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NORDIC SEPARATION SCIENCE SOCIETY



13<sup>th</sup> International Scientific Conference  
**THE VITAL NATURE SIGN**

May 16th – 17th, 2019

Kaunas, Lithuania

**ABSTRACT BOOK**

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# 13<sup>th</sup> International Scientific Conference "The Vital Nature Sign"

## Programme

Thursday, 16<sup>th</sup> of May 2019

Venue: Vytautas Magnus University, Small Hall, S.Daukanto 28, Kaunas, Lithuania

8:30	09:00	<b>Registration</b>	
9:00	<b>Welcome speech: Prof. habil. dr. Audrius Maruška</b>		
9:15	10:45	<i>I session Chairmans: prof. Audrius Maruška, dr. Nicola Tiso</i>	
9:15	prof. dr. (HP) Arvydas Povilaitis	Agriculture Academy Vytautas Magnus University	Nitrate Removal from Tile Drainage Water Using Woodchip Denitrification Bioreactors
9:45	dr. Vita Tilvikienė	LAMMC Institute of Agriculture	The Perspective of Growing Non-food Crops in Changing Climate Conditions
10:15	dr. Ina Jasutienė	Kaunas University of Technology	Fortification of Foodstuffs by Water and Oil Soluble Vitamins Using Emulsion as Delivery System
10:30	dr. Vytautė Šakienė	Lithuanian University of Health Science	Nutraceuticals Technology Based on Biomodified Lupine Protein Isolates
10:45	11:15	<i>Coffee Break</i>	
11:15	12:00	<i>II session Chairpersons: dr. Vita Tilvikienė, dr. Vilma Kaškonienė</i>	
11:15	dr. Vita Lele	Lithuanian University of Health Science	Cereal By-products Microbial and Enzymatic Conversion to Higher Value Products
11:30	Paulina Zavistanavičiūtė	Lithuanian University of Health Sciences	The Concept of Berries/Fruits and Dairy Industry By-products Valorization, in Combination with Antimicrobial Lactic Acid Bacteria, for the Preparation of Antimicrobial Coatings
11:45	dr. Arvydas Kanopka	Vilnius University, Institute of Biotechnology	Interplay Between Hypoxia and Pre-mRNA Splicing
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13:15	dr. Wojciech Łuczaj	Medical University of Białystok	The Effect of Cannabidiol on Keratinocytes Phospholipid Profile
13:30	Agnė Brazaitytė	Lithuanian University of Health sciences	Evaluation of Biological Activity of Different Extracts from Feverfew ( <i>Tanacetum parthenium</i> L.) to Glioblastoma Multiforme C6 Culture Cells
13:45	Yasaswini Kooniambedu Gunasekaran	Lithuanian University of Health Sciences	Technology and Characteristics of the Plant Based Proteinaceous Snack Prepared from Not Treated, Fermented and Ultrasonicated Peas
14:00	Eglė Zokaitytė	Lithuanian University of Health Sciences	Nutraceuticals Formulation Based on <i>Artemisia absinthium</i> Essential Oil, <i>Lactobacillus uvarum</i> LUHS245 Encapsulated in Whey and Blackcurrant Juice Preparation By-product
14:15	Baltramiejus Jakštys	Vytautas Magnus University	Investigation of Intracellular Content Release After Irreversible Electroporation In Vitro
14:30	dr. Paulius Ruzgys	Vytautas Magnus University	The Development of Anticancer Drug-controlled Gene Expression System
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15:15	17:00	<i>Poster session I at the stage (3 min oral speech max 3 slides)</i>	

# 13<sup>th</sup> International Scientific Conference "The Vital Nature Sign"

## Programme

Friday, 17<sup>th</sup> of May 2019

Venue: Vytautas Magnus University, Small Hall, S.Daukanto 28, Kaunas, Lithuania

<b>8:30</b>	<b>09:00</b>	<b>Registration</b>	
<b>9:00</b>	<b>10:30</b>	<i>IV session Chairmans: dr. Tomas Drevinskas, dr. Dmytro Volyniuk</i>	
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<b>9:30</b>	prof. Elena Bartkienė	Lithuanian University of Health Sciences	Lupine Seeds – Alternative Protein Source for The High Value Wheat Bread Preparation
<b>10:00</b>	dr. Marius Dagys	Vilnius university	Application of Bioanalytic and Bio(electro)catalytic Systems
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<b>11:45</b>	Kristina Laužikė	Institute of Horticulture, LAMMC	The Effect of Light Penetration Through Apple Tree Canopy on the Variation of Photosynthetic Indices
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## **ORAL PRESENTATIONS**

## Oral presentation

### Lupine Seeds – Alternative Protein Source for the High Value Wheat Bread Preparation

**Elena Bartkiene<sup>1</sup>, Vita Lele<sup>1</sup>, Ida Jakobson<sup>2</sup>, Grazina Juodeikiene<sup>3</sup>, Daiva Vidmantiene<sup>3</sup>, Iveta Pugajeva<sup>2,4</sup>, Vadims Bartkevics<sup>2,4</sup>**

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#### Abstract

There is a growing interest in industrial exploitation of new protein sources such as plant proteins to broaden the range and variety of foods [1-3]. Lupine products are valued for their GMO free status, functional food properties, nutritional and health benefits and seem to be particularly promising as a source of an innovative food ingredient for the food industry in Europe. Furthermore, lupine exhibits useful technofunctional properties allowing its use as an ingredient in the production of several palatable food products, such as biscuits, pasta and bread. For instance, the supplementation of wheat flour with high-protein legume flours improve the nutritional quality of baked goods, also lupine does not contain gluten thus it could be used as a functional ingredient in gluten-free foods. The aim of the study was to investigate the acrylamide formation during production of wheat bread supplemented with lupine flours fermented by *Lactobacillus* and *Pediococcus* strains. Additionally, bread texture, sensory characteristics and overall acceptability were analysed. The use of fermented lupine resulted in a lower specific volume and crumb porosity of bread on average by 14.1% and 10.5%, respectively, while untreated lupine lowered the latter parameters at a higher level (30.8% and 20.7%, respectively). The addition of lupine resulted in a higher by 43.3% acrylamide content compared to wheat bread (19.4 µg/kg d.w.). Results showed that acrylamide was significantly reduced using proteolytic *L. sakei* and *P. pentosaceus* strains for lupine fermentation. Although the bread supplemented with lupine spontaneous sourdough had the lowest level of acrylamide (15.6 µg/kg d.w.), but it has the malodorous flavour and was unacceptable to the consumers. Finally, the lactofermentation could increase the potential use of lupine as a food ingredient while reducing acrylamide formation and enriching bread with high quality proteins.

**Keywords:** bread, wheat, lupine, protein, sourdough, lactic acid bacteria.

#### References

- [1] E. Bartkienė, V. Bartkevics, J. Rusko, V. Starkutė, D. Žadeikė, G. Juodeikienė, *IJFST*, 2016, 51, 2049-2056.
- [2] E. Bartkienė, V. Bartkevics, J. Rusko, V. Starkutė, E. Bendoraitienė, D. Žadeikė, G. Juodeikienė, *LWT-Food Science and Technology*, 2016, 74, 40-47.
- [3] E. Bartkienė, V. Bartkevics, V. Starkutė, D. Žadeikė, G. Juodeikienė, *Frontiers in Plant Science*, 2016, 7, 1-5.

## Oral presentation

### Technology and Characteristics of the Plant Based Proteinaceous Snack Prepared from not Treated, Fermented and Ultrasonicated Peas

Yasaswini Kooniambedu Gunasekaran<sup>1</sup>, Egle Zokaityte<sup>1</sup>, Vytaute Sakiene<sup>1</sup>, Paulina Zavistanaviciute<sup>1</sup>, Dovile Klupsaite<sup>2</sup>, Vita Lele<sup>1</sup>, Elena Bartkiene<sup>1</sup>

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#### Abstract

Pulses (beans, peas, and lentils) have been consumed for at least 10 000 years and are among the most extensively used foods in the world. A wide variety of pulses can be grown globally, making them important both economically as well as nutritionally. Pulses provide protein and fibre, as well as a significant source of vitamins and minerals, such as iron, zinc, folate, and magnesium, and consuming half a cup of beans or peas per day can enhance diet quality by increasing intakes of these nutrients. In addition, the phytochemicals, saponins, and tannins found in pulses possess antioxidant and anti-carcinogenic effects, indicating that pulses may have significant anti-cancer effects [1]. More recently, the health benefits other than nutrition associated with pulse consumption have attracted much interest. Potential health benefits associated with the consumption of peas, *Pisum sativum* L., specifically green and yellow cotyledon dry peas, also known as smooth peas or field peas were demonstrated [2]. However, food should be attractive for consumers, in case of missing this characteristic, healthy products, as peas, are used not enough often. The aim of the study was to develop technology for plant (peas) based proteinaceous snack preparation and to evaluate characteristics of the created products. For snack preparation non treated, fermented and ultrasonicated peas were used. Additionally, different content of the salt (3.6% and 1%) and different fermentation conditions (submerged and solid state) were tried for the snack preparation. Acidity parameters, microbiological characterization, colour coordinates, texture hardness, overall acceptability and biogenic amines content in the developed products were established. It was found that the lowest pH and the hardest texture after 24 hours of solid state fermentation with *L. uvarum* strain in peas can be obtained ( $4.15\pm 0.01$  and  $1.30\pm 0.03$ , respectively). By using fermentation process, probiotic properties of the peas snack can be obtained (lactic acid bacteria count higher than  $6.0 \lg_{10}$  CFU/g, as well as ultrasonication of the raw material leads to enterobacteria reduction in products. Predominating biogenic amines in peas snack samples were phenylethylamine, putrescine and spermidine. Finally, by using peas lactofermentation and ultrasonication high biological value products can be prepared.

**Keywords:** peas, snack, lactic acid bacteria, fermentation, ultrasonication.

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## Oral presentation

# The Effect of Light Penetration on the Changes of Photosynthesis Indices in Apple Leaves

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### Abstract

Environmental factors strongly influence photosynthetic productivity and plants growth. Photosynthetic productivity depends on light, water, CO<sub>2</sub>, nutrients and other elements like leaf canopy size and architecture. Also, optical properties and photosynthetic indices were affected by seasonal changes.

The aim of this study was to analyze the impact of light penetration into crown and the effect of the agrotechnological tools and seasonality on photosynthetic behavior of apple trees. Apple tree cultivar 'Rubin' was grafted onto dwarfing rootstock P60 and different growth regulating methods were used (pruning, trunks incision, plant growth regulator). Nitrogen balance index and photochemical reflectance index were measured in 1.8 – 2.0 m above ground and 1.0 – 1.2 m above the ground; specific leaf area, fresh and dry weight were evaluated from all canopy in the beginning of apple maturity and harvest time. The significant effect of seasonality on all tested indices was observed. Dry and fresh mass ratio was significantly lower during harvest time compared to summer data, but specific leaf area was significantly lower in July compared to harvest time. Trunk incision had significantly negative effect on photochemical reflectance index (PRI). PRI was significantly lower during harvest time compared to summer results, but nitrogen balance index during harvest time was significantly bigger. Both indices, photochemical reflectance and nitrogen balance, were significantly bigger in higher canopy level (1.8 – 2.0 m) compared to measurements in lower canopy levels (1.0 – 1.2 m.), where light penetration was lower. Summarizing, it can be stated that decreasing light penetration into the canopy results the decrease in NBI and PRI and the plant maturation level has significant affect for all tested indexes.

**Keywords:** apple, nitrogen balance index, photochemical reflectance index, specific leaf area, seasonality

## Oral presentation

### Interplay between hypoxia and pre-mRNA splicing

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#### Abstract

RNA splicing takes place in the nucleus and occurs either co- or post-transcriptionally. Noncoding sequences (introns) in nuclear mRNA precursors (pre-mRNA) are removed by dedicated splicing machinery. The coding sequences (exons) are joined to generate the mature mRNA that is exported to the cytoplasm and translated into protein. Splicing events are tissue-specific.

Oxygen sensing is crucial for cell survival and for a living organism's ability to adapt to changing environments or physiological conditions. Cellular responses to hypoxia involve induction of transcription of a network of target genes, a process which is coordinately regulated by hypoxia-inducible transcription factors (HIFs).

Alternative splicing serves as regulatory platform that allows tissue specific expression and dramatically increases genomic complexity. In hypoxic cells pre-mRNA splicing plays an important role for their adaptation to hypoxic conditions. A striking change has been observed in alternative splicing pattern of genes and alterations in splicing factor expression under pathologic conditions especially in human cancers. Cancer cells are often confronted with a significant reduction in oxygen availability, which is a major reason for changeover of major cellular processes.

**Keywords:** hypoxia, pre-mRNA splicing, cell, oxygen.

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## Oral presentation

### Studies on Sema3C Function in Gliomagenesis

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#### Abstract

Secreted class 3 semaphorin (Sema3) proteins are involved in the regulation of cancer angiogenesis by interacting with components of the vascular endothelial growth factor (VEGF) signaling pathway. The relationships between the expression levels of individual semaphorins and their effectors and patient survival were determined in various cancer types. Our studies up to date were mainly focused on one of the Sema3 family member, Sema3C, since we recently detected a dramatic increase of Sema3C expression in gliomas of the highest grade (glioblastomas) and this increase was associated with the poor survival prognosis. With the aim to further investigate the role of Sema3C in gliomagenesis, we are currently exploring molecular mechanisms that underlie the tumor angiogenesis and the invasiveness of glioma cancer cells.

**Keywords:** semaphorins, Sema3C, VEGF, NRP1, gliomagenesis, angiogenesis

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## Oral presentation

# The Concept of Berries/Fruits and Dairy Industry By-products Valorization, in Combination with Antimicrobial Lactic Acid Bacteria, for the Preparation of Antimicrobial Coatings

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### Abstract

In this study the concept of the valorization of berries/fruits (B/F) (raspberries, blackcurrants, apples, rowanberries) and dairy industry (whey) by-products, in combination with antimicrobial lactic acid bacteria (LAB), for the preparation of antimicrobial coatings was analysed. Antimicrobial activities of the B/F by-products and LAB (thirteen LAB strains were estimated against fifteen pathogenic and opportunistic strains) were evaluated, while whey substrate for the selected and the highest antimicrobial activities showing LAB cultivation was used. It was established that B/F by-products can be promising ingredients for the preparation of antimicrobial coatings, and their antimicrobial activity can be enhanced in combination with the *Lactobacillus uvarum* LUHS245 and *Lactobacillus casei* LUHS210 strains. Whey is a suitable and sustainable substrate for selected LAB biomass preparation. Finally, in most cases, the highest antimicrobial activity was shown by the B/F–LAB coatings prepared with the LUHS210 strain, compared with LUHS245.

**Keywords:** berries/fruits, by-products, lactic acid bacteria, antimicrobials, valorization

**Acknowledgements.** This research is funded by the European Regional Development Fund according to the supported activity ‘Research Projects Implemented by World-class Researcher Groups’ under Measure No. 01.2.2-LMT-K-718.

## Oral presentation

# Fortification of Foodstuffs by Water and Oil Soluble Vitamins Using Emulsion as Delivery System

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### Abstract

Vitamins are a well-known group of compounds that are essential for human health. Our intake of vitamins depends on our diet. However, even foods that contain the necessary vitamins can have reduced vitamin content after storage, processing, or cooking. Therefore, scientists are looking for ways to protect vitamins from the harmful effects of technological factors. One of such possibility is encapsulation. Among different encapsulation systems, water in oil in water ( $W_1/O/W_2$ ) double emulsion is the good method for incorporation of sensitive biomolecules.

The emulsion, prepared from water, rapeseed oil, way protein isolate and polyglycerol polyricinoleate, was characterised by microscopy, droplets size and rheological characteristics measurements. Vitamin C, folic acid and B<sub>12</sub> were encapsulated into the inner aqueous phase ( $W_1$ ), vitamins D<sub>3</sub> and A were encapsulated to the oil phase. Reverse phase HPLC separation technique was used for vitamin quantification.

Composition of inner aqueous phase was studied after preparation. It was found that homogenization did not cause vitamin loss: obtained content was very close to the incorporated 21 mg/g vitamin C, 0.28 mg/g folic acid and 0.16 mg/g B<sub>12</sub>. Encapsulation efficiency of water-soluble vitamins was studied in freshly prepared emulsion and after storage for 20 days at 4 °C. Encapsulation efficiency of water-soluble vitamins was 76-87 %. Stability of vitamin D<sub>3</sub> and A during storage was also studied. Those vitamin C, A and folic acid are sensitive to heat treatment, the effect of pasteurization on the vitamin concentration in the emulsion were studied. Pasteurization did not have effect on the amount of vitamins, encapsulation was a good way to protect vitamins from thermal degradation. Release of vitamins during *in vitro* digestion, consisting on a gastric and small intestinal phase, was studied. Incorporation of vitamins B<sub>12</sub> and folic acid into inner aqueous phase of double emulsion gave them a significant protection in simulated gastric juice, whereas intensive release of vitamins (96-100 %) was observed after the intestinal phase of digestion. 50–70 % of incorporated vitamins A, D<sub>3</sub> and C were released during the gastric stage of digestion. Investigated double emulsion is good tool for the encapsulation of vitamins and has potential application in food fortification.

**Keywords:**  $W_1/O/W_2$  emulsion, encapsulation, water and oil soluble vitamins.

**Acknowledgments:** The research was granted by Research Council of Lithuania, project No. 01.2.2-LMT-K-718-01-0017.

## Oral presentation

### Nutraceuticals Technology Based on Biomodified Lupine Protein Isolates

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#### Abstract

As an addition to a normal diet, the use of food enriched with functional compounds, lately became very popular. Functional ingredients can be incorporated in more acceptable for consumers form, for example, gummy candies [1]. The aim of this study was to adapt lactofermented lupine protein isolates, *C. paradise* EO, and xylitol as a low glycemic index and antimicrobial properties showing sweetener for gummy candies preparation. For gummy candies preparation SMF lupine variety Vilniai protein isolate was selected, in which the highest protein content was observed ( $90.11 \pm 1.63\%$ ). According to our study, SMF Vilniai lupine protein isolate has one from the lowest TIA ( $19.40 \pm 0.48\%$ ), the highest protein digestibility ( $89.94 \pm 0.87\%$ ), and the highest genistein content ( $30.93 \pm 0.47 \mu\text{g g}^{-1}$ ) in comparison with other protein isolates. Also, to reduce a total energy value of the gummies, sugar was replaced by xylitol. To get a sour taste and hard texture of gummies, which is more acceptable for consumers, citric acid was changed to ascorbic acid. By using ascorbic acid and xylitol, texture hardness and overall acceptability of the gummy candies were increased ( $0.7 \text{ mJ}$  and  $108.7 \pm 2.9$ , respectively). The strong positive correlation was observed between gummies texture hardness and overall acceptability ( $r=0.8461$ ). For gummy candies prepared without and with grapefruit EO, in all the cases, higher acceptability was found of the gummies with EO, and it should be mentioned that grapefruit EO was a perfect agent for the lupine taste masking (overall acceptability of the gummies with  $0.5 \text{ g}$  of protein isolates without EO was  $93.6 \pm 4.2$  and overall acceptability of the gummies with  $0.5 \text{ g}$  of protein isolates with EO  $125.7 \pm 5.4$ ). The highest acceptability of the gummy candies prepared with the addition of EO and  $4.0$  and  $4.5 \text{ g}$  of the lupine protein isolate ( $140.0 \pm 2.5$  and  $140.0 \pm 3.2$ , respectively) was observed. In most of the cases fermentation with *L. sakei* strain increases the amino acids content in fermented lupine samples. Gummy candies formula consisting of the xylitol, ascorbic acid, grapefruit EO (up to  $0.2\%$ ), and lupine protein isolate (up to  $13.0\%$ ) allowed obtaining good texture and high overall acceptability products, containing desirable functional compounds.

