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17th International Scientific Conference

# THE VITAL NATURE SIGN

May 18th-19th, 2023

Kaunas, Lithuania

ABSTRACT BOOK

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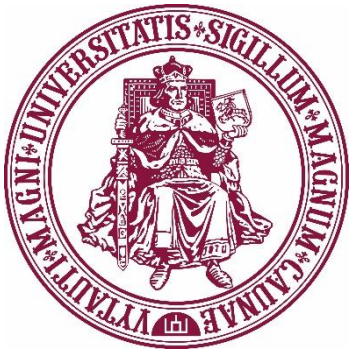



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# 17th International Scientific Conference "The Vital Nature Sign"

## Dedicated to the 95th birthday anniversary of Professor Stellan Hjertén

## Program

### Thursday, 18th of May 2023

**9.00-9.10** (Time zone GMT+3) **Opening of the conference:** ***Prof. Habil. Dr. Juozas Augutis (Rector of Vytautas Magnus university)***

***Prof. Dr. Saulius Mickevičius (dean of the Faculty of Natural Sciences)***

***Prof. Habil. Dr. Audrius Maruška (chairman of the conference)***

**Oral presentations.** ***Chairpersons: Prof. Habil. Dr. Ona Ragažinskienė; Prof. Habil. Dr. Audrius Maruška***

**9.15-9.30** **Congratulation speech dedicated to the 95th birthday anniversary of Professor Stellan Hjertén**
Audrius Maruška

**9.30-10.00** **Underwater Capillary Electrophoresis System: a Proof of Concept Instrument for Ocean Worlds**
Tomas Drevinskas, Aaron C. Noell, Mauro S. Ferreira Santos, Maria F. Mora, Peter A. Willis, Morgan L. Cable, Masahiro Ono

**10.00-10.15** **The Method Development for the Separation and Determination of Cyclitols and Carbohydrates from Biological Samples - As a Proposal for a New Diagnostic Method**
Magdalena Ligor, Małgorzata Szultka-Młyńska, Agnieszka Kucharska

**10.15-10.30** **Polyphenolic Compound Analysis in Alfalfa Using Supercritical Fluid Extraction and Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry**
Justyna Walczak-Skierska, Katarzyna Rafińska

**10.30-10.45** **Data Acquisition Strategies for Improved Proteome Profiling in Single Cell Proteomics**
Ákos Végvári

***10.45-11.00*** ***Coffee break***

**11.00-11.15** **Treatment of Freshwater Organisms with Polyethylene Microparticles: Toxicity and Trophic Transfer Evaluation in *Spirodela polyrhiza* (L.) Schleid. and *Echinogammarus veneris* (Heller, 1865)**
Valentina Iannilli, Laura Passatore, Serena Carloni, Francesca Lecce, Giulia Sciacca, Massimo Zacchini, Fabrizio Pietrini

**11.15-11.30** **Bioaccumulation of n-Perfluorooctanoic Acid (PFOA) in Basil (*Ocimum basilicum* L.) Leaves**
Enrica Donati, Fabrizio Pietrini, Laura Passatore, Isabel Nogues, Anna Wyrwicka-Drewniak, Massimo Zacchini

**11.30-11.45** **An Overview of Chitosan Extraction from Shrimp Waste through Chemical Methods and the Use of Artificial Neural Networks for Predicting Factors Influencing Chitosan Yield**
Ahmed Hosney, Sana Ullah, Karolina Barčauskaitė

**11.45-12.00** **The Influence of New Breeds Purple Wheat Wholemeal Flour Fermented with *Lactiplantibacillus plantarum* LUHS135 for the Quality Parameters and Acrylamide Formation in Wheat Bread**
Dovile Klupsaite, Aura Kaminskaite, Arnoldas Rimsa, Agne Gerybaite, Agne Stankaityte, Ausra Sileikaite, Elzbieta Svetlauskaite, Emilija Cesonyte, Giedre Urbone, Karolis Pilipavicius, Konstancija Vaiginyte, Marija Vaisvilaite, Vilte Prokopenko, Giedre Stukonyte, Emilis Radvila, Vytaute Starkute, Egle Zokaityte, Vita Lele, Darius Cernauskas, Ernestas Mockus, Zilvinas Liatukas, Vytautas Ruzgas, João Miguel Rocha, Elena Bartkiene

