphisms in genes involved in AMD pathogenesis: *SIRT1* rs12778366, *FGFR2* rs2981582, *STAT3* rs744166 and early AMD development.

Materials and methods. 284 patients with early AMD and 800 healthy control group subjects were examined. DNA was extracted from peripheral blood leukocytes using DNA salting-out method. Genotyping was carried out using real-time polymerase chain reaction (RT-PCR) method. Statistical analysis was performed with „SPSS version 20.0“.

Results. *FGFR2* rs2981582 AA genotype compared to GG and GA is associated with decreased risk of early AMD in overall group ($p=0.037$), as well as in males group ($p=0.025$). Also, AA compared to GG and to GG and GA genotypes together is associated with decreased risk of early AMD development in younger age ($p=0.035$ and $p=0.014$, respectively). GA genotype compared to GG and AA is associated with 1.7-fold increased risk of early AMD ($p=0.031$).

STAT3 rs744166 GG genotype compared to AA, also, to AA and AG genotypes together is associated with decreased risk of early AMD development ($p<0.001$ and $p<0.001$, respectively). AG and GG genotypes compared to AA are associated with decreased risk of early AMD development ($p=0.041$) as well as each G allele ($p<0.001$). GG genotype compared to AA is associated with decreased risk of early AMD in younger and older age groups ($p=0.022$, $p=0.039$, respectively). The same results remain when GG compared to AA and AG genotypes together ($p=0.018$, $p=0.013$, respectively).

Conclusions. *FGFR2* rs2981582 GA is associated with increased risk of early AMD in males while AA is associated with decreased risk in males and in older age. *STAT3* rs744166 AG and GG genotypes and each allele G are associated with decreased risk of early AMD in females. GG genotype is associated with early AMD development in younger and older age groups.

Keywords: *SIRT1*, *FGFR2*, *STAT3*, SNP, early age-related macular degeneration.

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Poster presentation

Variants of Collagen Matrix Pathway Genes In Exudative AMD Development

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Abstract

Introduction. One of the leading causes of irreversible vision loss in developed countries is age-related macular degeneration (AMD) [1]. The major marker for AMD is drusen formation between retinal pigment epithelium (RPE) and the inner collagenous zone of Bruch's membrane [2]. Recently was suggested that the collagen matrix pathway genes *COL10A1* and *COL8A1* are associated with advanced age-related macular degeneration [3]. According to those findings, we aimed to determine the association between *COL8A1* rs13095226 and *COL10A1* rs1064583 single nucleotide polymorphisms (SNPs) and exudative AMD development.

Materials and Methods. Our study involved 844 participants including 300 patients with exudative AMD form and 544 healthy persons from the control group. Peripheral blood samples from all study subjects were collected into EDTA collection tubes. DNA was extracted using DNA salting-out method. Genotyping was carried out using real-time polymerase chain reaction (RT-PCR) method. Statistical analysis was performed with „SPSS version 20.0“.

Results. Statistical analysis showed that *COL8A1* rs13095226 genotype (AA, AG, GG) distributions did not differ statistically significantly between exudative AMD and control groups (37.7%, 54.7%, 7.7% vs. 41.9%, 52.4%, 5.7%, respectively, $p=0.329$). On the other hand, *COL10A1* rs1064583 genotype (TT, TC, CC) distribution analysis revealed statistically significant differences between exudative AMD and control groups (60.0%, 33.0%, 7.0% vs. 65.6%, 31.4%, 2.9%, respectively, $p=0.015$). Binomial logistic regression analysis showed that genotype CC at rs1064583 compared with is associated with 2.6-fold increased risk of exudative AMD development under the codominant model (OR=2.6031 95% CI:1.326-5.111; $p=0.005$) and with 2.5-fold increased risk of exudative AMD under the recessive genetic model (OR=2.484; 95% CI:1.276-4.837; $p=0.007$). Also, each copy of allele C is associated with increased risk of exudative AMD development under the additive (OR=1.335; 95% CI:1.048-1.701; $p=0.019$).

Conclusion. *COL10A1* rs1064583 is associated with exudative AMD development and may be involved in the pathogenesis of exudative AMD.

Keywords: *COL10A1* rs1064583, AMD, collagen.

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rs5882 and rs708272 Association with Early Age-Related Macular Degeneration In Younger Age

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Abstract

Introduction. Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in elderly people [1]. The main pathological hallmark of the disease is drusen or lipid and protein deposits formation in the RPE and the Bruch's membrane (BrM) [2]. Genetic variants in genes encoding components of lipid metabolism have been found to result in the lipid particle deposition, formation of drusen in the retina as well as the BrM, thereby affecting retinal function [3]. Cholesteryl ester transfer protein (CETP) regulates the concentration of cholesteryl esters in high-density lipoproteins (HDL) and transfers oxidized lipids from the outer segments of the photoreceptors and other membranes to HDL-like lipoprotein particles [4-5]. Therefore, single nucleotide polymorphisms (SNPs) in *CETP* may lead to the accumulation of oxidized lipids, which contributes to the development of AMD [5].