**Keywords:** lupine, protein isolates, *Citrus paradise*.

## Oral presentation

### Evaluation of Biological Activity of Different Extracts from Feverfew (*Tanacetum parthenium* L.) to Glioblastoma Multiforme C6 Culture Cells

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#### Abstract

Glioblastoma multiforme (GBM) is the most common and lethal primary malignancy of the central nervous system (CNS). Temozolomide (TMZ) based chemotherapy is currently established for GBM patients. However, due to TMZ toxicity there is still a need for an agent that is more effective and safer. Parthenolide is considered to be the primary bioactive compound in feverfew and exhibits antitumor properties on glioblastoma cells. The aim of this study is to assess the effectiveness of three different *Tanacetum parthenium* L. extracts on rat glioblastoma cell culture viability and proliferation.

Three different *Tanacetum parthenium* L. extracts (1:5) were produced by classical maceration method. Extracts consisted of feverfew's dry herb and of three different solvents - purified water, 10% of dimethyl sulfoxide (DMSO) and 40% of ethanol. Total phenolic content of prepared feverfew's extracts was evaluated using Folin-Ciocalteu's spectrophotometric method. Cell viability and proliferation was assessed using Hoechst 33258 and propidium iodide assay.

Results have shown that investigated three feverfew's extracts induce dose-dependent reduction of cell viability. In aqueous extract statistically significant reduction of viability was determined at concentrations of 17.5 µg/µl – 25 µg/µl, in DMSO extract at concentrations of 15 µg/µl – 25 µg/µl, in ethanolic extract at concentrations of 10 µg/µl – 25 µg/µl. Furthermore, propidium iodide/Hoechst assay showed that low concentrations of phenolic compounds of *T. parthenium* extracts can reduce C6 cells proliferation. Incubation of cells with 0.25-1.5 µg of aqueous extract reduced cell proliferation after 48 hours by 16-46 %, incubation with 0.25-1.5 µg of DMSO extract reduced proliferation by 17-49 %, incubation with 0.25-1 µg of ethanolic extract inhibited the growth of cells by 17-44%.

Three different *Tanacetum parthenium* L. extracts showed cytotoxic and anti-proliferative effect on rat C6 glioblastoma cells in a concentration-dependent manner.

**Keywords:** glioblastoma, feverfew, *Tanacetum parthenium*, extracts

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**Oral presentation**  
**Ultrafast Laser-Based Fabrication of Functional 3D Objects: from  
Nanoresolution to Millimeter Size Devices**

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**Abstract**

We present a femtosecond laser-based workstation for high-speed (up to cm/s translation velocity) high-precision (tens of nm) hybrid additive-subtractive manufacturing of functional 3D micro-structures. We unveil the potential of this approach by creating example structures such as photonic elements, various microlenses, 3D scaffolds for cell growth, lab-on-chip devices, microneedles for drug delivery, free-movable 3D microrobots and similar. These objects are made out of multiple materials and/or combining several different additive-subtractive fabrication methods enabled by the use of femtosecond laser. Ways to increase structuring throughput sufficiently to push hybrid femtosecond laser processing from scientific laboratories to the wide-spread solution are also discussed, their effectiveness assessed. Overall, it is shown that hybrid laser 3D manufacturing is a powerful tool for advanced structure fabrication with far-reaching implications in the various scientific fields.

## Oral presentation

### Cereal by-products microbial and enzymatic conversion to higher value products

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#### Abstract

A higher economical advantage could be achieved with the use of by-products from agro-food industries, such as from flour milling industry, as potential resources to produce a higher nutritional and biological value food/feed stock. Wheat and barley are popular cereals for human consumption; however, processing of these cereals produces a high amount of by-products. The bran of wheat and barley is the highest producer of enterolignans in vitro (294.1–321.9 nmol/g) [1]. The aim of this study was to apply the enzymatic treatment and fermentation by *Pediococcus acidilactici* strain for industrial cereal by-products conversion to food/feed bioproducts with high amount of probiotic lactic acid bacteria (LAB). LAB propagated in potato media and spray-dried remained viable during 12 months (7.0 log<sub>10</sub> cfu/g) of storage and was used as a starter for cereal by-products fermentation. The changes of microbial profile, biogenic amines (BAs), mycotoxins, lactic acid (L+/D–), lignans and alkylresorcinols (ARs) contents in fermented cereal by-product were analysed. Cereal by-products enzymatic hydrolysis before fermentation allows to obtain a higher count of LAB during fermentation. Fermentation with *P. acidilactici* reduce mycotoxins content in fermented cereal by-products. According to our results, *P. acidilactici* multiplied in potato juice could be used for cereal by-products fermentation, as a potential source to produce safer food/feed bioproduct with high amount of probiotic LAB for industrial production.

**Keywords:** *Pediococcus acidilactici*, cereal by-products, fermentation, biogenic amines, alkylresorcinols.

#### References:

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## Oral presentation

# Genetical Identification of Antibacterial Agents Producing Microorganisms and Analysis of Bacteriocins and Killer Toxins Produced by Them

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### Abstract

Research on naturally occurring antibacterial agents has attracted considerable scientific attention worldwide over the last twenty years. Instead of chemical food additives, it is possible to use natural antimicrobials released by lactic acid bacteria and yeast. The pure antimicrobials produced by microorganisms or the microorganisms that produce these substances can be used for food bio-preservation or as probiotics in medicine and veterinary medicine. Searching for lactic acid bacteria and yeast in natural foods and researching their application possibilities are relevant worldwide. Depending on the environment, microorganisms adapt, resulting in different strains that can produce antibacterial substances with different properties. Such substances are bacteriocins, produced by lactic acid bacteria and killer toxins, produced by yeast [1, 2].

The aim of this work is genotyping of microorganisms, isolated from cottage cheese, obtained from local milk products producers, which shows antibacterial activity against *S. aureus*, *E. coli*, *M. luteus* and *P. vulgaris* strains. MALDI-TOF MS method was used for this task [3]. *Leuconostoc mesenteroides* lactic acid bacteria and *Kluyveromyces marxianus*, *Debaromyces hansenii*, *Candida zeylanoides*, *Candida inconspicua* yeasts were identified during this work. Additional PGR analysis showed that genotyped microorganisms by MALDI-TOF MS method are the same. To analyze what kind of bacteriocins and killer toxins were produced, capillary electrophoresis was used [4]. Detailed results of the work will be presented during the conference.

**Keywords:** bacteriocins, killer toxins, antibacterial activity, capillary electrophoresis.

**Acknowledgements:** This project was financed by Research Council of Lithuania, grant No. 09.3.3-LMT-K-712-10-0235.

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## Oral presentation

### LED's for Horticulture: Novel Insights in Plant Cultivation

**Giedrė Samuolienė, Akvilė Viršilė, Aušra Brazaitytė, Viktorija Vaštakaitė-Kairienė,  
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#### **Abstract**

Various aspects of cultivation technologies can be employed seeking to manage plant metabolic processes, including properties of light, derived by light emitting diodes (LEDs). Compared to electric incandescent or electric discharge lamps, solid-state lighting in the modern era of electricity reaches higher efficiencies still, with substantial increases expected in the future. This study explores the metabolic changes based on the aspects of technological development and conception of photophysiological point of view. As light is the only energy source for photosynthesis, thus photosynthetic flux density, photoperiod, spectral composition acts on plants as information source determining their growth and development, estimates effectiveness of photosynthesis and bioenergy exchange. Moreover, the spectrum changes during the day, thus, we seek to explore the effects of dynamic lighting spectrum and intensity parameters on plant physiological processes and create lighting conditions that would be more favorable for plants.

**Keywords:** light-emitting diodes, photophysiology, metabolic changes.

## Oral presentation

### Sustainable use of nitrogen of legume species - soybean, pea and clover - in organic crop rotation

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#### Abstract

Grain and forage legumes form root nodules which contain symbiotic bacteria fixing atmospheric nitrogen, which is a significant economic and ecological advantage for a sustainable crop rotation. Field pea and faba bean are the main protein crops cultivated in Lithuania, while soybean is common in the tropical climate countries. Attempts are made to reduce the reliance on the mineral fertilizers by investigating the impact of legume species to the crop rotations in organic agriculture. The organically managed field trials were conducted between 2015 and 2019 to compare the effect of three legume plants – soybean, field pea and red clover in 3-year crop rotation on soil biological and chemical properties and yield of subsequent spring wheat and winter wheat.

While field pea and red clover could be successfully infected by local *Rhizobium* strains, the un-inoculated soybean could not establish symbiosis with the native rhizobium bacteria. Therefore, two *Bradyrhizobium japonicum* strains were tested and results revealed that 16 % more effective nodulation was observed for ‘AGF78’ compared with ‘2490’. The inoculated soybean produced 98 % higher beans’ yield, 7% higher TKW and 6% higher protein content compared with un-inoculated, in the first year of rotation.

Soybean accumulated average 2090 kg ha<sup>-1</sup> of decided material, which was harrowed as the green manure after legume vegetation, pea – 2650 kg ha<sup>-1</sup>, red clover – 1140 kg ha<sup>-1</sup>. The green matter of inoculated soybean had the low nitrogen content (5.58 g kg<sup>-1</sup> DM) comparing with red clover (30.8 g kg<sup>-1</sup> DM) and pea (9.02 g kg<sup>-1</sup> DM), because nitrogen accumulation was directed into soya beans (50.57 g kg<sup>-1</sup> DM), e.g. pea grains – 29.97 N g kg<sup>-1</sup> DM.

The grain yield of spring wheat (II in rotation) and winter wheat (III in rotation) increased significantly following all legume species (I in rotation). The yield of subsequent crops was highest growing barley intercropped with red clover. The effect of soybean and field pea was positive but did not differ significantly from each another. Spring wheat cultivated after barley + red clover, accumulated significantly more protein in grains by 14 %, while other legume plants did not affect it significantly.

**Keywords:** nitrogen fixation, crop rotation, nitrogen accumulation.

## Oral presentation

### **Nutraceuticals Formulation Based on *Artemisia absinthium* Essential Oil, *Lactobacillus uvarum* LUHS245 Encapsulated in Whey and Blackcurrant Juice Preparation By-product**

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#### **Abstract**

The world's nutraceutical market has grown in the last few years, and this growth is expected to continue, yet the high costs of developing, manufacturing and marketing nutraceutical compounds remain a challenge. A nutraceuticals composition of *Artemisia absinthium* water extract and essential oil, *Lactobacillus uvarum* LUHS245 strain multiplied in a whey media, and blackcurrant juice preparation by-product was formulated as chewable tablets. In addition, two texture-forming agents for the nutraceutical preparations were tested (agar and gelatine), and the best formulation regarding quality parameters (colour coordinates, texture, total phenolic compounds content, antioxidant activity and sensory properties) was identified. The antimicrobial and antioxidant nutraceuticals composition of *A. absinthium* essential oil ( $\leq 0.1$  inhibited methicillin-resistant *Staphylococcus aureus* M87fox, *Enterococcus faecium* 103, *Bacillus cereus* 18 01, *Streptococcus mutans*, *Staphylococcus epidermidis* and *Pasteurella multocida*), *L. uvarum* LUHS245 encapsulated in a whey media (inhibited 14 of the 15 tested pathogenic strains) and blackcurrant juice preparation by-product (inhibited 13 of the 15 tested pathogenic strains) formulated as chewable tablets, can be prepared in a sustainable manner. The best formulation contained the *A. absinthium* essential oil, *L. uvarum* LUHS245 encapsulated in a whey media, and blackcurrant juice preparation by-product immobilised in agar, as this formulation showed a 2.1% higher content of total phenolic compounds and 17.7% higher overall acceptability in comparison with its gelatine counterpart. Compositions developed from antimicrobial ingredients of diverse origins can be very attractive for nutraceuticals formulations and, furthermore, can lead to dosereducing and other activities, such as antioxidant properties.

**Keywords:** antimicrobials, *Artemisia absinthium*, essential oil, lactic acid bacteria, blackcurrants, by-products.

## Oral presentation

### The Development of Anticancer Drug-Controlled Gene Expression System

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#### Abstract

Gene therapy for induction of immune response has been a cutting-edge technology for quite some years. Nevertheless, a major drawback of such technology was the control of foreign gene expression. In the case of an overexpression of immune system controlling genes may result in the induction of autoimmune diseases. This way the implementation damage of gene therapy for immune response activation does not meet the obtained benefits. Nevertheless, a specific stimulation of immune system has an immense potential in the field of cancer therapy, since an active and fully functional immune system can recognize and destroy the tumors within a body of the patient without a use of the conventional treatment.

Here we present an *in vitro* study that does show the feasibility of time-controlled gene expression when gene delivery to the cells is performed simultaneously with cytotoxic drug bleomycin. The method of electroporation was used for the delivery of gene and anticancer drug to the cells. The selection of electroporation as a vector of gene and drug delivery to the cell was based on the application properties that are already in use, namely clinical anticancer drug delivery to the tumors (electrochemotherapy) and electroporation gene therapy.

Previously, it has been shown that intracellular transfer of anticancer drug bleomycin via electroporation result in cells death only in between 24 - 72 hours [1]. Routinely, transfection efficiency after successful electrotransfer is measured after 24 hours. Therefore, it was decided to combine both methods in order to obtain temporal control of transfection efficiency. Results clearly indicate a successful cellular transfer of both molecules: bleomycin (anticancer drug), and plasmid DNA (pmax-GFP), thus obtaining a high transfection efficiency within 72 hours and death of the same transfected cells after 72 hours. Moreover, unexpectedly, a combined technique also induced a higher efficiency of anticancer drugs. This allows to achieve anticancer drug induced cell death with 20 times lower bleomycin concentration as compared to electrotransfer without plasmid DNA.

Further development of, this combination of described techniques has a high potential to be implemented in clinics as a therapy. Such innovation could combine targeted anticancer drug delivery to tumors and a gene therapy induced immune system activation with the potential of avoiding autoimmune response.

**Keywords:** controlled gene expression, electrotransfection, anticancer drug.

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## Oral presentation

### Investigation of Intracellular Content Release after Irreversible Electroporation *In Vitro*

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#### **Abstract**

Electroporation (EP) has become a widely used method these days because of its application in many different fields. It has been successfully employed for electro-based therapies for cancer treatment and tissue ablation, gene transfer into cells and tissues, extraction of intracellular compounds from plants, bacterial inactivation, cell-cell electrofusion and more. EP was associated with alterations in the cells plasma membrane that are believed to be electropores which facilitate migration of various molecules from the extracellular environment into cells and vice versa. These alterations lead to cell death in a short time, if cells were incapable to reseal pores or longer time periods if pores were resealed. Process of rapid and prolonged cell death after EP was named irreversible electroporation (IrEP). However, it still remains unclear what are the main reasons causing the cells death after IrEP. In this study, we related the cell death with the loss of important metabolite ATP and damage of the cells plasma membrane to investigate whether loss of ATP plays the main role inducing cell death after IrEP.

For experiments flow cytometry assay for cell number determination at any time after experiments together with the clonogenic assay for final cell viability estimation were employed. In addition, we evaluated the dynamics of the cells plasma membrane resealing. Luciferase-luciferin assay was performed for extracellular ATP determination.

It was found that there is a range of the electric pulse intensity that shrank if an increasing number of pulses was used in which the cells were capable of restoring their plasma membrane and remained vital. Moreover, it was determined that ATP was extracted more due to electrophoresis that occurred during lower intensity but a higher number of pulses rather than after application of more intensive but fewer pulses. After comparison of the results we evaluated that loss of ATP could influence the cell viability after IrEP only at the certain conditions. In addition, after IrEP release of proteins was observed which release efficiency depended on EP parameters.

**Keywords:** electroporation, irreversible electroporation, cell viability, protein extraction, ATP.

## Oral presentation

### The New SI in Chemical Measurements

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#### **Abstract**

On 16th November 2018, a unanimous decision was made by the BIPM Member States, to approve the revision of the International System of Units (SI). Most importantly the definitions of the kilogram, the ampere, the kelvin and the mole. This revision means that all SI units will be defined by the constants that describe our physical world. Thus a set of physical constants got fixed numerical values: the Planck constant ( $h$ ), the elementary charge ( $e$ ), the Boltzmann constant ( $k$ ), and the Avogadro constant ( $N_A$ ), while the speed of light in vacuum ( $c$ ), the luminous efficacy of monochromatic radiation of frequency  $540 \times 10^{12}$  Hz ( $K_{cd}$ ) and the unperturbed ground state hyperfine transition frequency of the Cesium 133 atom (Cs) were defined earlier. This will assure the future consistency of the SI and opens the opportunity for the use of new technologies to implement the definitions. These new definitions will come into force on 20th May 2019.

Although for most common measurements this will have no direct implications, some specific fields of science will have to adapt to the new SI. In terms of chemical measurements, the kelvin, the mole and the kilogram can be considered to be the most important units. In the New SI Avogadro constant will have a fixed numerical value, thus having no relative standard uncertainty. While the molar mass of C-12 (used for current mole definition) will have a relative standard uncertainty of  $0.070 \cdot 10^{-8}$ . Because of this change the molar mass constant will become and experimentally determined value with an estimated relative uncertainty of  $1.4 \cdot 10^{-9}$ . Being of such a small value, these uncertainties does not affect most analytical measurements. Fixing the Avogadro number puts clarity into the definition of the mole, so that it can be considered to be only a scaling factor for relating atomic scale entities. Similar scenarios happen with the kilogram and the kelvin.

Lots of criticism can be found in scientific publications regarding the revised kilogram, mole and kelvin. But with the 2019 redefinition, the SI is for the first time wholly derivable from natural phenomena, with units based on fundamental physical constants.

**Keywords:** the new SI, measurements, metrology.

## Oral presentation

### The effect of cannabidiol on keratinocytes phospholipid profile

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#### **Abstract**

Endocannabinoids interactions with the immune system are involved in the suppression of the body's inflammatory response. Therefore, it was suggested that exogenous cannabinoids, primarily phytocannabinoids, can be the basis to propose possible new pharmacotherapy of inflammatory diseases, including skin diseases. The unique phytocannabinoid which has no psychoactive properties is cannabidiol. Therefore, it can be considered as a potential therapeutic agent in the treatment of skin diseases. One of the problematic skin diseases with an immunological and inflammatory basis is psoriasis, in which the skin is irradiated with UV radiation, and patients are treated with anti-inflammatory drugs. However, UV irradiation also changes skin metabolism including phospholipid metabolism. Therefore, the aim of presented study was to investigate the cannabidiol effect on UVA and UVB-induced alteration in phospholipid profile of human main epidermal cells - keratinocytes cultured *in vitro*. Keratinocytes lipid extracts were analyzed by LC and MS/MS using HILIC chromatography to characterize phospholipid profiles. Phospholipid species responsible for observed changes in the PL profile were selected by use of multivariate statistics (PCA, PLS-DA VIP). Principal discriminant phospholipids belonged to lysophosphatidylcholine, lysophosphatidylethanolamine and phosphatidylinositol classes. Moreover, changes in the ceramide profile of keratinocytes were also examined. Nevertheless, our results provide data on utility of cannabidiol as potential therapeutic compound.

**Keywords:** cannabidiol, phospholipids, ceramides, keratinocytes.

**Acknowledgement:** This study was financed by the National Science Centre Poland (NCN) grant no. 2016/23/B/NZ7/02350.

## Oral presentation

### The Perspective of Growing Non-food Crops in Changing Climate Conditions

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#### Abstract

Non-food crops play significant role in bioeconomy. The interest in such crops is increasing with the new technologies linked to the reduction of non-biodegradable materials in our life. Many warm climate countries are known for their high-yielding crops which could be used for the production of bioplastics, bioliquids, biofuels or other products, but in Northern climate countries this section is new. The main reasons are the short vegetation season and cold weather conditions during the winter. More than ten years ago we could not imagine growing such crops as *Miscanthus* in our country but changing climate conditions and progress in science may be beneficent for new crop integration in our country. It is expected that new crops could positively affect the bioeconomy as well as contribute to the climate change mitigation.