***12.00-13.00*** (time zone GMT+3) ***Lunch break***

**Flash presentations I.** ***Chairpersons: Dr. Mantas Stankevičius; MSc. Kristina Bimbiraitė-Survilienė***

**13.00** **The Impact of Enzymatic Hydrolysis to Ovine Meat By-Products Antioxidant and Antimicrobic Properties**
Ignė Juknienė, Agnė Jankauskienė, Irena Mačionienė, Gintarė Zaborskienė

**13.07** **The Study of Bystander Effect After Combining Calcium Electroporation, Electrotransfer of Anticancer Drug Bleomycin or Irreversible Electroporation**
Neringa Barauskaitė, Paulius Ruzgys, Vitalij Novickij

**13.14** **Factors Determining the Severity of COVID-19 in Pakistani Population**
Hina Tabassum, Numan Javed

**13.21** **Nutritional Value and Functionality of Beef Sausage Enriched with Omega-3 Fatty Acid Sources in Tandem with Fruit, or Vegetable Additives**
Agnė Šeputytė, Gintarė Zaborskienė, Vaida Andrulevičiūtė, Ingrida Sinkevičienė, Ignė Juknienė, Artūras Kašauskas, Artūras Stimbirys

**13.28** **Spectrometric and Chromatographic Assessment of Radical Scavenging and Bioactive Compound Accumulation in Medical Plants**
Domantas Armonavičius, Mantas Stankevičius, Ona Ragažinskienė, Hirotaka Ihara, Makoto Takafuji, Yutaka Kuwahara, Shoji Nagaoka, Elżbieta Skrzydlewska, Audrius Maruška

**13.35** **Determination of Potentially Skin-Sensitizing Compounds of Commercial Sweet Birch (*Betula lenta* L.) Essential Oil in Different Potential Nanoemulsions**
Modesta Čaplikaitė, Kristina Bimbiraitė-Survilienė

**13.42** **Analysis of Biologically Active Compounds of Medicinal Plants of the Notreli Family**
Martina Kazočiūnaitė, Kristina Bimbiraitė-Survilienė, Audrius Maruška

**13.49** **White Rot Fungus Enzymes Laccase and Manganese Peroxidase Influence on the Bioremediation**
Redas Dzekunskas, Mantas Stankevičius, Rūta Mickienė, Audrius Maruška

**13.56** **Composition of Volatile Compounds of Roman Chamomile at Different Vegetation Phases**
Renata Baranauskienė, Monika Bertašiūtė, Petras R. Venskutonis, Ona Ragažinskienė

**14.03** **Mathematical Modelling of Drying Kinetics of Forced Convection Drying Method of Potato Peels Waste**
Fatiha Brahmi, Inmaculada Mateos-Aparicio, Khokha Mouhoubi, Sara Guemouni, Farid Dahmoune, Ferroudja Belmehdi, Chafiaa Bessai, Khodir Madani, Lila Boulekbache-Makhlouf

***14.10-14.25*** ***Coffee break***

**Flash presentations II.** ***Chairpersons: Dr. Rūta Mickienė; Assoc. Prof. Dr. Vilma Kaškonienė***

**14.25** **Examining the Impact of Stainless Steel and Titanium Electrodes on Cell Transfection, Permeability, Pore Resealing and Viability**
Dominyka Gabulaitė, Baltramiejus Jakštys, Saulius Šatkauskas

**14.32** **In vitro studies on drug and gene transfer into cells**
Lukas Giedrimas, Saulius Šatkauskas

**14.39** **Analysis of Biologically Active Compounds in Natural Food Supplements**
Laura Šimaitytė, Kristina Bimbiraitė-Survilienė, Manats Stankevičius, Audrius Maruška

**14.46** (time zone GMT+3) **Evaluation of the Effect of Enzymatic Hydrolysis on Bee Bread and Bee Pollen**
Liveta Muliuolytė, Vilma Kaškonienė, Audrius Maruška

**14.53** **Comparison of Phenolic Content and Antioxidant Activity of *Bjerkandera adusta* Extracts Before and After Heat-Shock Treatment**
Karolis Klusas, Nicola Tiso

**15.00** **The Impact of Fermentation Using Symbiotic Cultures Combined with Ultrasound Treatment on Antioxidant and Antibacterial Activity of Common Oak (*Quercus robur*) Bark**
Greta Raškauskienė, Vilma Kaškonienė, Rūta Mickienė, Audrius Maruška