Materials and methods. A total of 296 subjects aged <65 years were examined, including patients with early AMD (n=74) and healthy controls (n=222). DNA was extracted from 200 µL venous blood using the silica-based membrane technology utilizing a genomic DNA extraction kit according to the manufacturer's recommendations. The genotyping of *CETP* polymorphisms (rs5882, rs708272) was carried out using the real-time PCR.

Results. Statistical analysis showed that rs5882 and rs708272 genotype distributions in patients with early AMD and healthy controls were similar and did not reveal any significant associations between rs5882 and rs708272 SNPs and early AMD development in patients aged <65 years.

Conclusions. rs5882 and rs708272 variants in *CETP* gene do not contribute to early AMD development in younger age.

Keywords: rs5882, rs708272, age-related macular degeneration, single nucleotide polymorphisms.

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Poster presentation

Sustainable Technology for *Pediococci* Immobilization and Immobilized Cell Using for Higher Value Bread Preparation

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Abstract

The aim of this study was to evaluate the potential use of *Pediococcus acidilactici* LUHS29 immobilized in apple pomace in the case to apply in barley sourdough fermentation for functional bread production. The strain was phenotypically characterized by the growth and acidification rate, carbohydrate metabolism and resistance to acidic conditions. The effect of immobilized bacterial cells on antioxidant properties of barley sourdough and on the acrylamide content in wheat-barley bread was analyzed. The phenotypic and molecular testing indicates the *P. acidilactici* having a versatile carbohydrate metabolism and acid resistance, showing 42.7% of viable cells surviving after incubation at low pH as compared to the initial number (7.5 log₁₀ CFU/g). Fermentation with immobilized strain increased by 15.3% the production of LA compared to spontaneous fermentation (24.2 g/kg), and the ability to produce L-lactic acid contents up to 92.7% from the total LA. The use of *P. acidilactici* for barley sourdough fermentation increased b-glucan solubility by 1.3 - 5.1%, moreover, the total phenolic compounds (TPC) content and radical scavenging activity were found higher up to 34.6% and 79.7%, respectively. Addition of barley sourdough at a level of 10% could reduce acrylamide content in bread up to 44% and retard bread staling process. The application of immobilized in apple pomace bacterial cells could have the future impact for the food industry due to the bioactive potential.

Keywords: *P. acidilactici*, immobilization, apple pomace, fermentation, bread, acrylamide.

Poster presentation**Estimation of The Antitumor Activity Of Synthesized Peptides Z-Gly-Val-D-Ala-OH And Val-Val-Leu-Arg-OH 2HCl**

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Abstract

The antitumor activity of the synthesized peptides Z-Gly-Val-D-Ala-OH and Val-Val-Leu-Arg-OH 2HCl on the model of Ehrlich ascites carcinoma (EAC), as well as the antitumor effect of the combined action of these peptides and the pharmacopeia substance Doxorubicin was investigated in vivo.

Material and methods. Male ICR mice (n = 100) weighing 19-21 g subcutaneously were grafted onto the external surface of the thigh (1×10^6 cells / 0.2 ml of medium 199) of EAC. Z-Gly-Val-D-Ala-OH or Val-Val-Leu-Arg-OH·2HCl alone at doses of 70 mg/kg or 100 mg/kg or with Doxorubicin at dose 2 times per 4 mg/kg was administered for 5 days. A group of EAC tumor-bearing mice (n = 10) served as a control. Linear tumor dimensions (in mm), the tumor volume (in mm³) and the inhibition of tumor growth (ITG, %) are calculated on the 1st, 7th, 14th days.

Results. The inhibition of tumor growth (ITG) was observed during whole experiment with maximum effect on 7 day of administration as Z-Gly-Val-D-Ala-OH (ITG-22% at a dose of 70 mg / kg and 33% at 100 mg / kg) and Val-Val-Leu-Arg-OH 2HCl (ITG - 42% at a dose of 70 mg / kg and 48% at 100 mg / kg). Combined usage of the studied peptides with doxorubicin revealed an increase in inhibition of tumor growth, which indicates the absence of antagonism between the investigated substances.

Keywords: peptides, antitumor activity, Ehrlich ascites tumor model.