The present study includes the information about the growing and use of non-food crops in different countries (project PANACEA data) and evaluation of the growing intensity of non-food crops such as *Miscanthus giganteus*, *Artemisia dubia*, *Sida hermaphrodita*, *Silphium perfoliatum* in Northern part of temperate climate conditions. As the result of the experiments it is indicated that those crops could be perspective in our country. The highest yield is produced by *Miscanthus giganteus*, but other ones, for example *Artemisia dubia* could be beneficent for its chemical components.



## Oral presentation

### Evaluation of the *In Vitro* Decolorization of Textile Dyes by White Rot Fungi

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#### Abstract

The increased usage of textile dyes creates worldwide environmental problems, since during the dyeing process high amounts of wastewaters are released. It has been estimated that the textile dye industry uses up higher amounts of water than any other industry and that all the released wastewaters are highly polluted [1]. A cost effective and eco-friendly approach for this problem is the biological degradation using microbial systems with ligninolytic enzymes [2].

This study describes the ability of five different isolates of *Basidiomycetes* to decolorize a set of three types of textile dyes: reactive, acid and direct. The isolates were inoculated on Petri dishes containing malt extract agar supplemented by textile dyes. The ability to decolorize the textile dyes present in the medium was evaluated during a 7-day incubation period, during which the most effective isolate was selected. Using the obtained results, it was determined that *I. lacteus* was the most effective fungal strain for textile dye biodegradation among all the investigated fungal strains and further quantitative analysis was performed with *I. lacteus*. The decolorization efficiency in malt extract liquid medium was evaluated by measuring the absorbance of the samples and the control at a determined wavelength for each textile dye using a UV-vis MILTON ROY spectronic 1201 spectrophotometer (USA). It was determined that *I. lacteus* is able to decolor up to 99% of the textile dye contained in the liquid medium within 7 days after inoculation.

**Keywords:** decolorization, textile dyes, *Basidiomycetes*, *Irpex lacteus*.

**Acknowledgements:** The tested textile dyes were kindly provided by dr. Rima Klimavičiūtė from the Department of Polymer Chemistry and Technology, Faculty of Chemical Technology, Kaunas University of Technology.

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**Oral presentation**  
**Recent Advancements in Portable and Autonomous Analytical Instrumentation**

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**Abstract**

Portable analytical instrumentation is interesting due to its applicability for *in situ* analysis. What is more important, current scientific trends show that it is possible to develop autonomous (performing all necessary functions without the intervention of the operator) instrumentation from portable systems.

One of the perspective directions of investigations in chemical analysis are separation techniques due to their versatility analysing different origin substances and capability of determining multiple analytes simultaneously. In this group of works special attention will be given to capillary electrophoresis and (digital-droplet) microfluidics.

During the presentation advances, peculiarities and applications developing portable and autonomous instrumentation will be reviewed. Software, hardware, sampling, signal compensation, sensitivity enhancement, automation and integration aspects will be discussed.

**Keywords:** miniaturization, autonomization, capillary electrophoresis, calculations.

**Acknowledgements:** This research was funded by a grant (Nr. 09.3.3-LMT-K-712-02-0202) from the Research Council of Lithuania.

## Oral presentation

# Synthesis of New Gels of Mixed Mode Separation Mechanism and Their Application in Capillary Electrochromatography

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### Abstract

Capillary electrochromatography, combining the advantages of high performance liquid chromatography and capillary electrophoresis, is one of the most successful separation techniques that can be applied for the separation of complex biological compounds such as pharmaceuticals, natural compounds and chiral molecules. Separation of neutral molecules is also a possible application of capillary electrochromatography. In size exclusion, separation is possible due to molecular mass difference and uncharged molecules are driven through the column by electroosmotic flow. To our knowledge there is no report of capillary electrochromatography for size exclusion separation of the macromolecules reported yet. Aim of this work was to create the stationary phase for the separation of neutral molecules and anions with capillary electrochromatography using contactless conductivity detector. Agarose is a linear polysaccharide polymer that can be used in analytical methods in order to separate molecules [1]. Agarose gel was modified by attaching ionic groups, creating a stationary phase that acts as a molecular sieve - because it is a porous structure that holds up a larger molecular weight analytes, which results in a faster movement of molecules having lower molecular weight through the capillary column in the electric field. The reaction was performed for 24 hours. Modified and 4% pure agarose gel were mixed and washed with electrolyte. 75 µm I.D. fused silica capillary was filled with molten agarose gel mixture using pressure and was used for the analysis of 1% dextran solutions using electrical field. Results showed the ability to perform mixed mode separation of neutral molecules and anions simultaneously. Conditions of analysis were: background electrolyte 30 mM acetic acid, pH 3.3; applied voltage 3.6 kV; total capillary length 40 cm, effective capillary length 35 cm; separation temperature 21 °C. The results obtained will be reported in the presentation.

**Keywords:** capillary electrochromatography, contactless conductivity detector, agarose gel, neutral molecules, anions.

**Acknowledgements:** Financial support from Research Council of Lithuania project Nr. 09.3.3-LMT-K-712-10-0233 is acknowledged.

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## Oral presentation

### Evaluation of Antimicrobial, Insecticidal and Allelopathic Properties of Extracts of *Artemisia dubia* Wall.

Dovilė Jurevičiūtė<sup>1</sup>, Audrius Sigitas Maruška<sup>1</sup>, Vita Tilvikienė<sup>2</sup>, Renata Žvirdauskienė<sup>2</sup>, Laisvūnė Duchovskienė<sup>2</sup>, Aušra Bakšinskaitė<sup>2</sup>

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#### Abstract

Plant biotechnology is one of the most fast-moving areas of biotechnology. The area of plant biotechnology also includes energy crops. These plants are popular because of producing a lot of biomass. This is one of the fastest growing areas of alternative energy. In Lithuania, one of these plants is from the *Artemisia* tribe - *Artemisia dubia* Wall. The aim of this investigation was to evaluate the antimicrobial, insecticidal and allelopathic properties of *Artemisia dubia* Wall., in order to maximize the plant's potential for use. The plant was collected from two different regions of Lithuania: Akademija, Kedainiai district. (55.3896° N, 23.8624° E) and Trakų Vokė, Vilnius district. (54.6238° N, 25.1113° E). Samples were obtained from three different experimental areas with fertilization type: non-fertilized, N90 and N180 fertilizes (fertilizers with different nitrogen amount), during different time of harvest [1]. Collected samples were air-dried in Lithuanian Research Centre for Agriculture and Forestry. In this research, the antimicrobial activity of extracts was evaluated using modified antimicrobial analysis methods [2]. Different bacteria were selected for this research, such as gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*. Two plants were selected for evaluation of insecticidal properties: agave (*Agave americana*) and oleander (*Nerium oleander* L.). The scale insect was sprayed on the selected plants with a biological insecticide from *A. dubia* extract [3]. For the evaluation *A. dubia* allelopathic properties [4] the most popular used plant lettuce (*Lactuca sativa* L.) was chosen. The report will include an evaluation of the antimicrobial, insecticidal and allelopathic properties of the *Artemisia dubia* Wall., the conditions of the experiments (bacterial strains and media, concentrations, methods, etc.) and a statistical analysis.

**Keywords:** *Artemisia dubia*, plant biotechnology, antimicrobial activity, insecticidal properties, allelopathy.

**Acknowledgements:** This project was financed by Research Council of Lithuania project No. 09.3.3-LMT-K-712-10-0236.

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## Oral presentation

# Evaluation of Antibacterial and Antioxidant Activities of Fermented Bee Pollen from Different Europe Regions Using Chemometric Analysis Methods

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### Abstract

Bee pollen is one of the most appreciated natural products, which chemical composition represents a source of biologically active substances [1]. The aim of this research was to determine the effect of fermentation process of bee pollen from different Europe regions on its antioxidant and antibacterial activity by chemometric analysis methods. Also an impact of fermented bee pollen to antibiotic activity was evaluated. Previous studies show that solid-state fermentation has positive impact on antioxidant and antibacterial activities [2]. To our knowledge, this is the first study about the evaluation of antibacterial and antioxidant activities of fermented bee pollen from different Europe regions and also the first research about synergistic effect of fermented bee pollen. Nine samples of bee pollen from Spain, Italy, Netherlands, Sweden, Poland, Lithuania, Denmark, Malta and Slovakia were fermented using optimized fermentation conditions with *L. rhamnosus* bacteria for 8 days. For comparison purposes, spontaneous fermentation was applied on bee pollen samples for 12 days. Synergistic effect was evaluated using penicillin, gentamycin, ciprofloxacin, oxytetracycline, ceftazidime and erythromycin.

It was noticed that, antibacterial and antioxidant activities increased after fermentation. Hierarchical clustering analysis and principal component analysis were applied to help grouping of bee pollen from different Europe regions based on their different antioxidant and antibacterial activities.

The study revealed that both antibacterial and antioxidant activities, and impact to antibiotic activity were strongly related with geographic origin of bee pollen.

**Keywords:** bee pollen, solid-state fermentation, antibacterial activity, antioxidant activity, chemometric analysis.

**Acknowledgements:** This project was financed by Research Council of Lithuania project No. 09.3.3-LMT-K-712-10-0232.

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## Oral presentation

### Towards Highly Efficient Organic Light-Emitting Devices Based on Emitters Exhibiting Thermally Activated Delayed Fluorescence

**Dmytro Volyniuk, Oleksandr Bezikonnyi, Karolis Leitonas, Matas Guzauskas, Ausra Tomkeviciene, J. Simokaitienė, Dalius Gudeika, Juozas Vidas Grazulevicius**

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#### Abstract

Organic light-emitting diodes (OLEDs) nowadays are widely used in displays and lighting devices. Further enhancement of OLED performance is possible due to discovery of new materials with unique properties such as thermally activated delayed fluorescence (TADF). TADF is established as emission mechanism which allow to achieve theoretical 100% internal quantum efficiency of OLEDs. Therefore, TADF emitters are good candidates for highly efficient electroluminescent devices entering the OLED industry. The aim of this presentation is to describe promising approaches towards highly efficient OLEDs based on emitters exhibiting thermally activated delayed fluorescence. Recent progress on TADF emitters based on both intramolecular and intermolecular (exciplex) charge transfer will be discussed. In addition, the most interesting OLED approaches, developed at our laboratories, will be presented including versatile exciplex-forming materials for simplified non-doped white OLEDs [1].

**Keywords:** electroluminescence, organic light-emitting diode, thermally activated delayed fluorescence, exciplex.

**Acknowledgements:** This work was supported by Research Council of Lithuania (Project “PolyTADFer” No S-LLT-19-4).

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# **POSTER PRESENTATIONS**

## Poster presentation

# Environment Impact on Plant Respiration in Ecological Agroecosystems

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### Abstract

Plant respiration forms small inputs to CO<sub>2</sub> emissions in agroecosystem' C cycle. Nonetheless, agricultural land area occupied 4889 Mha, and thus respirational emissions from agroecosystems became evident at global scale [1]. Agriculture and plant respiration are influenced by environment conditions such as temperature and precipitation [2].

This paper was aimed on the validation of effects of environment conditions on plant respiration in different crops of ecological farming.

Investigations of soil respirational emissions in ley, wheat, vetch + oat mixture and barley + ley undercrop agroecosystems were carried out at the Training Farm of Agricultural Academy (former Aleksandras Stulginskis University) in 2014–2016.

Plant respiration rates (R) depended on crop species and ranged between 0.354 μmol m<sup>-2</sup> s<sup>-1</sup> in ley and 1.593 μmol m<sup>-2</sup> s<sup>-1</sup> in wheat agroecosystems due to different biological peculiarities. The observed seasonal variation in respiration strongly correlated to environmental changes. Between them meteorological conditions generally forced the plant respiration rates. Optimal temperature stimulates biological processes, therefore a strong positive correlations were determined between plant respiration and air and soil temperature (r=0.3-0.6, p=0.004-0.01). While increased precipitation and soil moisture decreased temperature and compose anaerobic conditions in soil and thus had negative impact on respiration. Thus, negative correlation between plant respiration and precipitation and soil moisture confirmed this unfavourable impact.

**Keywords:** plant respiration, emissions, environment, crop.

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## Poster presentation

### Effect of Bee Products Extracts on Contamination of Nutrient Grains with Microscopic Fungi

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#### **Abstract**

Bee propolis, bee bread and bee pollen are rich in phenolic compounds, which have antifungal properties and their extracts can be used as natural fungicides. The aim of this research was to determine total phenolic compound (TPC) in bee propolis, bee bread and bee pollen and evaluate the use for the protection of nutrient grains from microscopic fungi.

TPC in bee products was determined using Folin-Ciocalteu reagent with UV/VIS spectrophotometer. Stored wheats, oats and corns grain (100 units) were treated with bee product extracts prepared in ethanol and dimethyl sulfoxide (DMSO) solvents (5 g grain: 1 ml twice diluted extracts). Nutrient grains treated with extracts were plated in Petri-dishes with potato dextrose agar and incubated for 7 days at  $23\pm 1$  °C. Microscopic fungi colonies were identified, and the contamination percentage was estimated according to the contaminated grain number.

The highest TPC of all bee products extracts was in propolis –  $15.5\pm 0.5$  mg/g dry weight; however significant ( $P<0.05$ ) differences in antifungal activity between bee products were not determined. Propolis extract in ethanol was effective inhibitor of the microscopic fungi on wheat grains: they were 9% less contaminated, compared with control. All bee products extracts in ethanol had a significant ( $P<0.05$ ) impact on the oat grains contamination with microscopic fungi. The most effective antifungal activity was determined of bee bread extract in ethanol: microscopic fungi was found 21% less on oats grains, compared with control. The antifungal activity of bee products extracts on contamination of corn grains with microscopic fungi was insignificant.

Ethanol solvent is recommended for antifungal activity studies. Its impact on the growth of microscopic fungi on the nutrient grain was minimal. However, the antifungal properties of bee products in ethanol were more effective compared to bee products in DMSO solvent. Results show that it is impossible to prevent grain contamination completely. The inhibition of naturally dominated microscopic fungi on nutrient grain creates conditions to reproduce other pathogenic fungi.

**Keywords:** bee products, microscopic fungi, antifungal activity, grains.

## Poster presentation

### Changes of Chlorophyll Fluorescence Parameters and Plant Performance Induced by Pre-sowing Treatment of *Fagopyrum esculentum* Seeds with Cold Plasma

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#### Abstract

The effects of cold plasma (CP) are under extensive investigation as an alternative to the traditional pre-sowing seed treatment technologies. Numerous studies provide evidence that seed treatment with CP improves agricultural crop performance. The objective of this study was to evaluate the effects of seed treatment with CP on growth and physiological activity of buckwheat (*Fagopyrum esculentum*) variety 'Nojai' plants. Seeds processed with capacitively coupled plasma device at a pressure of 60 Pa for 5 and 7 minutes (CP5 and CP7 treatments) were sown four days after CP treatment and grown in the field for 14 weeks. At the beginning of flowering growth stage, the chlorophyll *a* fluorescence measurements were performed by a Plant Efficiency Analyser (PEA) with randomly selected youngest fully expanded leaves. The results showed, that cold plasma treatment had statistically significant effect on several fluorescence parameters of *Fagopyrum esculentum*. The differences in the efficiency of second photosystem (Fv/Fm) between control and treated groups were statistically significant: this parameter was higher by 10.3 and 11.2 % after CP5 and CP7 treatments, respectively. Electron transport flux per cross section (ETo/CSm) was 2.6 and 3.2 times higher in CP5 and CP7 groups as compared to the control. The increase of electron transport and efficiency of the PSII might be explained by 37 and 39 % increased trapped energy flux per cross section (TRo/CSm) after CP treatment. 10 and 11% higher maximum quantum yield of primary photochemistry (phi(Po)) and 2.2 and 2.6 times higher quantum yield for electron transport (phi(Eo)) was detected for plants growing from CP exposed seeds. Thus, the obtained results indicate strong activation of photosynthetic system induced by seed exposure to CP and these changes were associated with stimulation of growth. In comparison to the control plants, the weight of seedlings, their leaves, stems and regenerative organs 8 weeks after sowing was by 76, 103, 68, 78% and by 94, 99, 89, 105% larger in CP5 and CP7 groups, respectively. Harvest was collected 14 weeks after sowing. Seed number and seed weight in CP5 group was by 85 and 97% larger, and in CP7 group - by 45 and 55 larger in comparison to the control.

**Keywords:** pre-sowing seed treatment, *Fagopyrum esculentum*, chlorophyll fluorescence, cold plasma, morphometric plant analysis.

## Poster presentation

### **Biosynthesis, Purification and Characterization of a Newly Produced Fungal Laccases Isoenzymes from *Didymocrea sp.***

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#### **Abstract**

Laccases (E.C 1.10.3.2) are widely distributed in higher plants, bacteria, fungi and insects showing specific function in each of them. Many laccase-producing fungi secrete isoforms of the same enzyme, which can differ markedly in their stability, optimal pH and temperature, affinity for different substrates. Fungal laccases are responsible in mechanism for removing toxic phenols from the medium in which these fungi grow under natural conditions. These enzymes are able to oxidize complex polyphenol structures and similar aromatic compounds through one-electron transfer mechanism using oxygen as oxidizing agent. Laccases have various biotechnological applications due to their strong catalytic properties. Keeping these in mind, we have initiated studies on purification and characterization of newly laccases.

J61-2 laccases electrocatalytic properties were researched previously, wired to gold nanoparticles via the trinuclear copper cluster. During enzyme purification it was noticed, that at the existing synthesis conditions *Didymocrea sp.* produces two laccase isoenzymes. In this research, both isoenzymes were extracted and characterized.

The optimum pH of enzyme activity was found at 3.5 using ABTS as substrate. J61-2 is more resistant to higher temperature, maintains most of its activity at 50°C while J61-1 nearly completely denaturates. However, J61-1 is more stable in wide pH range (4.5-7.2), which suggest potential application in bio-bleaching. J61-1 in 4 °C temperature after 25 days remained about 60% of its activity while J61-2 had only about 40%. In room temperature both isoforms activity decreased drastically.

Laccase substrate specificity and effect of pH was determined spectrophotometrically.  $K_m$  values of J61-1 for ABTS, DMP are 0.4, 6.4 mM respectively,  $K_m$  values of J61-2 for ABTS, DMP are 0.01, 0.98 respectively. It is assumed that purified isoenzymes potentially useful for decreasing phenol concentration in a model wastewater solution. J61-1 and J61-2 have the same optimal pH of 3.5 on PPA, but differ on syringaldazine and potassium ferrocyanide, J61-1 having optimal pH of 7.0 and 3.5 and J61-2 having pH of 8.5 and 5.0 respectively.

Moreover, the effect of metal salts on enzymatic activity was tested using ABTS as substrate. General laccase inhibitors  $N_3^-$  and  $Hg^{2+}$ ,  $Na^+$  and  $Zn^{2+}$  acetates completely inhibited both enzymes activity, but at different concentrations. The sodium sulfate caused about 60 % inhibition of J61-2 activity but increased J61-1. The binding of halides (such as  $NaN_3$ ) to the types 2 and 3 copper sites effect internal electron transfer, thus inhibiting the activity of the laccase.