**15.07** **Evaluation of the Impact of Various Treatment Methods on Bee-Collected Pollen Biological Activity**
Lukas Asanavičius, Vilma Kaškonienė, Nicola Tiso, Audrius S. Maruška

**15.14** **Distribution of Micromicetus in Fresh Medicinal Material of Rosehip (*Rosa canina* L.) and Guelder-rose (*Viburnum opulus* L.)**
Paula Batutytė, Kornelijus Micka, Rūta Mickienė, Audrius Maruška

**15.22** **Monitoring Research of Genotype to Scrapie in Lithuanian Sheep Breeds**
Oksana Ilina, Edita Meškinyte, Jūratė Staveckienė

**15.30** **Antimicrobial Activity and Phytochemical Composition of Oregano, Peppermint, Thyme, and Lavender Essential Oils**
Deimantė Jurevičiūtė, Ugnė Gabrytė, Rūta Mickienė, Audrius Sigitas Maruška

**15.37** **Novel N-substituted Aminopyridine Derivatives: Synthesis and Antibacterial Activity**
Gabrielė Juraitytė, Simona Gelažunaitė, Paulius Ruzgys, Vida Malinauskienė

**15.44** **Assessment of the Effect of Agrotechnologies on Total Respiration in Agroecosystems**
Ligita Baležentienė

**15.50** **Potential of Agrotechnologies to Reduce Carbon Footprint Facility in Agroecosystems**
Ligita Baležentienė

**15.57** **Assessment of Invasive Species' Photosynthetic and Transpiration Response to Invaded Habitat**
Ligita Baležentienė

**16.05** **Comparative Efficacy of *Nicotiana tabacum* and *Azadirchta indica* Extracts Along with Their Green Synthesized Nano-ZnO Particles Against *Brevicoryne brassicae* L.**
Muhammad Irfan Shan, Muhammad Hamid Bashir, Faisal Noor, Rana Muhammad Mazhar Ali, Farwa Alsam

## Closing of the Session

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# 17th International Scientific Conference "The Vital Nature Sign"

## Dedicated to the 95th birthday anniversary of Professor Stellan Hjertén

## Program

### Friday, 19th of May 2023

**Oral presentations.** ***Chairpersons: Prof. Dr. Elena Bartkienė; Assoc. Prof. Dr. Vilma Kaškonienė***

**9.00-9.15** (time zone GMT+3) **The Influence of Different Treatment Time and Marinades Based on Valorized Food Industry By-Products on the Quality and Safety Parameters of Lamb Meat**
Paulina Zavistanaviciute, Jolita Klementaviciute, Dovile Klupsaite, Modestas Ruzauskas, Vilija Buckiuniene, Pranas Viskelis, Elena Bartkiene

**9.15-9.30** **Functionalised Rice Bran as A Promissing Substrate Supplement to Enhance Horticulture Plant Growth and Resistance Against Stem and Root Rot-Associated Pathogens**
Ruta Vaitkeviciene, Natalija Burbulis, Ramune Masiene, Rimgaile Degutyte, Daiva Zadeike

**9.30-9.45** **Application of Bio-preservatives in the Production of Hot-smoked Pork Sausages, Evaluation of the Effect on the Formation of Carcinogenic Substances**
Gintarė Zaborskienė, Sonata Gustienė, Ignė Juknienė, Artūras Stimbirys, Vaida Andrulevičiūtė, Aistė Kabašinskienė, Ingrida Sinkevičienė

**9.45-10.00** **Influence of Salting Method on Chemical Parameters and Sensory Indices of Cold Smoked Atlantic Salmon (*Salmo salar*)**
Artūras Stimbirys, Diana Vasiliauskaitė, Gintarė Zaborskienė, Ingrida Sinkevičienė, Ignė Juknienė

**10.00-10.15** **Formation of Bioactive Compounds in Fermented Microalgae Spirulina**
Ernesta Tolpeznikaite, Vadims Bartkevics, Anna Skrastina, Romans Pavlenko, Modestas Ruzauskas, Vytaute Starkute, Egle Zokaityte, Dovile Klupsaite, Romas Ruibys, João Miguel Rocha, Elena Bartkiene

**10.15-10.30** **The Mechanisms of Molybdenum Toxicity**
Sylwia Terpilowska

***10.30-10.45*** ***Coffee break***

**10.45-11.00** **Determination of Radical Scavenging Activity in Postharvest Microgreens *Brassica juncea* and *Amaranthus tricolor* Grown Under Different Light Spectrum**
Ieva Gudžinskaitė, Giedrė Samuolienė, Kristina Laužikė