Poster presentation

L-Lactic acid production from wheat straw using combined lactic acid bacteria strains belonging to *Lactobacillus* genera

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Abstract

Recently, considerable interest has arisen to bio-recycle of the agro by-products such as straw into the valuable chemicals such as lactic acid. Cheap cellulosic materials are regarded as economically attractive feedstocks for lactic acid fermentation, which allow the utilization of agro waste as source of carbohydrate [1]. Lactic acid is a platform chemical, and its salts have a long history of commercial uses and applications [2]. Higher economical effect has been found by using biological conversion of wheat biomass to lactic acid vs chemical synthesis by increasing the energy efficiency by 47% and decreasing the total costs by 17% [3]. The chemical route produces a racemic mixture of DL-lactic acid, while optically pure L(+)- or D(−)-lactic acid can be obtained by microbial fermentation. Since elevated levels of the D-isomer are harmful to humans, L-(+)-lactic acid is the preferred isomer in food and pharmaceutical industries [4], therefore the search of microorganisms producing high content of L-lactic acid from lignocellulosic material such as wheat straw has outstanding importance.

The aim of the research was to investigate the usability of wheat straw in the production of L-lactic acid via fermentation applying by newly isolated lactic acid bacteria (LAB) strains belonging to *Lactobacillus* genera and its combinations.

Agro-industrial by-products were fermented with thermophilic and mesophilic LAB belonging to *Lactobacillus sanfranciscensis*, *Lactobacillus delbrueckii*, *Lactobacillus rossiae*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus* and other species. Before fermentation enzymatic treatment of by-products was carried out using carbohydrases. An enzymatic test K-DLATE 08/11 (Megazyme Int. Ireland, Wicklow, Ireland) was used for lactic acid and D/L-lactates determination.

The results show that the proposed mixed LAB starter cultures of mesophilic *L. sanfranciscensis* MW15, *L. crustorum* W19 and *L. sanfranciscensis* MR29 strains and combination of thermophilic *L. delbrueckii* subsp. *bulgaricus* DSM 20081 and *L. delbrueckii* subsp. *bulgaricus* MI strains can be successfully used to enhance lactic acid production from bio-treated wheat straw. Moreover, *L. crustorum* W19 and *L. sanfranciscensis* MR29 strains as well as a mixture of those strains could be used for pure L-lactic acid isomer from wheat straw medium production by revealing the possible synergistic effect of combined LAB on L-lactic acid production.

Keywords: Cellulase, *Lactobacillus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, lactic acid, lactic acid bacteria, wheat straw.

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Poster presentation

Introduction of *Geranium macrorrhizum* L. and evaluation of biologically active compounds in overground parts during different plant vegetation periods

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Abstract

The issue of improving the quality of life and health of the World Health Organization (WHO) and biodiversity is being resolved. XIX century an important role in the solution of this problem is played by medicinal, herbal (aromatic) plants, their medicinal plant material and bio-active compounds accumulated therein [1].

The aim of these chemical analyses is to determinate total content of phenolic compounds, the total content of flavonoid compounds and radical scavenging activity of *Geranium macrorrhizum* L. methanolic extracts in the different vegetation periods: intensive growth, flower budding, beginning of blossoming, massive blossoming and the end of blossoming. The object of these analyses is *G. macrorrhizum* L. It is a perennial herbaceous plant of *Geraniaceae* family which is widely spread in Central Europe. In Lithuania *G. macrorrhizum* L. doesn't grow naturally, but it is grown in flower beds, botanical gardens [2]. The extracts studied come from Sector of Medicinal Plants, Kaunas Botanical Garden of Vytautas Magnus University were harvested during the year 2016.

According to the literature, *G. macrorrhizum* L. extracts made from broad-leaved herbal remedies act anti-inflammatory, antiseptic and anesthetic, reduce blood pressure, and have a sedative effect. It also inhibits bleeding, cures, acute chronic colitis, gastric ulcer and gastritis. It is used in the treatment of hypertension in patients with kidney stones, rheumatism, gout, diarrhea, oral inflammation of the oral cavity, bleeding in the gum and bronchitis. Externally, the roots of *Geranium macrorrhizi* radix are used to wash swelling wounds, to nourish swabs, to treat opal, skin diseases, itching, dermatitis of allergic origin and phlegmons [3].

In this study determination of phenolic compounds was performed using spectrophotometry methods. The total amount of phenolic compounds was evaluated using modified Folin–Ciocalteu reagent spectrophotometry method. Modified colorimetric aluminum chloride method was carried out to determinate the total content of flavonoid compounds. Lastly, the radical scavenging activity was estimated by DPPH (2,2-diphenyl-1-picrylhydrazyl) [4].

Keywords: *Geranium macrorrhizum* L., phenolic, flavonoids, radical scavenging activity, spectrophotometry methods.