**Keywords:** laccase, properties, oxidation.

## Poster presentation

### High Performance Liquid Chromatography Method for Qualitative and Quantitative Analysis of Ginger-containing Food Supplements

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#### Abstract

Ginger (*Zingiber officinale* Rosc.) is the most widely cultivated and used spice around the globe next to the black pepper. Nowadays spices are known not only for their taste and flavour, but also for their medicinal value. Ginger contains several principal substances which are mainly 6-gingerol and 6-shogaol, they are responsible for the biological activities of ginger which could be used for the treatment of conditions such as nausea of motion sickness, loss of appetite, indigestion and common cold. The object of this investigation was four different multivariate food supplements from Lithuania's community pharmacies, containing ginger as the main component in their composition. All test solutions were extracted in ultrasonic bath for 60 min. Reference solutions of 6-gingerol and 6-shogaol were prepared with methanol using both substance's standards. HPLC analysis was performed using The Waters Alliance 2695 Separations Module, chromatographic separations were performed on ACE C18 column (250x4.6 mm) using a variable mobile phase consisting of acetonitrile and water. Data analysis and other operations were controlled using the Empower2 software. For quantitative evaluation calibration curve of 6-gingerol was obtained using 9 prepared reference solutions, which concentrations were between 0.000883 mg/ml and 0.226 mg/ml. Calibration curve of 6-shogaol was obtained using 6 prepared reference solutions, which concentrations were between 0.0125 mg/ml and 0.4 mg/ml. The experiment was carried out at triplicate and the values were expressed as a Mean  $\pm$  Standard deviation. The 6-gingerol and 6-shogaol peak's identifications were based on the retention times of the standards and further confirmed by comparing their photodiode array spectra to those of the individual standards. After the qualitative analysis, 6-gingerol and 6-shogaol were quantitated using external standards. Our results suggest that ginger-containing food supplements and medicines contains two major constituents which leads to ginger biological active properties. Chromatographic analysis might be useful in providing information about quality of ginger rhizomes and commercial ginger products.

**Keywords:** Ginger, *Zingiber officinale* Roscoe, food supplements, high-performance liquid chromatography, HPLC, qualitative analysis, quantitative analysis.

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## Poster presentation

### Biotechnological Potential of Secondary Metabolites Produced by Cyanobacteria from Curonian Lagoon

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#### Abstract

The Curonian Lagoon is the largest and one of the most severely impacted by harmful cyanobacteria blooms in Europe. In summer, cyanobacterial biomass reaches over 100 mg/l [1,2] and is dominated by *Aphanizomenon flosaquae*, *Planktothrix agardhii*, *Microcystis* and *Dolichospermum* spp. [3]. The goal of this study was to examine the activity of metabolites produced by cyanobacteria from the Curonian Lagoon. Bloom samples collected in 2018 over the season differed in species composition and cyanobacterial biomass. The extracts prepared in 75% methanol were preliminary fractionated, and the obtained material was tested using enzymatic, antibacterial and cytotoxicity assays. The content of the samples was determined using LC-MS/MS. All tested samples inhibited the activity of trypsin and thrombin (mean relative inhibition of 81,5%), however, the strongest activity was observed in samples dominated by *Aph. flosaquae*. In antibacterial assays, samples dominated by *Dolichospermum* and *Microcystis* showed strong (>70%) inhibition of *Staphylococcus aureus*, *Enterococcus faecium* and *Pseudomonas aeruginosa* antibiotic resistant strains. Cytotoxic effects against human breast adenocarcinoma cell line were also observed. LC-MS/MS analysis of active fractions revealed presence of several classes of cyanopeptides, including aeruginosamids, microginins, anabaenopeptins and cyanopeptolins. Preliminary studies indicated that apart from the known toxins, cyanobacteria from Curonian Lagoon produce many bioactive metabolites of potential pharmacological application.

**Keywords:** Secondary metabolites, cyanobacteria, bioactive fractions.

**Acknowledgements:** This study was partially financially supported by the COST Action ES1408: European Network for Algal Bioproducts (EUALGAE).

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## Poster presentation

### Raman Studies of Urea Reactions with Humic Acid Model Compounds in the Presence of Relative Humidity

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#### Abstract

In recent years, it has become increasingly clear that synthetic nitrogen (N) fertilizers, such as urea ( $\text{CO}(\text{NH}_2)_2$ ), not only provide a reliable supply of the major nutrient essential for plant growth, but also result in adverse environmental and human health effects due to the loss of reactive N from agricultural soils [1]. Literature data provides information that  $\text{NH}_3$  volatilization from urea could be reduced up to 16 % when amendment with organic acid. In particular, humic substances play an important role in the global nitrogen cycle by influencing the distribution, bioavailability and ultimate fate of organic nitrogen. Two possible mechanisms of urea-humic substance interactions have been suggested using field tests with 1) part of the ammonium generated from urea mineralization incorporated into humic substances thus reducing the net loss due to volatilization and 2) humic substances inhibit the activity of urease which decomposes urea to  $\text{NH}_3$ , resulting in a lower rate of urea hydrolysis. Humic substances can incorporate nitrogen into their structure either directly through chemical reactions or indirectly through microbial activities and subsequent decomposition of microbial biomass [2]. To analyze molecular interactions between humic acids model compounds and urea molecules Raman spectroscopy was used. Humic acids model compound (SA, CA or CAT) were mixed with urea at a mass ratio (1:1) and stored in a laboratory incubator (CLIMACELL 707, MMM Medcenter Einrichtungen GmbH, Munich, Germany). Laboratory incubator parameters were set as follows: temperature  $25 \pm 0.5$  °C, RH  $40 \pm 2$  % or  $80 \pm 2$  %, fan mode on (100 %), light off. After 15, 30, 60, 90, 120, 180, 240, 360, 1380 min samples were collected and placed into stainless steel sample holder for Raman analysis. After 1380 min reaction samples were transferred into another laboratory incubator with RH 25 %. After 420 minute another Raman spectrum was recorded to find out the reversibility of the reaction. Obtained results between urea and humic acids model compounds interactions step by step will be presented at the conference.

**Keywords:** Raman spectroscopy, urea, humic acid

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## Poster presentation

### Determination of Protein Quantity Isolated from *Glycyrrhiza Glabra* L. and *Desmodium canadense* (L.) DC. Roots during Blooming and Seed Maturity Vegetation Phases

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#### Abstract

*Glycyrrhiza glabra* L. and *Desmodium canadense* (L.) DC – are the herbaceous perennial species of *Fabaceae* family. Both plants are commonly used herbs in ayurvedic medicine. Studies have shown that extracts of plants roots possess antibacterial, antioxidant, antimalarial, anti-inflammatory properties. It was found that preparations of both plants contain a big amount of proteins, but the quantity has not been studied during vegetation phases. The aim of the experiment is to compare protein content in liquorice and Canadian tick-trefoil roots during blooming and seed maturity vegetation phases.

Experiment material and methods: the object - *Glycyrrhiza glabra* L. and *Desmodium canadense* (L.) DC raw root material, collected in the Botanical Garden of Vytautas Magnus University in Kaunas. Protein fractions were obtained from liquorice and Canadian tick-trefoil roots by extraction and fractionation with ammonium sulphate and protease inhibitor - ε – amino-capronic acid.

The quantity of protein was measured by the Bradford method using bovine serum albumin BSA protein standards.

Proteins of *Glycyrrhiza glabra* L. and *Desmodium canadense* (L.) DC fresh root material was obtained in blooming and seed maturity vegetation phases. Experiment results shown that the biggest yield of protein was defined in liquorice root extract, while Canadian tick-trefoil root extract had a significant lower quantity of protein in tested phases. Protein quantity in blooming phase extracted from liquorice roots material was four times higher than in Canadian tick-trefoil roots. At the end of the vegetation, in seed maturity phase, protein amount increased in both plants roots material and has become approximately equal.

In conclusion, we can state that independently of the similarity of the botanical classification, analyzed plants had a different protein content in roots during vegetation.

The highest content of protein was on seed maturity phase, where both plants accumulate the similar amount of proteins.

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## Poster presentation

### The Effects of Timentin on Oxidative Stress in Tobacco Shoots *In Vitro*

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#### **Abstract**

Oxidative stress is a complex chemical and physiological response to external stimulus or an event that causes stress in higher plants. Oxidative stress originates as an overproduction and accumulation of molecules containing activated oxygen - reactive oxygen species (ROS). ROS are produced in plants by various enzymes, they trigger complex signalling and defensive reactions that influence plant growth and its defence against stressful factors. In some cases, accumulation of ROS may lead to reversible or irreversible modifications of molecules such as proteins, nucleic acids, carbohydrates and lipids, that may cause serious damage to plant cells, even induce cell death [1].

The most common physiological effect of oxidative stress is reduced crop growth and productivity. A similar effect, poor plant growth, is observed in plant regeneration following *Agrobacterium* mediated plant transformation during and after the antibiotic Timentin treatment. Timentin is the most efficient antibiotic for *Agrobacterium* elimination after the transformation, and least deleterious for the plant. *Agrobacterium* mediated plant transformation is the most productive way of introducing new genes into various plant species [2]. However, it's crucial to remove bacteria after plant transformation, because prolonged plant exposure to *Agrobacterium* infection has a detrimental effect to plant health and in some instances may result in cell apoptosis or plant tissue necrosis [3].

The aim of this study was to evaluate stress related biochemical characteristics of several tobacco shoots grown on media supplemented with Timentin. Stress related production of  $O_2^{\cdot-}$  and  $H_2O_2$  were measured using Nitro blue tetrazolium (NBT) and 3,3'-diaminobenzidine (DAB) staining, respectively. In addition, lipid peroxidation was evaluated by detecting malondialdehyde (MDA) concentration with the Thiobarbituric acid reactive substances (TBARS) assay. Our results showed that exposure to Timentin affects the accumulation of the oxidative stress related byproducts. As these products have the capacity to cause oxidative damage, it is an important factor for tobacco regeneration and overall growth. The results suggest a possible alternative way of improving shoot regeneration and growth while still using the same antibiotic for efficient elimination of *Agrobacterium* from plant tissues by reducing antibiotic induced plant stress.

**Keywords:** Oxidative stress, ROS, Timentin, *Agrobacterium*, tobacco shoots, *in vitro*.

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## Poster presentation

# Resin Gland Fluorescence of *Chamaecyparis lawsoniana* as an Additional Feature for Species Identification

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### Abstract

Identification of conifer species and cultivars in the cypress family *Cupressaceae* is sometimes complicated as there is a wide variety of colours and shapes of branches looking alike, especially when the plants are young and immature for fruiting. Fluorescent features of conifer resins, absorbing of Ultra Violet (UV) light and emitting it at the same time in a longer wavelength, are well known. This study aimed to verify any fluorescent under UV (395 nm) light and easily noticeable for human eyes distinctive features for species or cultivar identification of *Cupressaceae* family. Cultivars with yellow coloured foliage did not show any distinctive signs of glowing. Underside of young branches of 16 different *Cupressaceae* species (of *Thuja*, *Chamaecyparis*, *Juniperus*, *Microbiota* and *Cryptomeria* genus) and 29 cultivars were checked for fluorescent patterns. Resin glands of only *Chamaecyparis lawsoniana* glowed in a bright blue color and the glowing was very clear if additional yellow filter was used. Mean length of measured *C. lawsoniana* resin glands was the largest (0.52 mm) in comparison with *C. pisifera* (0.44 mm), *Thuja occidentalis* (0.29 mm) and *T. plicata* (0.35 mm). Blue-green and grey-green cultivars of *C. lawsoniana* had also fluorescent resin glands, while yellow-green did not. Fluorescence was not noticed on *C. lawsoniana* 'Blue surprise' cultivar underside branches, which shape and leaf position on a shoot was similar to *C. pisifera* and not to *Thuja* spp. Resin gland fluorescence may be used as an additional feature for identifying *C. lawsoniana* and some of its cultivars.

**Keywords:** fluorescence, resin glands, *Cupressaceae*, *Chamaecyparis lawsoniana*.

## Poster presentation

### Aromatic Constituents of Essential Oils as Antibacterial Agents

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#### **Abstract**

The extract with some ingredients (menthol, cinnamal, citral, eugenol, d-limonen, linalol) obtained by the conventional 60% vol. alcohol extraction. The chemical essential oils composition of the extract was analysed using GC-MS. The aromatic composition of the extract was tested for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus* by the disc diffusion method. Extract showed strong antibacterial activity against all tested strains. These extracts showed different activities when tested by direct application and in the vapor phase. This study provides novel approaches for assessing the antimicrobial potential of essential oils in direct contact and the vapour phase. These results suggest that the tested ingredients might be used anti-microbial agents for decontaminating an indoor environment.

**Keywords:** essential oils, antibacterial, extract, bacteria

## Poster presentation

### Disease Agents and Pest Species in Recreational Greeneries in Lithuania

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#### Abstract

In Lithuania the evaluation of state of plants growing in green plantations was carried out extempore with no systematical order. From 2009 in two Lithuanian cities (Kaunas and Alytus) has proceeded a long-term monitoring of woody plants. 4000 plants are examined in 11 parks and squares annually.

The intensity of fungal diseases and pests' abundance are evaluated according to 0 to 4 grades scale.

Climatic conditions have a direct influence on the alter of plants' diversity and intensity of pathogenic injuries. 17 plant species were affected by fungal diseases by 0.02–2.07 grades: mildew fungi – *Acerginalla* and *A. tatarica* (agents *Sawadaea bicornis*), *A. platanoides* 'Globosum' (*Sawadaea tulasnei*), *A. negundo* (*Phyllosticta negundinis*), *Caragana arborescens* (*Erysiphe palczewskii*), *Berberis thunbergii* 'Purpurea' (*Erysiphe berberidis*), *Euonymus europaea* (*Erysiphe euonymi*), *Fraxinus exelsior* (*Phyllactinia fraxini*), *Quercus robur* (*Erysiphe alphitoides*), *Spireae arguta* (*Erysiphe penicillata*), *Syringa vulgaris* (*Erysiphe syringae*), *Rosa* sp. (*Podosphaera pannosa*); rust fungi – *Larix decidua* (*Melampsora laricis-populina*), *Pinus strobus* (*Cronartium ribicola*), *Pinus sylvestris* (*Cronartium flaccidum*), *Pyrus pyraeaster* (*Gymnosporangium sabine*), and spot diseases – *Tilia cordata* (*Didymosphaeria petrakiana*, *Apiognomonina errabunda*, *Mycosphaerella microsora*).

*Erysiphe flexuosa* annually injure *Aesculus hippocastanum*. In 2002 these invasive species were described for the first time in Lithuania. In 2013 leaf spots (agents *Phyllosticta paviae* Desm. (syn. *Guignardia aesculi*) on hores–chestnut trees were observed repeatedly at places where *Cameraria ohridella* have less affected chestnut trees.

Since 2013 the damage of leaf spot agent *Asteromella tiliae* were noticed on *Tilia cordata* and *T. platyphyllos* located near ponds. The spread of *Schizotetranychus tiliarum* was also observed and noticed sever damages on *Tilia platyphyllos* (0–3 grades).

The outspread of *Caliroa annulipes* have started since 2010, it expanded from 0.08 to 1.13 grades. *Chalara fraxinea* (officially detected in 2010 in Lithuania) have injured *Fraxinus* sp. (*Phyllactinia fraxini*).

In 2013 injuries of *Pristiphora subbifida* were recorded to be to 0.29 grades. In 2014 *Taxus baccata* was injured by the pest *Parthenolecanium pomeranicum* by 3 grades.

## Poster presentation

# Endophytic Bacteria in Cold Plasma Treated Sunflower (*Helianthus annuus*) Seeds

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### Abstract

Endophytic microorganisms are adapted to grow within plant tissues without causing apparent symptoms of plant disease but some of them can promote plant growth by production of plant hormones, modulation of hormone production in plants, increasing nutrient availability or suppressing plant pathogens.

Treatment with cold plasma (CP) can inhibit growth of various bacteria, therefore it is applied for food decontamination. Short term seed treatment with CP was reported to improve agronomical crop performance, however the mechanism of such effects is unknown. Possible outcomes of seed CP treatment to some extent may be dependent on changes in composition of endophytic microflora.

The aim of our study was to characterize microflora diversity and plant growth promoting properties of endophytic bacteria in control and CP treated sunflower seeds. Seeds of sunflower were treated with CP for 9 and 12 minutes (CP9 and CP12). After treatment the surface of affected and control seeds was sterilized, sunflower kernels were separated from hulls and homogenized. Fresh nutrient medium was added to samples and after vigorous shaking for 30 min samples were inoculated on plates with nutrient medium.

Bacterial isolates were characterized by the number of morphological and biochemical properties, including – catalase, oxidase activity, gram staining, secretion of amylolytic and DNA degrading enzymes, ability to reduce nitrate, growth on selective XLD and MSA media. Bacteria isolates were also screened for traits specifically associated with direct and indirect plant growth promotion – auxin production, nitrogen fixation, siderophore production.

Gram-positive bacteria dominated in all samples, both cocci and bacilli were present. Gram-negative bacteria were absent in CP9 samples although were found in control and CP12. The ability to fix nitrogen and to produce auxin were common among endophytic isolates of sunflower seeds. There was not much variation in distribution diazotrophic activity and auxin production between CP treated and untreated samples. Production of siderophores was detected only in 10 percent of isolates.

**Keywords:** cold plasma, endophytic bacteria, *Helianthus annuus*.

## Poster presentation

### The Response of Humic Substances on Egg Quality

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#### Abstract

Public concerns about food and environmental safety (e.g. antibiotic residues or antibiotic-resistant pathogens) have driven researchers to look for natural agents in poultry nutrition [3]. One of the strategies can be application of humic substances. Humic substances (HSs) are derived from organic matters decomposed by bacteria in the soil. HSs (includes humus, humic acid, fulvic acid, ulmic acid and microelements) are known as natural plant-growth stimulator, but in animal studies have been less evaluated [1, 2]. This research was designed to investigate whether the inclusion of HSs into diets of laying hens increases egg quality. The trial was carried out with laying hens of the crossbreed *Loman Brown* from 28 to 35 weeks of age. The total treatment duration was 60 days, without pre-treatment period. The compound feed was based on wheat-soybean and formulated to meet the nutrient and energy requirements for layer hens (NRC, 1994). Animals were assigned to the control and treatment aviaries. The laying hens of control group received the plain drinking water. The drinking water of treatment group was enriched with HSs (fulvic, humic, gallic, caffeic, shikimic, fumaric and other organic acids) at the rate of 1 l/m<sup>3</sup> H<sub>2</sub>O. It was found, that in addition HSs the egg shell strength was improved 5% and eggshell weight by 2%, compared with control (p>0.05). The internal egg quality parameters, such as egg albumen height and Haugh unit were decreased 2% and 3%, respectively, in water supplementation with HSs, compared with control (p>0.05). The slight effect on productivity parameters of laying hens was obtained. An egg weight and rate of lay of hens were similar in both groups, but feed:gain ratio to produce 1 kg of eggs was lower 3% in addition of HSs (p>0.05). Such finding could be explained, that HSs stimulate changes in intracellular divalent calcium levels and act as dilators increasing mucosal and cellular permeability. Increased permeability helps in assimilation of minerals from the gut and their transfer from blood to the egg of hens [1]. As it expected the results of our experiment demonstrated that the eggshell quality is increasing in addition of HSs to the drinking water of hens.

**Keywords:** humic substances, egg production, egg quality.

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## Poster presentation

### Evaluation of Electrochromic Properties of Methylene Blue Synthesized in Presence of Glucose, Sucrose and Lactose Dopants in Solutions of Different pHs'

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#### Abstract

Electrochromism is “the electrochemical variation of color in accompaniment with an electron-transfer (or ‘redox’) reaction”, while electrochromic materials are called electrochromes [1]. Electrochromic materials are categorized into two categories, according to the mode of operation: by reflection or by transmission [1]. These materials are used in production of smart glass [2], electrochromic windows, electrochromic devices such as digital watches, visual display unit (VDU) screens [1], protective eyewear for military [3], etc.

Electrochromic material used in the experiment was methylene blue (MB). MB is an organic compound based on phenothiazine origin, MB is used as a biomedical marker and electrochromic material. In its oxidative state it is blue while in its reductive state leucomethylene blue is colorless [1].

The aim of this research was to determine electrochromic properties of MB synthesized in the presence of polyanionic dopants. In this case such dopants were glucose, sucrose, lactose. Electrochromic properties were evaluated in solutions of different pHs' ranging from acidic to basic. The synthesis was performed electrochemically and the layer of poly-(methylene blue) (pMB) was formed onto ITO (Indium Tin Oxide) coated glass electrode. The electrochemical synthesis was performed by potential cycling based method, the potential was cycled in the range of -0.5 V – 1.2 V.

After the synthesis the absorbance of the layers was evaluated in solution with different pH values. The results show that optical absorbance of pMB/Lactose, pMB/Sucrose, pMB/glucose layers has been highest around  $\lambda=698$  nm in all pH values.

**Keywords:** Methylene blue, polyanionic dopants, electrochromism, indium tin oxide (ITO).

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## Poster presentation

### Determination of Green and Black Teas Secondary Metabolites using HPLC MS Method

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#### Abstract

Tea (*Camellia sinensis* L.) is one of the most popular beverages around the world. Green tea (unfermented) and black tea (fully fermented) are produced from the leaves of the *Camellia sinensis* plant. Tea possesses antimicrobial, antioxidative, antihypertensive and anti-inflammatory properties [1]. Green tea leaves contain secondary metabolite such as catechins and its derivatives [2]. The aim of this work is to identify secondary metabolites and to determinate metabolic profile differences between green and black tea. The commercially available green and black teas leaves (*Camellia sinensis* L.) were grounded into powder and 30 mg of each tea powder were mixed with 1.5 ml of methanol, ethanol and acetonitrile solutions (v/v) by vortexing for 20 seconds. The mixture was then ultrasonicated for 10 minutes followed by centrifugation at 10,000 rpm for 10 minutes. The collected supernatant with the major tea metabolome were passed through a 0.22 µm membrane filter for LC/Q-TOF/MS (Agilent 1260 and Agilent 6530B) analysis [3]. Twentytwo green and black teas samples (n=3) with different concentration of methanol, ethanol and acetonitrile were analysed. Based on the received PCA data analysis, for further secondary metabolites identification and metabolic profile differences, aqueous methanol solutions (3:1) were used. PC1 variance (%) 36.99; cumulative variance (%) 36.99; PC2 variance (%) 25.80; cumulative variance (%) 62.79. The optimized black and green teas extracts are compared and some compounds are identified, for example: theasinensin A (green tea 915.1624; -1.04 ppm; black tea 915.1622; -0,82 ppm; 915,1614; log<sub>2</sub>(ZA/JA)=1,24); theasinensin B (green tea 763,1512; -0,93 ppm; black tea 763,1509; -0,54 ppm; 763,1505; log<sub>2</sub>A(ZA/JA)=1,4) and other compounds groups, such as kaempferol, catechin and quercetin derivatives and oxidation products. Aqueous methanol teas extracts (3:1) showed largest amount of extracted secondary metabolites. In aqueous methanol green tea extracts were determined 110 different compounds; in black tea aqueous methanol extracts were determined 137 different compounds. Green tea secondary metabolites constituents are different than black tea secondary metabolites, because of oxidation process.

**Keywords:** *Camellia sinensis*, chemical composition, secondary metabolites, green tea, black tea.

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## Poster presentation

### Application of Miniature Retraction Force Measurement Device in Biological Sample Investigations

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#### **Abstract**

Nowadays, people are increasingly concerned about the environment in which we live. Consequently, new technologies that detect and remove pollutants are being developed and improved. One of those improved technologies is newly developed miniature wireless device which measures retraction force based on Du Noüy Ring Method. By measuring retraction force, we can indirectly determine liquid surface tension. Reduced or increased liquid surface tension is expected to predict the presence and quantity of fungal enzymes used in mycoremediation.

The aim of this research was the application of retraction force measurement device “Portabilus“ and the investigation whether the concentration of substances that reduce or increase surface tension of liquid in biological samples *Pleurotus ostreatus* and *Irpex lacteus* can be determined. In this study methodology of retraction force measurements was developed and optimized. Newly developed automatic wireless miniature device “Portabilus” was used to measure retraction force from liquid surface in the malt extract growth medium with *P. Ostreatus* and *I. Lacteus*. Fungi were grown in malt extract medium in 10 mL glass bottles with cotton-wool filters. Samples were diluted 1:1 (volume of medium: volume of water) 30 min before measurement. Specimens were diluted again 1:1 just before measurement. Samples were measured every few days in room temperature (20±3).

The investigation showed that retraction force, thus and liquid surface tension, was reduced by *P. Ostreatus* and *I. Lacteus* fungi grown in liquid medium. This indicates that investigated samples contained substances reducing surface tension such as biosurfactants.

**Keywords:** surface tension, retraction force, Du Noüy Ring method, biosurfactants, mycoremediation.



## Poster presentation

### Antifungal Effect of Some Herbal Ingredients: Menthol, Cinnamal, Citral, Eugenol, D-limonen, Linalol

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#### Abstract

The antifungal activities of extract with some ingredients (menthol, cinnamal, citral, eugenol, d-limonen, linalol) have been demonstrated against fungi *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Cladosporium cladosporoides*, *Alternaria alternata*. The composition of phytochemicals was analyzed by gas chromatography mass spectrometry. The minimum inhibitory concentrations (MIC) and minimum fungicidal concentration (MFC) were determined by serial microdilution method. The results indicated inhibitory effects of the extracts on the tested fungi. The minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) values of the tested agents were between 0.25 and 10%. This extract with some ingredients was found to have a wide spectrum of activity against all fungi examined in this study and may be proposed in therapeutic or hygienic contexts.

**Keywords:** antifungal activity, medicinal plants, fungi, plants extract.

## Poster presentation

### The Application of Bio-Conservation with Creatine, Taurine and Coenzyme Q10 for Safety and Quality Improvement of Minced Meat

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#### Abstract

The production of high quality meat and meat products is very important. The aim of this study was to analyze effect of some selected antioxidants and protective bacterial cultures on the safety and quality of minced meat. Selected antioxidants: rosemary extract, taurine, creatine, coenzyme Q10 and protective bacterial cultures *Staphylococcus carnosus*, *Staphylococcus vitulinus* were used to improve the safety and quality of minced meat (minced pork and minced beef).

Using physical chemical methods (the determination of antioxidant activity, phenolic compounds, peroxide value, fatty acids composition) and microbiological analysis (the evaluation of total number of coliform bacteria and the total number of aerobic bacteria) minced meat samples were tested during the production and storage period.

All antioxidants selected and used during the study positively effected antioxidant activity of minced meat, and reduced lipid oxidation in the samples. After 1 day of storage, the acid value in the minced pork sample with rosemary extract was  $1.19 \pm 0.00$  mg KOH/g, and the acid value in the sample without additives was  $1.29 \pm 0.01$  mg KOH/g. The peroxide value in the minced beef sample (after 1 day of storage) with rosemary extract was  $1.00 \pm 0.02$  meq./kg and in the sample without additives was  $1.57 \pm 0.02$  meq./kg. The least antioxidant activity was in the samples without additives. The best antimicrobial properties during the whole study were shown in the minced meat samples with rosemary extract- after 5 days of storage the total number of aerobic bacteria in pork:  $4.28 \pm 0.10$  log<sub>10</sub> CFU/g, in beef:  $4.23 \pm 0.17$  log<sub>10</sub> CFU/g, when  $P < 0.001$  in both cases. The higher number of aerobic bacteria (after 5 days of storage) were determined in the samples without additives: in the minced pork sample  $6.87 \pm 0.05$  log<sub>10</sub> CFU/g and in the minced beef sample  $5.73 \pm 0.02$  log<sub>10</sub> CFU/g, the less numbers were in the samples with rosemary extract and in the mixture of rosemary extract and coenzyme Q10. The the higher number of coliform bacteria was tested in the sample without additives-  $5.10 \pm 0.05$  log<sub>10</sub> CFU/g. The low amounts of phenolic compounds after 5 days of meat storage were in the minced meat samples without additives- in pork:  $0.16 \pm 0.02$  mg GAE/100g, in beef:  $0.17 \pm 0.00$  mg GAE/100g.

Based on our results, rosemary, taurine and coenzyme Q10 extracts together with protective bacterial cultures *Staphylococcus carnosus*, *Staphylococcus vitulinus* could be used in some fields of meat industry to lower the food spoilage and to improve minced meat safety and quality.

**Keywords:** antioxidant activity, bacterial cultures, rosemary extract, antioxidants.

## Poster presentation

# The Impact of Bacteria and Fungi Conditioners on Soil Humification and Biodiversity

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### Abstract

During the 2017 autumn – 2018 summer period the field experiments were carried out at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. The research object was *Endocalcari – Endohypogleyic Cambisol*. The aim of this study was to investigate the effectiveness of different biological products on degradation of vegetable residues, physicochemical and biophysical quality of the soil. The investigation on influence of these factors on soil microbial activity of rhizosphere and the development of roots of agricultural crops was performed. This study consisted of two research schemes – the first one included two factorial experiments consisting of residues handling (Factor A) and fertilization with mineral fertilizers (Factor B). The second research scheme consisted of selection of microorganism treatments (single or mixed). It was revealed that in the stand of winter wheat growing during extremely dry 2018 year the best efficiency for humification processes activation in the top-soil layer were obtained in the treatment which includes *Trichoderma reesei* combination with *Bacillus megaterium*. In this treatment soil C/N ratio, N-min accumulation and the viability increased. *Trichoderma reesei* in combination with *Bacillus megaterium* significantly increased the values of the AWCD and R index but did not have an effect on H index. Fungi and nitrogen fixing bacteria combination determined the highest OD values for carbohydrates, carboxylic acids, polymers, amino acids, amines and other soil C sources.

**Keywords:** soil nitrogen, soil carbon, rhizosphere bacteria, nitrogen fixing bacteria, soil microscopic fungi.

## Poster presentation

### Evaluation of Terbinafine Interaction with Human Serum Albumin by Circular Dichroism, Capillary Electrophoresis and Spectroscopy

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#### Abstract

Human serum albumin (HSA) is the most abundant protein in human plasma and constitutes about 60% of all proteins there. It is established as a versatile, non – specific transporter and plays a significant role in binding a wide variety of drugs that are delivered to their target tissues. The study about possible binding interactions between HSA and certain drugs can provide important information about medicine behavior in the human body and its pharmacokinetic characteristics [1]. Terbinafine is allylamine antifungal agent, which is well established for onychomycosis treatment. Considering the facts that terbinafine is widely used by administering it orally, and that more than 99% of the dose can be bound to plasma proteins, the binding mechanisms and intensity are still studied [2].

The aim of this study was to investigate possible interaction between HSA and terbinafine applying circular dichroism (CD), capillary electrophoresis (CE), Fourier transform infrared spectroscopy (FT-IR) and ultraviolet and visible absorption (UV-vis) spectroscopy. Considering that terbinafine is a basic hydrophobic agent (pKa=8,94) and is very slightly soluble in water, the investigation was performed under conditions of pH=7,4 and pH=3, because solubility of terbinafine increasing in acidic water [3].

The obtained results demonstrate no significant changes in the HSA molecule structure in presence of terbinafine. UV-vis spectroscopy shows the shift on the characteristic HSA absorption peaks. CD spectroscopy results indicated the decrease in  $\alpha$ -helix contents in proteins secondary structure and therefore unfolding of 1.9% when compared to CD spectra of not bound albumin. CE showed changes in migration time when albumin and terbinafine mixture was analyzed. FT-IR spectroscopy demonstrated spectra change dependency on the quantity of terbinafine in the analyzed mixture thus indicating possible changes of albumin secondary structure.

**Keywords:** Human serum albumin, terbinafine, UV spectroscopy, FT-IR spectroscopy, circular dichroism, capillary electrophoresis.

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## Poster presentation

### Study on Oxidation of Naturally Occurring Polyphenolic Compounds by Laccase Isolated from *Lithothelium* Sp.

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#### Abstract

Laccases are one of the few enzymes that have been studied since the nineteenth century. Laccases are copper-containing enzymes that catalyze the oxidation of a wide variety of organic and inorganic substrates, including mono-, di- and polyphenols, amino and methoxy phenols, etc. with the concomitant four electron reduction of oxygen to water. This makes laccases useful for their varied applications in several biotechnological processes, such as phenolics elimination for stabilization and browning of fruit juices, beer/wine, in bleaching of paper pulp, decolourization of dyes, wastewater treatment.

The aim of this study is to report biochemical characteristics of purified laccase from *Lithothelium* sp. and to demonstrate laccase-catalysed oxidation of different polyphenolic compounds.

The optimization of laccase production was selected by physical factors such as pH, temperature, cultivation period, different salts. Laccase isoenzymes were purified by using ion exchange, hydrophobic and ion exchange chromatography with a specific activity of 44.0 U/mg for isoenzyme L95-1 and of 1.2 U/mg for isoenzyme L95-2. Due the very small amount and instability of isoenzyme L95-2, in this research only isoenzyme L95-1 was investigated.

A wide range of phenolic derivatives as substrates for laccase isolated from the fungus *Lithothelium* sp. have been studied. The oxidation of the assayed substrates was monitored spectrophotometrically. Kinetic curves were recorded at the wavelength corresponding to the maximum of absorbance. The concentration of oxidized substrates was calculated by using molar absorption coefficients. Quercetin was the best substrate considering the  $k_{cat}/K_m$  values ( $1.15 \mu\text{M}^{-1}\text{s}^{-1}$ ). The apparent  $K_m$  of L95-1 was determined to be  $3.7 \mu\text{M}$  with syringaldazine as substrate. Compared with other substrates, laccase showed a much higher affinity with syringaldazine. The pH optimum of laccases is highly dependent on the substrate utilized. The determined bell-shaped profile and optimum pH was 5.0 using gallic, caffeic, ferulic, sinapic and syringic acids as substrates. However, for quercetin the optimum pH was determined 3.5 and exhibit sigmoidal profiles.

The influence of different metal ions on laccase activity was investigated.  $\text{NiCl}_2$ ,  $\text{KNO}_3$ ,  $\text{AlCl}_3$  salts showed a strong inhibitory effect on activity of laccase and the residual activity was found to be 20% while metal salts such as  $\text{Na}_2\text{SO}_4$  and  $\text{CuSO}_4$  have a weak inhibitory effect on laccase activity.

**Keywords:** laccase, oxidation, polyphenols.

## Poster presentation

# Investigation of Vascular Endothelial Growth Factor HBD Domain Effect on Angiogenesis

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### Abstract

The process of angiogenesis is one of the most important determinants of cancer. The suppression of this process is one way of stopping the spread and growth of the tumor, therefore, new anti-angiogenic molecules would provide hope to cancer patients. Knowing that the vascular endothelial growth factor (VEGF) is a master regulator of the angiogenesis process, the idea of our studies is to create a version of VEGF, termed FHBD, which is composed of the the signal sequence, FLAG tag and the heparin-binding domain of VEGF. Expectedly, the FHBD protein will compete with the native full-length VEGF in the living organism and displace it from the receptors (such as VEGFR2 and Nrp1) and thereby interfere with the spread of the vascular signal towards the tumor.

**Keywords:** angiogenesis, VEGF, HBD, NRP1 (Neuropilin 1), PCR, Western blot.

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## Poster presentation

# The Evaluation of the Particle Size Changes of Oil-in-water Microemulsions Containing Quercetin

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### Abstract

Microemulsion is thermodynamically stable dispersed system. Its quality is characterized by particle size (10–200 nm) and polydispersity index ( $PDI \leq 0.5$ ). Microemulsions can enhance solubility of poor water soluble drugs as quercetin. Quercetin is a natural flavonoid, known for its antioxidant, anti-inflammatory, antiviral, antifungal properties. The aim of this study was to evaluate the changes of the particle size of three different o/w microemulsions containing quercetin within 4 weeks.

Three oil-in-water microemulsions (QME-EtOH, QME-Pg, QME-PEG-400) consisted of 3% of isopropyl myristate, 33% of purified water and 64% of mixture of labrasol and co-surfactant (5:1). Ethanol (EtOH), propylene glycol (Pg) and PEG-400 were used as co-surfactants. Quercetin was dissolved in microemulsion components. Average particle size and PDI were measured applying light scattering method. Measurements were carried out after formulation, after 2 and 4 weeks.

After formulation, particle size of MEs was varying according co-surfactant type: ME containing EtOH was in the range of 1.5–5.6 nm, ME containing Pg – 28.2–141.8 nm and ME formulated with PEG-400 as co-surfactant was in the range of 78.8–615.1 nm. Particle size changes were observed in all ME after 2 and 4 weeks. It was determined, that after four weeks there were no 1.5–2.0 nm size particles in ME containing EtOH, but formation of 24.4–91.3 nm size new particles was observed. In the range of 2.3–2.7 nm were 1.8–9.7% less particles and in the range of 3.1–4.2 nm were 0.9–4.4% more particles than after formulation. Increase of particle size was observed in ME containing Pg: after four weeks 0.1–5.8% increase of particle number in the range of 50.8–164.2 nm was observed. It was obtained, that there were no particles in the range of 78.8–105.7 nm in ME containing PEG-400 after four weeks. In the range of 122.4–164.2 nm were 1.8–6.8% less particles than after preparation. The increase (0.1–4.5%) of particle number in the range of 190.1–712.4 nm was observed. PDI values of microemulsions were determined less than 0.4, although changes were observed within 4 weeks.

Changes of particle size were observed in all investigated microemulsions. Particle size of ME could be determined by nature of used co-surfactant: large particle size (>200 nm) of QME-PEG-400 could be attributed to polymers property to formulate micelles. According to observed PDI values, all formulated microemulsions were corresponding quality requirements.

**Keywords:** microemulsion, quercetin, particle size, polydispersity index, particle size.

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## Poster presentation

### Determination of Phenolic Compounds, Flavonoids and Radical Scavenging Activity in a Droplet Using Micro Colorimetric System

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#### **Abstract**

In today's science modern analytical instruments are becoming particularly important. Miniaturization, portability and autonomous operation are the key factors in developing novel and innovative devices. Therefore, in many cases application of such devices is still problematic. The following shortcomings have been identified: higher detection limits and less sensitivity, impact of environmental factors on device performance and high potential for field contamination. In this work we present an improved micro colorimetry system that can be customized for determination of biologically active compounds.

The determination of total phenolic compounds was performed using Folin - Ciocalteu method. Rutin standard solutions (0.01 and 1 mg/ml) were used for calibration curve. The received results prove linear dependency between concentration of measured samples and absorbance. Reaction with AlCl<sub>3</sub> was applied to determine the total flavonoid content. Antioxidant activity of the extract was assessed using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method. Results were expressed as rutin equivalents (mg/g).

The device was optimized for the miniaturized procedures, 15 µl droplet and the three different LEDs were adjusted for optimal operation. During this presentation obtained results, further investigation and obstacles associated with the novel design will be discussed.



## Poster presentation

### The Role of SRSF1 and SRSF5 in the Regulation of Hypoxia Dependant Alternative Splicing of Neurodegenerative Disease Related Genes

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#### Abstract

Alzheimer's disease is one of the most wide-spread forms of dementia, characterized by progressive loss of brain cognitive function. There are several identified causes of Alzheimer's disease: 1) accumulation of extracellular beta amyloid (A $\beta$ ) peptides in the brain cells, which are produced of amyloid precursor protein (APP); 2) accumulation of hyperphosphorylated microtubule-associated protein Tau that forms neurofibrillary tangles [1].

Latest literature data states that lack of oxygen in the brain cells (hypoxia) affects the development of neurodegenerative diseases. One of cellular responses to reduced oxygen tension are changes in alternative pre-mRNA splicing, when mRNA isoforms are produced, from which translated proteins promotes cell survival under unfavorable conditions. Multiple factors are involved in hypoxia dependent splicing regulation. It is shown that SR proteins are among them [2].