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Poster presentation**Evaluation and Comparison of Two Emulsion Systems for Semisolid Preparation Modeling**

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Abstract

Selection of semisolid base for dermatology products has an essential role in drug absorption and transdermal delivery of active substance. The aim of this study was to formulate stable emulsion system for semisolid drug form. There was made two emulsion systems with different content. The first ones aqueous phase contained glycerin, water and phenoxyethanol and oil phase contained grapeseed oil and cetyl stearyl alcohol (lanette). The second formulation was gelified emulsion and contained oil phase of grapeseed oil, the aqueous phase of polymeric emulsifier (pemulen), water, glycerin and phenoxyethanol. For this formulation triethanolamine was used as gelling agent.

The stability studies of these emulsion systems were conducted with centrifugate at 3000rpm for 5minutes. Texture studies were performed using Texture Analyzer. pH value was determined using pH meter.

Both emulsion systems were stable after centrifugation test. Texture Analyzer showed good texture properties such as firmness, consistency and cohesiveness for both emulsion systems. The first emulsion system had 5,6 pH, its preparation required heating and could be used as a semisolid base for thermostable substances. On the other hand, the second formulation – gelified emulsion, had higher pH value – 6,7 and its preparation doesn't involve heating, so it can be used for thermolabile substances.

Keywords: emulsion system, semisolid base.

Poster presentation

Cosmetic applications of fungal extract

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Abstract

As trend to use natural products in cosmetics and cosmeceuticals grows, the need to produce new cheap, natural, safe and effective bioactive compounds from natural sources increases too [1], [2]. The aim of this study is to make a crude extract with antioxidant and/or enzymatic properties using solid-state fermentation (SSF) of wheat bran with two fungal strains and prepare a cosmetic product for desired purpose. Two *Pleurotus* spp. edible fungi – *P. ostreatus* and *P. eryngii*, are used in this research. Those fungi are considered as safe for medicinal properties and are attractive sources of natural antioxidant, antimicrobial, anti-inflammatory compounds and oxidative enzymes which could protect respectively from cell aging and oxidative damage of free radicals, microorganism growth in cosmetic products and they may improve skin tone and texture, lighten skin [3]. It is known that solid-state wheat fermentation can enhance the extraction yield, total phenolic, flavonoid and antioxidant activity [4]. This study first examines optimal conditions for the wheat bran fermentation. Two different speeds (6 rpd and 12 rpd) for the rotating drum bioreactor and three different wheat bran moisture levels (67%, 75%, and 80%) are set (6 jars with *P. eryngii* homogenized inoculum and 6 jars with *P. ostreatus*). Crude extract will be prepared by hot water extraction. Spectrophotometric methods such as determination of total phenolic compounds using Folin-Ciocalteu reagent, the total content of flavonoids, antioxidant activity measurement by radical scavenging activity (DPPH), laccase and peroxidases activity measurement by monitoring ABTS enzymatic oxidation will be performed. After determining the optimal conditions, they will be used for the obtainment of crude extracts and the formulation of a skin cream considering extract physicochemical properties. DPPH radical scavenging and ABTS enzymatic oxidation will be measured to examine antioxidant and enzymatic changes in cream formulation. Skin penetration tests, toxicity effect and dermatological assays will be performed as well. Comparable studies have shown that *P. ostreatus* mycelium extract at 10 mg/ml have 96% of DPPH and 55% of ABTS scavenging activity [3]. Wheat bran fermented with *P. eryngii* for poultry feed showed increasing lignocellulolytic enzymes activities in fermented wheat bran [5]. Fermented wheat bran with two *Pleurotus* spp. mushrooms could be used as a source of bioactive compounds for cosmetic applications considering that all the used material is safe and demonstrates antioxidant and enzymatic activity however further investigation has to be made.

Keywords: *P. ostreatus*, *P. eryngii*, solid-state fermentation, antioxidants, enzymatic activity, cosmetics

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Poster presentation

Airborne Microorganisms Sampling

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Abstract

According to Baron and Willeke [1] an aerosol is an assembly of solid or liquid particles suspended in a gaseous medium like air. Bioaerosols contain a heterogeneous mixture of particles from animal, microbial origin and plant. In our study fungi and bacteria are main particles of bioaerosols. The size bacterial cells (0.5-40µm), fungal spores (1.0 - 40µm). The methods used in airborne sampling – filtration, impaction or liquid impingement [2]. Before calculating or identification microbes are mainly based on cultivation, microscopy, molecular biological, immunochemical or biochemical methods. Method for fungi or bacteria - cultivation methods, based on direct impaction on a growth media. After incubation, microbial colonies identified by their morphological – genus or species level. The microbes composition and concentration differ with the weather, season, location, time of the day. By cultivation of air samples from homes dominant fungi in indoor air are *Penicillium*, *Aspergillus*, *Cladosporium* spp.

Keywords: bioaerosols, fungi, bacteria, airborne sampling

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