The aim of this study is to investigate cellular hypoxia influence on APP and MAPT gene splicing profiles in the brain (U-87 MG) carcinoma cells cultivated under normoxic or hypoxic conditions and to elucidate the role of SRSF1 and SRSF5 proteins in hypoxia dependent pre-mRNA splicing regulation.

**Keywords:** Alzheimer's disease, alternative splicing, APP, hypoxia, Tau, CRISPR.

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## Poster presentation

### Sampling and Chromatographic Analysis of Airborne Linalool

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#### **Abstract**

The protection of the environment and the reduction of the pollution is of utmost importance. One of the main trends in instrumental analysis is miniaturization. In this work miniaturized solid phase microextraction instrument is discussed. The mechanism is based on adsorbing the substance on the prepared sorbent in fused silica capillary. During sorption, the sample is concentrated and can be desorbed using small amount of solvent or without solvent if heated. This method reduces sample preparation time and consumables.

During this work the sorbent for the absorption of volatile compounds in the air was synthesized in the fused silica capillary. The adsorbent is used in a device that has been designed specifically for the purpose. The applicability of the designed instrument using volatile compound – linalool was demonstrated.

The hand-held solid phase microextraction device was placed in a closed model system for evaluating it. Gas chromatography with flame ionization detection was used to quantify linalool. Results and further research will be presented and discussed during the presentation.

**Keywords:** miniaturization, solid phase microextraction, fused silica capillary, Gas chromatography with flame ionization detection.

## Poster presentation

### **Analysis of the Effects of Storage to the Quantities of Flavonoids, Phenolic Compounds and Allelopathic Qualities in *Thuja standishii* (Gord.) Carr., *Thuja occidentalis* L., *Thuja occidentalis* ‘Aurescens’**

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#### **Abstract**

The object of interest for this research were leaves and bark of *Thuja standishii* (Gord.) Carr., *Thuja occidentalis* L. and *Thuja occidentalis* ‘Aurescens’. The aim was to measure the quantities of flavonoids and phenolic compounds, the radical scavenging activities, compare the results to find out which parts of the plant and which species produce the most of the compounds, evaluate the effects of storage and allelopathic properties.

The quantities of the compounds were measured using spectrophotometric methods [1].

The quantities of flavonoids in leaves varied from 1.02 % to 2.36 %, of phenolic compounds in bark from 4.51 % to 8.97 % fraction mass and radical scavenging activity was determined to be from 0.81 % to 1.23 % rutin equivalents in leaves.

Leaves turned out to be the richest part in flavanoids and bark in phenolic compounds. *T. occidentalis* ‘Aurescens’ had the largest quantities of both of the desired compounds. Radical scavenging activity was highest in the bark of *T. occidentalis*.

1.51 - 1.76 times more phenolic compounds and flavonoids were found in extracts made from primary samples kept in refrigerator for 6 months at -18°C comparing to extracts kept at 5°C. Comparing fresh extracts and after keeping at 5°C for 6 months, fresh extracts had 1.53 - 1.77 more phenolic compounds. Radical scavenging activity had little dependence to keeping.

Allelopathic properties against *Lepidium sativum* L. were highest in the leaves of *T. occidentalis*.

**Keywords:** Phenolic compounds, flavonoids, radical scavenging activity, storage effects, *Thuja standishii*, *Thuja occidentalis*, *Thuja occidentalis* ‘Aurescens’.

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## Poster presentation

# Electrically Conductive Pressure Sensitive Adhesive for Biomedical Application

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### **Abstract**

Pressure-sensitive adhesives were in wide use since the late 19th century, starting with medical tapes and dressings and now with added electrically conductive filler gaining popularity in electronics, biomedicine and automotive. This adhesive is a specific composition of polymeric materials (epoxy, silicone, acrylic, polyurethane, etc.) for which electrical conductivity can be provided by inserting electrically conductive particles - filler. It can be found in the literature that metal particles (silver, gold, nickel, etc.) and inorganic materials (graphite fiber, soot, metallized glass spheres, etc.) are used for this purpose. The electrical conductivity occurs when the percolation threshold is reached, i.e. when the filler particles come into contact with each other and form a conductive path. This factor is heavily influenced by their nature, the number of particles, size, morphology and distribution. By increasing the concentration of the filler, the adhesive passes from the insulator to the conductor. This report analyzes new potentially available fillers that would be more cost-effective, but no less efficient than the currently used in conventional application. The future perspectives of pressure sensitive electrically conductive adhesives for biomedical application is discussed as well.

**Keywords:** pressure sensitive adhesive, electrically conductive filler, biomedicine.

## Poster presentation

# The Search of Plant Dihydroquercetin Sources and Evaluation of Their Extracts Characteristics

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### Abstract

Dihydroquercetin is described as one of the strongest naturally occurring antioxidants. It protects the liver from the effects of toxic substances, strengthens the cardiovascular system and is important in cancer treatment [1]. This compound can be found in plants and their parts, such as milk thistle (*Silybum marianum* L.) seeds, Siberian larch (*Larix sibirica* Ledeb.) core, red clover (*Trifolium pratense* L.) seeds, an aerial part of sweet clover (*Melilotus* Lam.), and red onion (*Allium cepa* L.). In this study, extracts of these and similar plant sources of dihydroquercetin were selected to investigate the amount of phenolic compounds and flavonoids, and their antioxidant activity [2]. High performance liquid chromatography with electrochemical detection was chosen for identification of dihydroquercetin in the plant extracts [3]. Antibacterial activity against pathogenic bacteria of examined extracts was evaluated using the agar-well diffusion assay [4]. Considered the obtained data the correlation between different parameters of the plant extracts was also evaluated.

**Keywords:** dihydroquercetin, plant extracts, phenolic compounds, flavonoids, antioxidant activity, high performance liquid chromatography, antibacterial activity.

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## Poster presentation

### Determination of Synthetic Musks in Perfumes by GC-MS

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#### Abstract

Synthetic musks exhibit a strong, warm, sensual and long-lasting odour, which makes them essential in modern perfumery and form the base note foundations of most perfume formulas. Nevertheless, synthetic musk fragrances have been described as a new group of bioaccumulative and persistent xenobiotics. Synthetic musks are generally divided in three subgroups: nitro musks, polycyclic musks and macrocyclic musks. Nitro musks dominated the market for many years but declined significantly in the 90s due to their bioaccumulative properties and health adverse reactions, which led to the prohibition of musk tibetene, musk moskene and musk ambrette. At the present, other two nitromusks, musk ketone and musk xylene are still permitted but with restrictions. There was a parallel increase in the use of polycyclic musks which comprises several high volume use products, such as tonalide and galaxolide. Research indicate that the polycyclic musks are environmentally persistent, can accumulate in human bodies, and they are suspected hormone disruptors. Macrocyclic musks were more recently introduced to the market. Since customers and fragrance industries detected problems regarding human health and the environment related to some of the fragrance compounds used, a search for substitutes have been intensified. Within the musk family of fragrances synthetic musks have replaced the natural musk, mainly for economic reasons. Among the synthetic musks, the trend is to replace the nitrocyclic musks with macrocyclic and polycyclic musks. This is due to the superior fragrance qualities of the newer materials and to the concerns about potential toxicity of the nitro musk. The objective of this study is to determine the presence of nitro musks, polycyclic musks and macrocyclic musks in perfumes. The identification of musks were carried out directly with minimal sample preparation required on 15 samples of brand name and imposter fragrances using Shimadzu (TQ-8040) series GC-MS system (Tokyo, Japan) equipped with an AOC-6000 auto-sampler. Chromatographic separation was achieved on a Restek Rxi-5SilMS column (30 m x 25 mm (i.d.) x 25 µm film thickness). The oven temperature gradient began at 150 °C, held for 1 min, ramped to 200 °C at 30 °C/ min, followed by a ramp to 260 °C at 5 °C/ min where it was held for 1 min and a final ramp to 280 °C at 20 °C/ min which was held for 5 min. A 1 µL volume of each sample was injected in split mode at a split ratio of 20:1. The inlet temperature was set at 250 °C. Detector parameters used for GC-MS analyses were as follows: interface temperature, 280 °C; ion-source temperature, 210 °C; scan time, 0.2 s/scan. For single scan analysis, the scan range was set from m/z 33 to 400. The lowest amount giving well detectable peaks is 1 mg musk compound per kg sample.

**Keywords:** Synthetic musks, perfumes, gas chromatography-mass spectrometry.

## Poster presentation

### Detection of Phthalates in Perfumes by GC-MS

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#### **Abstract**

Phthalates (phthalic acid esters), especially diethyl phthalate, are commonly used as solvents and fixatives in perfumes. Phthalates have received special attention in the last years due to their ubiquitous presence in the environment, the clear evidences of their reproductive toxicity and their estrogenic activity. Most perfumes contains non-negligible amounts of diethyl phthalate. Rapid and sensitive detection of diethyl phthalate in perfumes is thus of increasing importance. The identification of phthalates were carried out directly with minimal sample preparation required on 20 samples of brand name and imposter fragrances using Shimadzu (TQ-8040) series GC-MS system (Tokyo, Japan) equipped with an AOC-6000 auto-sampler. Chromatographic separation was achieved on a Restek Rxi-5SilMS column (30 m x 25 mm (i.d.) x 25 µm film thickness). The oven temperature gradient began at 150 °C, held for 1 min, ramped to 200 °C at 30 °C/ min, followed by a ramp to 260 °C at 5 °C/ min where it was held for 1 min and a final ramp to 280 °C at 20 °C/ min which was held for 5 min. A 1 µL volume of each sample was injected in split mode at a split ratio of 20:1. The inlet temperature was set at 250 °C. Detector parameters used for GC-MS analyses were as follows: interface temperature, 280 °C; ion-source temperature, 210 °C; scan time, 0.2 s/scan. For single scan analysis, the scan range was set from m/z 33 to 400. The major phthalate was diethyl phthalate that was found in 18 samples with concentration ranging from 0.2 to 21583 mg/kg.

**Keywords:** Phthalates, perfumes, gas chromatography-mass spectrometry.

## Poster presentation

### ***In vitro* Analysis of the Possibility to Simultaneously Transfer Bleomycin and Plasmid DNA in Cells via Electroporation.**

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#### **Abstract**

Electroporation is a process when as a result of electric fields effect induced transmembrane potential create transient pores in the plasma membrane, leading to a large increase in drug transport, delivery of macromolecules, a quick drug effect onset. Hence, anticancer drugs can be easily transported into targeted tumour cells by using this technique. Nowadays, electrochemotherapy is used in clinics for the cancer treatment. Electrochemotherapy is a process, when electroporation is combined with the injection of cytotoxic drugs (e.g. bleomycin (BLM)). A single molecule of bleomycin can cause around 8–10 DNA breaks causing the cell death, which explains its high cytotoxicity when present inside the cell. However, it is only a local response. In order to initiate a systemic response rate, one has to induce immune response. One way to do it is to transfect a gene that express a compound that enhance immune response. In this study, we show the possibility to simultaneously transfer bleomycin and plasmid DNA in cells via electroporation.

Chinese hamster ovary (CHO) cell line was used for experiments. The final concentration of BLM in experiments ranged from 0.1 ng/ml to 20 ng/ml. For that different concentrations of anticancer drugs were used together with pDNA transfection. pMAX FGP coding plasmid in concentraton of 200 µg/ml was used. Electroporation was performed by using combination of 1 electric pulse that induced electric fields at the amplitude of 1400 V/cm for the duration of 100 µs. Cells were electroporated in laboratory made EP medium (pH 7.1, conductivity 0.1 S/m, osmolarity 270 mOsm). Afterwards, comet assay was performed to evaluate DNA damage. In addition, clonogenic assay was done to evaluate cell viability. Transfection efficiency was measured using flow cytometry (BD accuri C6).

Experimental results showed a significant increase in DNA breakage leading to cell death using BLM electrotransfer after combination of bleomycin, pDNA and electroporation. In conditions of 1HV electroporation intensity, 200µg/ml pDNA and 20 ng/ml BLM concentrations, DNA cleavage reaches up to 10%. Meantime, while electroporating the cells together with the highest BLM concentration, DNA damage reaches only circa 5%. At the same time transfection efficiency was measured reaching 50% of transfected cells.



## Poster presentation

### Analysis of Antimicrobial and Antioxidant Properties of Bee Bread

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#### Abstract

Bee bread is a product of the hive obtained from pollen collected by bees, to which they add honey, enzymes and is stored in the combs. Increasing evidence suggests bee bread's potential therapeutic benefits, including antimicrobial, antioxidant and anticancer properties [1]. Most of the bee bread properties are influenced by phenolic compounds such as flavonoids and phenolic acids, which is considered to be the main reason for its antimicrobial and antioxidant properties [2].

The aim of this research was to determine the effect of different extraction process of bee bread on its antioxidant, antibacterial and antifungal activity. Eight samples of bee bread were collected from different locations of Lithuania and prepared using different solvents (methanol and water) and using enzyme assisted extraction method. The antimicrobial activity of bee bread extracts was evaluated by agar well diffusion assay against three different types of bacteria and two different types of molds. Total phenolic compound content was evaluated by spectrophotometric Folin-Ciocalteu test, total flavonoid content by colorimetric  $AlCl_3$  assay and antiradical activity using the spectrophotometric DPPH bleaching assay [3]. The antifungal activity against mould species, namely *Aspergillus niger* and *Monilinia fructicola*, and antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* were also evaluated. Study showed, that both antioxidant and antimicrobial activity were depended on sample geographic origin, what may be strongly related with botanical origin of bee bread.

**Keywords:** bee bread, antibacterial activity, antifungal activity.

**Acknowledgements:** This project was financed by Research Council of Lithuania project No. 09.3.3-LMT-K-712-10-0248. Wilara, Ltd for providing bee bread samples.

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## Poster presentation

### Genetic Diversity of *Monilinia* spp. Species within $\beta$ -tubulin Gene

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#### Abstract

The main disease of pome and stone fruit plants in *Rosaceae* family is brown rot. This disease reduces the fruit quality during and post-harvest period and cause about 30% yield losses each year. Brown rot is caused by worldwide spread *Monilinia* spp. pathogens: *Monilinia laxa*, *M. polystroma*, *M. fructigena* and *M. fructicola*. *Monilinia laxa* and *M. fructigena* are considered indigenous in Europe, *M. polystroma* – origin was first identified in Japan, while *Monilinia fructicola* is named “New World” pathogen and is indigenous in USA and Australia. Despite *M. fructicola* has been discovered in thirteen European countries, the pathogen is in the list of quarantine pathogens in Europe. Appearance of the mycelium of this fungus is similar to other *Monilinia* pathogens. Therefore, only molecular methods are reliable for identification of this pathogen. In Lithuania this invasive pathogen is still absent according to results of molecular analysis. Knowledge of the genetic diversity of *Monilinia* spp. is required for the control of this fungal disease spread. The genomes of all four *Monilia* spp. pathogens are sequenced, using the next generation of “Illumina” platform and uploaded to *National Center for Biotechnology Information* (NCBI) GenBank database. Relationship between pathogens can be investigated according to polymorphism of certain genes. Tubulin coding gene is major components of microtubules that are important for cell division and intracellular transport.  $\beta$ -tubulin forms the building block of the microtubule by assemble in a head-to-tail heterodimers. In the NCBI GenBank data base 1879 records for *Monilinia* spp. were found while only 2 records in NCBI Refseq secondary data base. Thirty-two  $\beta$ -tubulin sequences from the NCBI GenBank database were used for genetic diversity analysis of *Monilinia* spp. During this study multiple sequence alignment (MSA) and phylogeny analysis were performed by using Molecular Evolutionary Genetics Analysis (MEGA X) platform [1]. Transitions, transversions, deletions and insertions were established in 402-1630 bp length *Monilinia* spp.  $\beta$ -tubulin sequences. Intraspecific and interspecific differences among *Monilinia* spp. pathogens were established. Highest level of intraspecific genetic differences was observed in  $\beta$ -tubulin sequences of *M. fructicola* pathogen during MSA analysis. Geographical area and the host - plant were evaluated as important factors for the differences among pathogens. The results showed that quarantine pathogen *M. fructicola* is genetically the most distant from all other *Monilinia* spp., particularly *M. laxa* fungal pathogen. Furthermore, genetically closest species are *M. polystroma* and *M. fructigena* according to  $\beta$ -tubulin gene sequences.

**Keywords:** *Monilinia*, Brown rot, beta-tubulin, genetic diversity.

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## Poster presentation

### The Effects of Soil Properties and Contamination on Toxicity to *Eisenia fetida*

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#### Abstract

Soil contamination by trace elements is a common problem around the world raising concerns on the potential effects to human health and environment. Shooting activities is a source of remarkable environmental pollution. The negative impact of the shooting ranges on environment occurs through shot and bullets containing metals that are harmful to the environment. The aim of the study was to evaluate the toxicity of trace elements to *Eisenia fetida* in recreational and military shooting range soils. The earthworms were exposed to soils, and mortality, growth and reproduction endpoints were determined. In the recreational shooting range total Pb concentrations were 6151 mg kg<sup>-1</sup> and in soil of the impact berm of military shooting range was 653 mg kg<sup>-1</sup>. These Pb contaminated soils caused significantly higher mortality, weight loss and lower reproduction than the reference grassland soil. The most sensitive indicator was reproduction rate - a significantly lower cocoon production was found in shooting range soils than in reference soil. Among soil parameters, content of soil organic matter and bulk density had influence on the survival of earthworms.

**Keywords:** ecotoxicity; lead; earthworms; shooting range.

## Poster presentation

### Different Fertilizers Effect on Photosynthetic Resistance to Drought of Barley (C<sub>3</sub>) and Millet (C<sub>4</sub>) under Two Climate Conditions

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#### Abstract

The aim of this research was to evaluate the effect of different fertilization on photosynthetic resistance of C<sub>3</sub> plant barley and C<sub>4</sub> plant millet to drought stress under different climate conditions. Half of investigated plants were sown in peat substrate with mineral fertilizers and other in substrate with organic fertilizers. All plants were grown in automatically controlled climatic conditions under current (400 ppm CO<sub>2</sub>, 21/14 °C, day/night) and elevated (800 ppm CO<sub>2</sub>, 25/18 °C) climates, and well-watered and drought stressed conditions. There was obtained, that elevated climate increased the growth of both investigated plant and the effect was more pronounced for barley. Drought stress was decreasing growth parameters of barley, while for millet no statistically significant effect was detected. Single effect of different fertilization on dry biomass of both plants was weak and statistically insignificant in almost all treatment variants, with exception for barley grown under current climate, when dry biomass of plants fertilized with mineral nutrition increased ( $p < 0.05$ ), compare to plants fertilized with organic fertilizers. Water deficit decreased photosynthetic rate of barley plants ( $p < 0.05$ ), while the effect on photosynthetic rate of millet was statistically insignificant ( $p > 0.05$ ) under both investigated climate conditions. Lower losses of photosynthetic rate of drought stressed barley were detected for plants grown with organic fertilization, and the effect was more pronounced under elevated climate. Lack of the water decreased transpiration rate of barley under both climates. Under current climate higher decreases were detected for barley plants fertilized with organic fertilizers, while under elevated climate conditions the changes were opposite. Whereas drought stress caused variations of transpiration rate of millet were different, i.e. under current climate conditions it decreased ( $p > 0.05$ ), and under elevated it increased ( $p < 0.05$ ) in both fertilization variants. Mineral nutrition and elevated climate increased electron transport rate of barley, while for millet the effect was a little bit different. Water deficit decreased both: trapped energy flux per cross section and amount of active PSII reaction centers in barley leaves, equally the changes were lower under organic fertilization and elevated climate conditions, however for millet no statistically significant changes were detected.

**Keywords:** climate change, fertilizers, drought, photosynthesis, chlorophyll a fluorescence.

## Poster presentation

### The Influence of Meteorological Factors on Vegetation Rhythms of *Artemisia absinthium* L.

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#### Abstract

The seasonal development rhythms of the medicinal, spice (aromatic) plants (MAPs) match with the length of vegetation period and reflect seasonal and periodic climatic conditions. These changes are important in the context of plant species introduction from their natural habitats to *ex situ* collections [4].

Previous studies have found that Lithuania's largest number of (MAPs) species – 46 are *Astraceae* (Bercht. & J. Presl) family [2]. In the plant kingdom, family *Asteraceae* is endowed with prospective plants and among these plants, the genus *Artemisia* L. occupies top position for its bio – prospection. The genus consists of small herbs and shrubs, found in northern temperate regions and comprises of about 500 species [1].

The investigations were conducted in Spice – Melliferous plants collections of Scientific sector of Medicinal and Aromatic Plants of Botanical Garden at Vytautas Magnus University during vegetation periods in 2017-2018. The object of investigations was *Artemisia absinthium* L. – medicinal, spice (aromatic) perennial herbaceous plant of the largest *Asteraceae* (Bercht. & J. Presl) family, native of Europe, Caucasus, North India, North and South America, Asia Minor [3].

The results of a study on the dependence of growth and vegetation process of *Artemisia absinthium* upon meteorological factors are presented that the earliest beginning of vegetation and optimal climatic conditions for growth are when hydrothermic coefficient reaches 1.50-1.0 (optimal climatic conditions). Then conditions of excessive humidity for massive flowering and the end of flowering maturation are observed when hydrothermic coefficient increases to 1.6-2.0. Research found that 2017 all phenological phases of *Artemisia absinthium* started earlier than 2018 about 10 days.

*Artemisia absinthium* L. passes the whole development cycle under climatic conditions of Central Lithuania.

**Keywords:** *Artemisia absinthium*, meteorological factors, vegetation period.

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## Poster presentation

### Single and Combined Effect of Drought and Fertilization on Accumulation of Nitrates in Garden Lettuce

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#### Abstract

Leafy vegetables are characterized by ability to accumulate nitrates ( $\text{NO}_3^-$ ) - toxic and carcinogenic compounds. Overfertilization of agricultural plants increases potential harvest, however the risk of harmful concentrations of nitrates also increases. The aim of this study was to investigate the single and combined effects of drought and complex fertilizers on yield of garden lettuce (*Lactuca sativa* L.), as well as accumulation of nitrates in the harvested leaves. A pot experiment was carried out in phytotron growth chambers. Seedlings of lettuce were grown in commercially bought vegetable substrate; at the 3.3. BBCH growth stage 3 levels of fertilization were applied: recommended amount, 2 times and 4 times higher than recommended amount. Water shortage was applied at the 3.7. BBCH growth stage and lasted 7 days. At the end of this treatment, plants were harvested, dry and fresh biomass per plant, moisture content in leaves, concentration of photosynthetic pigments and accumulation of nitrates in leaves were investigated. Recommended fertilization level under control conditions had positive effect on plants growth and moisture content in leaves; whereas 2 and 4 times higher than recommended fertilization did not have significant positive effect on lettuce growth and reduced the relative leaves humidity. Drought remarkably reduced growth parameters and relative leaves humidity in both non-fertilized and fertilized plants; however, the lowest negative effect was observed in plants fertilized with 2 times higher than recommended amount of fertilizers. Unfertilized plants accumulated relative low amounts of nitrates (0,9 mg/g FW), independent of water availability. Recommended fertilization did not stimulate nitrates accumulation statistically significantly; however, 2 and 4 times higher than recommended amount of fertilizers increased nitrates concentration in lettuce leaves by ~4,5 and ~5 times, respectively. Apart from this, water shortage drastically increased  $\text{NO}_3^-$  concentration in plants fertilized with recommended amount of fertilizers (67 %, up to 3,3 g/kg FW). Drought-induced stimulation of nitrates accumulation was not detected in over-fertilized plants. In contrast, water shortage significantly reduced  $\text{NO}_3^-$  concentration in lettuce leaves at the highest level of fertilization.

**Keywords:** *Lactuca sativa*, nitrates accumulation, overfertilization, drought stress.

## Poster presentation

# Combination of Advanced Oxidation and Biological Processes for Removal of Organic Matter and Nutrients from the Secondary Wastewater Treatment Plant Effluent

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### **Abstract**

Water scarcity is one of the major problems that exist in the countries, located close to the equator. Use of all the possible water resources is under the demand in these territories. However, together with water being used, production of the wastewater becomes an issue. Purification and reuse of secondary wastewater treatment plant (WWTP) effluent becomes an attractive option, since the recovered water can be repeatedly reused for technological purposes or land irrigation. At the moment, membrane processes are popular for the purification of the secondary effluent. However, membrane scaling and biofouling limits the application of this purification method. However, application of biological activated carbon (BAC) prior the membrane filtration can help to reduce the biofouling and scaling [1]. In this study we applied advanced oxidation and BAC treatment for the reclamation of WWTP secondary effluent.

From the batch experiments we can conclude, that ozonation enhanced the formation of easy biodegradable organic matter; therefore, the biodegradation of organic matter also increased 24 – 42%. The removal of nitrogen and phosphorous was not greatly influenced by the ozonation itself. However, removal efficiencies of 42% and 36% of nitrogen and phosphorous were observed in a BAC reactor, respectively. Moreover, higher than 24-hour hydraulic retention time and higher concentration of suspended biomass concentration ( $> 1$  g/L) resulted in greater removal efficiencies of total organic carbon, nitrogen and phosphorous. Based on the findings of this study we can conclude that ozonation coupled together with BAC can be used successfully as a pretreatment of the secondary WWTP effluent prior the membrane filtration [2].

**Keywords:** Wastewater, secondary effluent, biological activated carbon, nitrogen, phosphorous.

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## Poster presentation

### Validation Procedure of 96-Well Plate Spectrophotometric Method for the Quantification of Flavonoids and Total Phenolic Compounds

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#### **Abstract**

Validation procedure for determination of flavonoids and total phenolic compounds (TPC) was prepared for Biosan HiPo MPP-96 Microplate Photometer (Latvia). The advantage of new assay based on 96-well plate procedure is simple, fast and inexpensive measurement decreasing total assay solution volume up to 4 times in comparison with the previously optimized non-automated spectrophotometric assay using manual pipetting and spectrophotometer (Spectronic, Milton Roy, USA) [1, 2].

Calibration standards of rutin solution were prepared within the range of 0.01–1.00 mg/ml. For determination of flavonoids absorbance was measured at 407 nm (Milton Roy) and 405 nm (Biosan) and for determination of TPC was measured at 760 nm (Milton Roy) and 694 nm (Biosan). Calibration curve was built of 5 concentrations.

To evaluate the linearity within described concentration range and determination coefficient  $R^2$ , accuracy, precision, detection limit and quantification limit new assay was validated according to the recommendations of ICH Guideline Q2(R1) [3].

Accuracy was evaluated using known intermediate rutin solutions. The interday precision was evaluated following the same procedure for different days. The precision of the measurements was reported as the relative standard deviation (RSD%). Detection limit and quantification limit were determined based on the standard deviation of the response and on the slope of the calibration curve [3].

**Keywords:** Validation, flavonoids, total phenolic compounds, 96-well plate spectrophotometry, miniaturization, automation of assay.

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## Poster presentation

### Modeling of Rutin Species Distribution and Molybdate Complex Forming in Reaction with Folin-Ciocalteu (F-C) Reagent Using PHREEQC Tool

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#### Abstract

PHREEQC is a computer program developed by the US Geological Survey (USGS) and has become the standard for water chemistry. At present PHREEQC is implementing in biochemistry and environment science where a computational tool is needed.

This study is an attempt to model the distribution of rutin species at different pH, acidic pH shifting after addition of sodium bicarbonate instead of sodium carbonate and molybdate-rutin complex formation in F-C reagent.

This study used a simplified model of pH 6.8 rutin solution with 5 species  $H_4Rut$ ,  $H_3Rut^-$ ,  $H_2Rut^{2-}$ ,  $HRut^{3-}$  and  $Rut^{4-}$  corresponding to  $pK_a$  values. Reaction mixture reaches pH 10.63 value by mixing 0.311 M sodium carbonate solution, 0.5 mg/mL rutin solution and 2 N Folin-Ciocalteu reagent with prescribed proportions 30:1:1 [1, 2].

At pH value 10.63 anionic rutin species  $H_3Rut^-$  (1%),  $H_2Rut^{2-}$  (12 – 98%),  $HRut^{3-}$  (1 – 54%) dominate depending on  $pK_a$  constants [3–5]. Neutral species  $H_4Rut$  is minor (less than 0.001%). Two molybdate-rutin complex constants  $pK_1 = -8.01$  and  $pK_2 = -3.14$  were used [6]. An alkaline environment is critical to the reaction [7]. Alkaline pH is reached by adding sodium carbonate [8]. Complexes forming below pH value 6.0 was not taken into consideration by the simplified model.

Computation result of 3<sup>rd</sup> model revealed that at relatively wide pH interval (8 – 11) a concentration of molybdate-rutin complex is within the range of 26.88 – 27.0  $\mu M$ , and approximately 99.98 – 99.99 % of the total rutin amount was found in the form of molybdate-rutin complex.

**Keywords:** PHREEQC, rutin, molybdate-rutin complex, Folin-Ciocalteu reagent.

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## Poster Presentation

### Variation of Biologically Active and Volatile Compounds of Lilacs (*Syringa vulgaris* L.) During Different Vegetation Periods

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#### Abstract

Lilac (*Syringa vulgaris* L.) belongs to Oleaceae family; it is a small bush or tree, blooming with a vivid appearance, having blossoms from white to deep purple in color. It is one of the oldest and the most popular decorative bushes. Lilac extracts may be used as natural remedies because of their antioxidant, antibacterial and anti-inflammatory properties [1].

The aim of this research was to evaluate total amount of biologically active compounds, antioxidant activity and volatile compound composition of *Syringa vulgaris* L. collected during different plant vegetation periods. Raw material was collected from two different *Syringa vulgaris* L. color bushes: white and purple. Buds and leaves were collected in spring, blossoms in summer, leaves and seeds in autumn.

The total amount of phenolic compounds and flavonoids together with antiradical activity were evaluated in methanolic extracts using spectrophotometric analysis methods [2]. The highest amount of phenolic compounds were found in white lilac buds and purple lilac leaves collected in spring, i.e. 105.1±4.4 mg/g (expressed in rutin equivalents (RE)) and 102.8±4.0 mg/g, respectively. The highest content of flavonoids was also determined in white and purple lilac leaves collected in spring, 35.6±1.4 and 38.0±1.3 mg/g, respectively. Antiradical activity varied from 306.4±1.5 to 636.7±1.8 mg/g in the tested samples, the highest activity was observed in the buds extracts of purple lilacs.

Gas chromatography with flame ionization detector (GC-FID) was used for analysis of volatile compound composition and identification was performed by Kovats retention index. Hydrodistillation was applied for buds and blossoms of purple lilacs and obtained hydrolat was used for GC-FID analysis. Methanolic extracts were re-extracted with n-heptane and obtained samples were also tested by GC-FID. Lilac aldehyde A, lilac aldehyde B, estragole, benzaldehyde, β-pinene and lilac alcohol were identified in hydrolats of buds and blossoms from purple lilac, while phytol, farnesyl acetate - (2E,6E), methyl octadecenoate, hexadecanoic acid, oleic acid were predominant in n-heptane extracts.

**Keywords:** *Syringa vulgaris*, lilac, phenolic compounds, antiradical activity, volatile compounds.

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## Poster presentation

### Possibilities for Biorefining of Food Waste and Non-Conditional Products

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#### Abstract

Biodegradable waste and their correct management are some of the most sensitive issues nowadays. Throwing out biodegradable waste along with other wastes causes biogas to be released as an output during the biodegradation process that increases global greenhouse effect, pollution of environment and many other problems. Nowadays the great attention is paid to recycling of different biomass, including food wastes, which can be a potential raw material to produce different value-added products. The separation of organic waste parts and reprocessing of them, could lead to valuable products and materials, for example, biofuel, fertilizers, composting, biopolymers or even antimicrobial or antioxidant agents [1]. Biomass used for renewable energy is an attractive option received during biorefining. Biorefining is not a process of fossil raw materials but bio-raw materials reprocessing into various products [2]. Other biorefining processes may be applied for biomass, such as extraction, ultra/nanofiltration, distillation, chromatographic separation. The purpose of this project was to evaluate biorefining potential of some food wastes and non-conditional food products.

The following raw food waste materials have been selected for the project: banana peel, mandarin peel, apple, non-conditional carrot and coffee grounds. Total content of flavonoids, phenolic compounds and antiradical (DPPH) activity was determined in extracts of the tested food wastes. Fermentation by *Sacharomyces cerevisiae* of analyzed food wastes was performed without sample pretreatment and after enzymatic hydrolysis. Bioethanol content was evaluated after fermentation using gas chromatography with flame ionization detector.

The results showed that the highest amount of phenolic compounds was found in coffee grounds, while the lowest in carrot pomace. The highest amount of flavonoids was also determined in coffee extracts and mandarin peels extracts. Extracts from apple pomace showed the highest antiradical activity. The highest amount of bioethanol was obtained in the fermented mandarin peels after enzymatic hydrolysis.

**Keywords:** food waste, bioethanol, enzymatic hydrolysis, biorefinery.

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## Poster presentation

### Influence of Organic Acids on Formation of Methanol and Other Alcohols in Home-Made Apple Wine

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#### Abstract

In many countries, including Lithuania, wine produced of locally grown apples, a variety of fruits and berries is considered as a part of the national culture.

Methanol is natural ingredient of alcoholic beverages and soft drinks, produced by hydrolysis of methyl ester groups of pectin, however, larger amount of methanol is highly toxic to humans [1]. The lethal dose is usually from 100 ml to 200 ml of methanol, while the long-term blindness can be inflicted by taking only 10 ml [2]. Higher alcohols are present in wines and are formed in small amounts by yeast metabolism during alcoholic fermentation process. Some of them may possess positive and others negative effect on the aromatic wine profile [3]. However, Hou et al. pointed out that adding gallic acid or coumaric acid during winemaking process is a potential method for reducing methanol content, improving wine quality, as well as increasing healthy compounds in wine production [4].

The aim of this research was to determine the influence of organic acids on methanol formation in home-made apple wine. Different amounts of gallic and trans-p-coumaric acids were added into apple juice during fermentation process. Quantitative analysis of methanol was evaluated in the prepared apple wine by gas chromatograph with flame ionization detector.

The reduction of methanol content was determined in all cases after fermentation. Determined quantities of methanol in apple wines did not exceed the permitted limits. After fermentation, methanol concentration reached 0.052%. Organic acids in apple wine allowed to decrease the methanol content up to 1.4 times. The reduction level was also depended on the organic acid and its quantity.

**Keywords:** methanol, organic acids, apple wine, gas chromatography.

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## Poster presentation

### **The Dependence of the Cell Electrotransfection Efficiency on the Electrosensitization Induced Membrane Changes and the Time of DNA Addition to the Electroporation Medium**

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#### **Abstract**

Electroporation – a physical method that temporarily disrupts the membranes and increases the cell membrane permeability – has been known for a few decades. The application of short, high voltage (kV/cm) electric pulses leads to the formation of transient pores that allow the passage for different types of molecules into the cell. It has been applied for numerous uses in the technology and medicine. One of these applications is the gene electrotransfection, when the electroporation is used for the delivery of DNA to the cells. It is well known that the delivery of the DNA to the cells is much more complex in comparison to the delivery of small molecules. The aim of this work was to compare the dependence of cell electrotransfection efficiency on the electrosensitization induced membrane changes and the time of DNA addition to the electroporation medium. All the experiments were done with Chinese Hamster Ovary (CHO) cells. The cells were electroporated using combinations of 1400 V/cm, 100  $\mu$ s electric pulses. 100  $\mu$ g/ml of the plasmid coding for green fluorescent protein (pGFP) was used to evaluate the cell electrotransfection efficiency. Cell fluorescence was measured 24 hours after the transfection using flow cytometer (BD Accuri C6).

The cells were electroporated once and then again after 10, 15 or 20 minutes, and pGFP was added to the solution either before the electric pulses or during the waiting time. The results show that the addition of the time-delayed electric pulses increases the transfection efficiency in comparison to the settings used for the initial electroporation only if the plasmid is present in the medium before the first electroporation. If the plasmid is added to the solution during waiting time, the electrotransfection efficiency is reduced, signaling electro de-sensitization.

**Keywords:** electroporation, DNA, electrosensitization.

## Poster presentation

### ***Artemisia* Genus and its Contribution on Treatment of Modern Diseases**

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#### **Abstract**

With the increase of different diseases, medicinal plants have been the most successful to combat these diseases and most of the world's population uses medicinal plants as their primary health care (WHO, 2011). In this review we look at one of the species, genus of *Artemisia* with their very many pharmacological functions.

The aim of this review was therefore to analyse scientific research data published on a few different species of *Artemisia* Genus and their contribution to modern diseases.

In this review, we have compiled data of recent scientific publications (2008–2018) on the pharmacognostic aspects, chemical constituents of some species of *Artemisia* Genus.

The genus *Artemisia* L. consists of more than 500 species throughout the world. *Artemisia* species are well known for the treatment of malaria all over the world. These species are widely used for the treatment of diabetes, epilepsy and for psychoneurosis. Since they have hepatoprotective activity and they can be used for various liver disorder from traditional validation. They exhibit anesthetic, cosmetic, antiseptic, antispasmodic, CNS stimulant, cancer preventive, analgesic, nematicidal, pesticidal, antiasthmatic, antibacterial, perfumery and sedative activities. These species reported to exhibit repellency about 90% against *Aedes aegypti* mosquito that transmits dengue and yellow fever. Because of anti-inflammatory properties *Artemisia* species are being used more in the treatment of skin diseases like atopic dermatitis, psoriasis and more. *Artemisia* species are widely distributed of over the world but mostly in Africa and Asia. This review aimed at describing the different uses of these species in treatment of modern diseases of our century. Our focus mainly was to review available scientific research that has been conducted on this species. Due to the different components found in different species of the same genus, scientists are finding more ways and conducting more experiments to find out other modern diseases these species can treat, hence more investigation is still needed.

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## Poster presentation

### Investigation of Various Electroporation Conditions for Efficient Gene Transfection

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#### Abstract

Gene electrotransfection (GET) is a widely used gene delivery method which is based on the application of electric fields. The phenomenon of gene delivery into cells by means of electric pulses were proved to be more complicated compared to electrotransfer of low molecular mass molecules through the cells plasma membrane and involve endocytosis. GET already demonstrated itself as a relatively inexpensive and safe method in clinical trials because plasmid DNA is introduced into the cells without viruses or additional chemicals. However, the efficiency of GET is still low when carrying large genes and cell death after GET.

The aim of this research was to increase transfection efficiency without reducing cell viability. Experiments were done with Chinese hamster ovary (CHO) cell line, using different electroporation parameters to check the efficiency of GET. Green fluorescent protein (pmaxGFP) plasmid transfection efficiency was studied by measuring number of transfected cells and their fluorescence intensity with a flow cytometer (Accuri™ C6, USA) 24 h after application of different number of high voltage (HV) pulses. Cell viability was evaluated using a clonogenic assay 6 days after the experiment.

It was determined that using a different number from 800 to 1600 V/cm in increments of 200 V/cm HV pulses most of the cells were transfected at field intensities of 1200- 1400 V/cm. These parameters also gave the highest cell viability and fluorescence intensity of transfected cells compared to other parameters of pulses that gave similar transfection efficiency. Our results suggest that using less number of more intensive HV pulses was more efficient for GET and preserving cell viability than using more but less intensive HV pulses. In addition, using too much intensive electric field (e.g. 1600 V/cm) was inefficient for GET, although reduced cell viability drastically.

**Keywords:** gene electrotransfection, transfection, cell viability, GFP, electroporation.

## Poster presentation

### Investigation of ATP Influence on Cells Viability After Electroporation

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#### **Abstract**

Using high-voltage (HV) short (ns,  $\mu$ s) electrical pulses induce alterations in the cells plasma membrane that facilitates nonselective molecular transfer into the cells. Such a phenomenon was called electroporation (EP). Using very high electric field pulses cause instant cell death by necrosis and was called irreversible electroporation (IrEP). IrEP has already demonstrated a remarkable effect on treating tumors by induction of immune response. However, it is still not well understood why and how cells after EP and IrEP die. Moreover, it was proved that molecules such as ATP, proteins either DNA can move out of the cells through the electroporated membrane. The loss of intracellular biomolecules is thought to be one of the reasons that the viability of electroporated cells decreases. The aim of this study was to find out whether compensating the loss of ATP with extracellular ATP can help to preserve cell viability after EP. For experiments, we used Chinese hamster ovary (CHO) cell line. Electroporation was performed by using 9 HV 1200 V/cm electric field pulses with a duration of 100  $\mu$ s repeating at 1 Hz. ATP was weighed before each experiment to avoid its degradation. The final concentration of ATP used was 20  $\mu$ M/ml. Cells were electroporated in the presence or absence of ATP and kept for different periods in different temperatures again in the presence or absence of ATP. The viability of cells was assayed 24 hours after the experiment by Flow cytometry and after 6 days by colony formation test.

Results have revealed that addition of ATP before EP has slightly increased the viability of the cells suggesting that loss of energetic resources during the pulses has a low impact on cell death in the short term. However, keeping the cells in growth medium supplemented with ATP for additional 2 hours in an incubator (37° C, 5 % CO<sub>2</sub>) increased viability of the cells for almost 30 % compared to the viability of cells that were electroporated without ATP. In addition, it was determined, that incubating the cells in ATP supplemented growing medium at room temperature (23°C) did not increase the viability of the cells significantly. In conclusion, our results demonstrate that the viability of cells can be preserved by adding ATP into the extracellular environment medium.

**Keywords:** irreversible electroporation, ATP, cell death, cell viability, flow cytometry.



## Poster presentation

### Detection of the Whey Proteins by RP-HPLC

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#### **Abstract**

The aim of this study was to detect whey proteins  $\alpha$ -lactalbumin ( $\alpha$ -LA),  $\beta$ -lactoglobulin ( $\beta$ -LG) and bovine serum albumin (BSA) by reversed-phase high-performance liquid chromatography (RP-HPLC).

In total 120 milk samples were collected individually from normal lactating dairy cows. Milk samples were collected once during the spring, summer and autumn (10 cows/40 quarter samples).

For evaluation of the content of whey proteins, i.e.  $\alpha$ -LA,  $\beta$ -LG and BSA, samples from cow milk were prepared as follows: 25 mL of raw milk was adjusted to pH 4.6 with 0.1 mol·L<sup>-1</sup> HCl and allowed to stand at room temperature for about one hour for acid precipitation of caseins. Consequently, whey (7 mL) was taken from each of the samples separately and then centrifuged at 10000 rpm for 15 min. Finally, whey solutions were filtered through quality filters and then through 0.20  $\mu$ m disposable sterile filters (Millipore). The supernatants in vials were refrigerated until further analysis and, when appropriate, injected into the chromatograph (20  $\mu$ L).

A reverse-phase analytical column C18 (Nucleosil C18, 300 Å, 5  $\mu$ m particle size, 250 X 4.6, Macherey-Nagel, Düren, Germany) and UV detector were used for the analysis. The separation was carried out at 37°C using the gradient system.  $\alpha$ -LA,  $\beta$ -LG and BSA were detected and separated in 92.31 min, 98.47 min and 93.99 min respectively; purified bovine milk protein genetic variants were employed in calibration. A linear relationship ( $R^2 > 0.99\%$ ) between concentration and peak areas of  $\alpha$ -LA,  $\beta$ -LG and BSA were observed 0.9997, 0.9975 and 0.9996 respectively. Calibration curve designed over a concentration range for  $\alpha$ -LA 0.300-1.500 mg/ml, for  $\beta$ -LG 0.241-1.207 mg/ml and for BSA 0.030-0.253 mg/ml. Data collection and evaluation was performed by using LG Solution (Shimadzu Corp., Kyoto, Japan) operating system. Calibration of the chromatographic system for determination of proteins was carried out by the external standard method. Each protein was calibrated individually by injecting solutions of the standards (20  $\mu$ L).

Purified proteins from bovine milk ( $\alpha$ -LA,  $\beta$ -LG and BSA) were purchased from Sigma (Germany). All chemicals were of HPLC analytical grade. The  $\alpha$ -LA,  $\beta$ -LG and BSA compounds were quantified by comparison between peak area of  $\alpha$ -LA,  $\beta$ -LG and BSA compound in sample and peak area of this compound in standard solution.

In conclusion, in this study was achieved simple and sensitive method for proteins detection in cow's whey.

**Keywords:**  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, bovine serum albumin, milk, RP-HPLC.

## Poster presentation

### The Influence of Various Organic Solvents and Their Concentrations for the Extraction Efficiency of Phenolic Compounds from Hop (*Humulus lupulus* L.) Leaves and Cones

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#### Abstract

The medicinal properties of hop (*Humulus lupulus* L.) have been known for a very long time. However, this plant, which is extensively used in brewery, have recently gained a lot of attention, because hops contain compounds which have antibacterial, antioxidant and even anticancer properties [1]. Usually methanol is the most frequently used solvent for the extraction of biologically active compounds of hops [2]. However, other solvents are sometimes used [3]. The studies of biologically active compounds of hops would probably allow to discover new applications of hop harvest while the use of most effective solvent would allow to exploit the harvest in the most efficient way. The aim of this study was to analyze the influence of various organic solvents and their concentration for extraction efficiency of biologically active compounds from hops. To our knowledge it is the first study about the influence of different solvents for the extraction efficiency of phenolic compounds from Lithuanian hops. The concentration of phenolic compounds and flavonoids was analyzed using spectrophotometric methods [4]. The results were expressed as rutin equivalents mg/g of plant (mg RE/g). The analysis has shown that the highest concentration of phenolic compounds from leaves of hops was extracted using 25 % acetone solution as a solvent (92.55 mg RE/g) whereas the lowest extraction efficiency was achieved using pure acetonitrile (1.84 mg RE/g). In contrast, the highest extraction efficiency of flavonoids was achieved using 25 % N,N-dimethylformamide solution in water (25.75 mg RE/g) however the lowest amount of flavonoids was extracted using pure acetonitrile (2.03 mg RE/g). The highest amount of phenolic compounds from cones of hops was extracted using 75 % N,N-dimethylformamide solution in water (118.09 mg RE/g) while the lowest extraction efficiency was achieved using pure acetonitrile (13.45 mg RE/g). The best extraction efficiency of flavonoids from cones of hops was achieved using pure methanol (27.19 mg RE/g) while the lowest efficiency was achieved using 25 % acetonitrile solution in water (9.53 mg RE/g).

**Keywords:** *Humulus lupulus*, phenolic compounds, organic solvent extraction, spectrophotometry.

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## Poster presentation

### Decontamination of Biological Waste: Means and Possibilities

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#### Abstract

Nowadays, more and more of the nature getting polluted because of the human interference. In order to neutralize and remove pollutants it is important to choose appropriate and environmentally friendly methods. Biological waste is a biohazardous product, which could potentially be harmful to living organisms. In this context, bioremediation is a waste management technique that introduces microorganisms, plants or fungi to eliminate environmental pollutants. The aforementioned techniques can be used in two ways: “*in situ*”, when infected material is treated in place, and “*ex situ*”, when it is processed elsewhere. There are a few types of bioremediation that currently are used. Phytoremediation is a method when plants are used to remove biological waste from soil and water. Plants can remove metals, pesticides, solvents, crude oil and use it as nutrients or accumulate it. The use of fungi in bioremediation is called mycoremediation. Fungi break down waste via secretion of enzymes. Besides this, mushrooms’ fruiting bodies can be utilized as a source of food proteins [0]. In addition, microbial biomass can be used to absorb heavy metals’ ions [0]. There is a possibility to combine these methods to make bioremediation more effective. An example would be neutralization of polycyclic aromatic hydrocarbons (PAH) using bioremediation by microorganisms, biosorption, phytoremediation, etc. [0]. The mentioned strategies may help to make the elimination of biological waste effortless and low-cost.

**Keywords:** Bioremediation, mycoremediation, phytoremediation.

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## Poster presentation

### Analysis of Royal Jelly by Spectrophotometric and Capillary Electrophoretic Methods

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#### Abstract

Royal jelly is one of the most attractive bee products, due to its broad composition and wide range of biological activity [1]. It has been used since ancient times for care and human health and it is still very important in traditional and folk-medicine, especially in Asia [2]. Royal jelly is produced by hypopharyngeal and mandibular glands of worker honeybees [1]. It is composed of bioactive substances such as free amino acids, proteins, sugars, fatty acids, minerals, vitamins and phenolic compounds. It also contains physiologically active substances such as 10-hydroxy-2-decenoic acid (10-HDA) [3]. Royal jelly has been demonstrated to possess a number of pharmacological and biological activities [1]. It is noteworthy that 10-HDA is considered as one of the most important components from which the royal jelly biological activity derives. Considering the antioxidant activity, very important ingredients of the royal jelly are flavonoids and phenolic compounds [4]. This study aims to evaluate the antioxidant activity of royal jelly by spectrophotometric methods and to determine 10-HDA by capillary electrophoresis.

Five Lithuanian and one German sample of royal jelly were investigated in this study. Lithuanian royal jelly samples were collected in different time in June-August. Pure royal jelly was dissolved in distilled water; different dilutions were used in different analyzes. Total phenolic compounds content, total flavonoid content and antiradical (DPPH) activity of the samples was evaluated by spectrophotometric methods. 10-HDA was determined by capillary electrophoresis, using previously developed capillary electrophoresis system [5]. The separation of compounds was carried out using 300 mM, pH 9.0 TRIS borate buffer.

The results showed that the highest content the phenolic compounds and 10-HDA together with the highest antiradical activity were detected in the sample collected in the beginning of August. The highest content of flavonoids was found in the sample collected in middle of July. The phenolic compounds content varied from 8.10 to 9.23 mg/g, while antiradical activity varied from 2.44 to 3.04 mg/g. The flavonoid content ranged from 2.13 to 2.76 mg/g. 10-HDA content ranged from 1.52 to 3.73 percent.

**Keywords:** royal jelly, capillary electrophoresis, spectrophotometric tests, 10-HAD.

**Acknowledgements:** to Ignas Jackevičius from “Brolių medus” for providing royal jelly samples.

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## Poster presentation

### Evaluation of Phytochemical and Allelopathic Properties of *Artemisia dubia* Wall. Extracts and Investigation of Heating Value and Elemental Composition of the Solid Residue

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#### Abstract

In recent years, there has been increasing interest in the use of agricultural biomass for energy purpose in many northern countries. This has created demand for novel, high biomass yielding, specific quality crops for sustainable use [1]. The aim of the research is to investigate *Artemisia dubia* Wall. multistep solvent extraction fractions phytochemical, allelopathic capabilities and the solid phase leftovers biofuel characteristics before and after the extraction. Phytochemical analysis was done using the modified spectrophotometric analysis methods [2] to evaluate the total amount of phenolic compounds using Folin-Ciocalteu reagent, the total amount of flavonoids using AlCl<sub>3</sub> reagent and the antioxidant activity using the DPPH reagent. The results are as follows: largest phenolic content and antioxidant activity was in the third increasing polarity fraction of 50% methanol extract – 66.5 mg RE/g and 48.0 RE mg/g respectively; largest amount of flavonoids were in the decreasing polarity water and methyl tert-butyl ether fractions – 10.4 mg RE/g and 11.4 RE mg/g. In the research, using a modified allelopathic capabilities evaluation method [3], it was determined that small concentrations of both polar and nonpolar fractions are required to trigger noticeable allelopathic activates on Garden cress (lat. *Lepidium sativum*) seeds germination and growth. The most noticeable fraction – decreasing polarity secondary 50% methanol fraction – showed 15% germination and 0.7 mm seedling length, compared to the control group of 85% germination and 20.4 mm seedling length. As for the solid phase biomass, left from solvent-solvent extraction, thermal equipment research and testing laboratory standards [4] and equipment were used to determine thermal capabilities, organogenic composition, moisture and ash content. Ash content before the extraction was 11.6%, afterwards – 6.8-6.5%. Same proportional decrease was shown in the Sulphur content – from 0.241% to 0.138-0.132%. Finally, the caloric value decreased insignificantly – from 16.9 MJ/kg to 15.9-16.1 MJ/kg.

**Keywords:** Solvent fractionation, *Artemisia dubia* Wall., biomass, biofuel, phenolic content, flavonoid content, antioxidant activity, allelopathy, spectrophotometry.

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## Poster presentation

### **Analysis of Malt Extract Growth Medium Incubated with *Pleurotus ostreatus* Strain by Capillary Zone Electrophoresis and Photometric Methods**

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#### **Abstract**

Polycyclic aromatic hydrocarbons (PAHs) form high proportion of creosote, which are used to impregnate wooden railway sleepers. Some of PAHs are potential carcinogens. Some strains of white root fungi are known to produce non – specific extracellular enzymes, which are capable of degrading PAHs. One of those enzymes is laccase. The aim of this research was to develop an optimized capillary zone electrophoresis (CZE) analysis method and photometric analysis method for determination of the activity of laccase enzyme in growth medium.

0,5 M acetic acid was used as background electrolyte, separation voltage – 7.4 kV, sample injection – 20 kPa \* 40 sec, analysis duration – 45 minutes, detector type – contactless conductivity detector, 50 µm inner diameter, coated silica capillary was used for the separation. To determine enzymatic activity of laccase, photometric analyses were conducted at 415 nm wavelength, 50 µl of malt extract grown medium, containing *P. Ostreatus* was diluted with 1350 µl sodium acetate buffer (pH – 4.5), 100 µl of 30 mM ABTS solution was added to the reaction mixture as substrate for enzymatic reaction.

The highest enzymatic activity (0.00236 IU/ml) was determined after six days of *P. Ostreatus* incubation in malt extract growth medium. CZE analysis results showed highest growth medium changes during incubation period of 6 days.

**Keywords:** capillary zone electrophoresis, laccase, photometric analysis.

**Acknowledgements:** Financial support from Research Council of Lithuania project Nr. 09.3.3-LMT-K-712-10-0223 is acknowledged.

## Poster presentation

### Determination of Phenolic Compounds and Coumarins of *Lavandula angustifolia* Mill. by High-Performance Liquid Chromatography

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#### Abstract

Polyphenols and coumarins are the common antioxidant natural products found in medicinal plants. Literature review shows that herbal medicines (especially from Lamiaceae families) have been used from ancient times as remedies for the treatment of diseases because they contain pharmacological and biological active ingredients [1].

The plant material of *L. angustifolia* Mill. herb harvested during the plant's flowering was collected in July 2018 from Botanical Garden of the National university of pharmacy. The products were naturally dried in shadow and stored in controlled laboratory conditions.

In this study, HPLC with a photodiode array detector (HPLC-PAD) method for the determination of phenolic compounds and coumarins of herb of *Lavandula angustifolia* Mill. was applied and would be useful for quality control applications to self-heal and other plants associated with these ingredients. The impact of the solvent on the extraction of these components was also tested [2]. Identification of compounds was achieved by comparing their spectra and retention times with those of standards and literature data. The self-heal phenolic compounds can be successfully separated and identified by HPLC-PAD method with a gradient elution mode. HPLC method was applied for separation and quantification of the phenolic compounds and coumarins of herb of *Lavandula angustifolia* Mill. The optimized HPLC-PAD method was used for the simultaneous analysis (mg/g) of four phenolic acids (rosmarinic acid (13.100), caffeic acid (0.158), ferulic acid (0.029), gallic acid (0.009)), two flavonoid glycosides (rutin (0.031), apigenin-7-O-glucoside (1.080)), two flavonoid aglycones (luteolin (0.121), apigenin (0.424)), one catechin (6.420) and two coumarins (scopoletin (0.468), umbelliferone (19.459)).

A simple, accurate and reliable method for the simultaneous determination of fourteen major phenolic compounds from *P. vulgaris* L. by HPLC-PAD has been developed. Four phenolic acids, two flavonoid glycosides, two flavonoid aglycones, one catechin and two coumarins were determined and quantified of herb of *Lavandula angustifolia* Mill. Methanol and water was the most efficient solvents for extracting phenolic compounds from *L. angustifolia* Mill. herb. Rosmarinic acid was the most abundant phenolic compound and umbelliferone was the most abundant coumarins determined in *L. angustifolia* Mill. herb.

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## Poster presentation

# Phenolic Compounds, Flavonoids and Radical Scavenging Activity of Tetraploid and Diploid Forms of Leaves, Berries and Stalks of Black Currant (*Ribes nigrum* L.)

**Monika Simanavičiūtė<sup>1</sup>, Audrius Maruška<sup>1</sup>, Gintarė Naujokaitytė<sup>1</sup>, Vidmantas Stanys<sup>2</sup>**

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### **Abstract**

The blackcurrant berries are known to have high nutritional value, with a high level of polyphenols, namely anthocyanins, phenolic acids, flavanols and proanthocyanidins. Spectrophotometry was used to characterize total phenolics, flavonoids and radical scavenging capacity in leaves samples collected at different vegetation phases of blackcurrant as well as berries and stalks. Blackcurrant was cultivated in Institute of Horticulture of Lithuanian Research Centre of Agriculture and Forestry. The level of ploidy of analysed blackcurrants was diploid or tetraploid. Using phytochemical analysis of tetraploid blackcurrant was determined that this form accumulates higher amounts of phenolic compounds than diploid form. Higher radical scavenging activity was determined of the leaves of tetraploid blackcurrant comparing to the leaves of diploid form of blackcurrant.



## Poster presentation

### Portable Photometer for Enzymatic Measurements *in situ*

**Tomas Drevinskas<sup>1</sup>, Jūratė Balevičiūtė<sup>1</sup>, Gediminas Dūda<sup>1</sup>, Audrius Maruška<sup>1</sup>, Nicola Tiso<sup>1</sup>, Mantas Stankevičius<sup>1</sup>, Kristina Bimbraitė-Survilienė<sup>1</sup>, Jurgita Mikašauskaitė<sup>1</sup>, Vilma Kaškonienė<sup>1</sup>, Paulius Kaškonas<sup>1</sup>, Tomas Tekorius<sup>1</sup>, Donatas Levišauskas<sup>1</sup>, Mykolas Kazlauskas<sup>1</sup>, Vita Tumosaitė<sup>1</sup>, Ona Ragažinskienė<sup>2</sup>, Vilija Snieškienė<sup>2</sup>, Antanina Stankevičienė<sup>2</sup>, Saulius Grigiškis<sup>3</sup>**

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#### Abstract

Miniaturized instruments offer a possibility of using them *in situ*. Such approach in (bio)chemical analysis greatly reduces the time needed for an analytical task, because no sample transportation is needed resulting in faster analytical process and possibility to spoil the sample is reduced.

In this work, previously developed miniaturized colorimeter has been upgraded [1]. Using the designed system, the enzymatic activity has been determined following previously published procedure [2]. The fungi were grown according to the previously optimized downscaled format [3].

In this work a design of miniaturized photometer and application determining laccase activity in malt extract fermentation medium will be discussed.

**Keywords:** portable, enzymatic assay, laccase, photometer

**Acknowledgements:** Financial support from Research Council of Lithuania project Nr. 01.2.2-LMT-K-718-01-0074 is acknowledged.

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