**11.00-11.15** **Scalded and Scalded Fermented with *Lactiplantibacillus paracasei* Strain Rye Wholemeal Flour Influence on the Quality and Acrylamide Formation in Bread**
Dovile Klupsaite, Vytaute Starkute, Egle Zokaityte, Darius Cernauskas, Ernestas Mockus, Evaldas Kentra, Rugilė Sliazaite, Gabriele Abramaviciute, Paulina Sakaite, Vitalija Komarova, Ieva Tatarunaite, Sandra Radziune, Paulina Gliaubiciute, Monika Zimkaite, Julius Kunce, Sarune Avizienyte, Milena Povilaityte, Kotryna Sokolova, Emilis Radvila, João Miguel Rocha, Fatih Özogul, Elena Bartkiene

**11.15-11.30** **Nutraceutical Gummy Candies Formulations with Biomodified *Arthrospira platensis* as a Source of L-Glutamic and Gamma-Aminobutyric Acids**
Elena Bartkiene, Ernesta Tolpeznikaite, Dovile Klupsaite, Vytaute Starkute, Vadims Bartkevics, Anna Skrastina, Romans Pavlenko, Ernestas Mockus, Vita Lele, Gabija Batkeviciute, Ausrine Budrikyte, Rusne Janulyte, Ieva Jomantaite, Auguste Kybartaite, Karolina Knystautaite, Aiste Valionyte, Emilis Radvila, Romas Ruibys, João Miguel Rocha

**11.30-11.45** **Green Synthesis of Natural Colchinoids Using Electrochemistry**
Yi Du, Shakib Kazmi, Andrei V. Malkov

**11.45-12.00** **Lactic Acid Bacteria Isolation, Identification, Characterization and Applications in The Food/Feed Industry**
Vita Lėlė, Modestas Ruzauskas, Konrad Domig, Vadims Bartkevics, João Miguel Rocha, Elena Bartkiene

***12.00-13.00*** ***Lunch break***

**Flash presentations I.** ***Chairpersons: Dr. Paulius Ruzgys; Dr. Nicola Tiso***

**13.00** **Effect of ZnO Nanoparticles of Different Sizes on Growth Parameters of Swiss Chard (*Beta vulgaris* ssp. *cicla* L.)**
Eimantas Andrikis, Aušra Brazaitytė, Rūta Sutulienė, Martynas Urbutis, Simona Tučkutė

**13.07** **Abscisic acid: antioxidant response of different growth strategy crops**
Martynas Urbutis, Giedrė Samuolienė

**13.14** **Independent and Simultaneous Electrotransfer of Large Charged Molecules**
Rūta Palepšienė, Saulius Šatkauskas

**13.21** **MALDI/TOF MS Technique as A Method for Identifying Microorganisms of Forensic Significance with Simultaneous Indication of Differences in Protein Profiles of Strains From Different Sources**
Monika Radosińska, Aleksandra Radtke, Paweł Pomastowski, Michał Złoch, Aleksandra Florkiewicz, Ewelina Maślak

**13.28** **Analysis of High- and Low-Energy Electroporation Effect on Molecule Transfer**
Aras Rafanavičius, Rūta Palepšienė, Saulius Šatkauskas

**13.35** **Application of Highly Ordered π-electron Materials Incapsulating in Monolithic Columns**
Mantas Stankevičius, Kristina Bimbiraitė-Survilienė, Domantas Armonavičius, Audrius Maruška, Hirotaka Ihara, Makoto Takafuji, Elžbieta Skrzydlewska, Ona Ragažinskienė, Yutaka Kuwahara, Shoji Nagaoka, Nanami Hano, Yongxing Hu, Vilma Kaškonienė

**13.42** **Determination of Radical Scavenging Activity in Postharvest Microgreens *Brassica oleracea* Grown Under Different Light Spectrum and Intensity**
Eimantas Kielius, Giedrė Samuolienė, Ieva Gudžinskaitė

**13.49** **Size of Hypoxic Regions in Glioblastoma Spheroids Influence mRNA Isoform Formation**
Aistė Semionovaitė, Karina Šapovalovaitė, Eglė Jakubauskienė

**13.56** **Estonian Honey as an Anti-Borrelia Agent**
Pille-Riin Laanet, Piia Jõul, Piret Saar-Reismaa, Olga Bragina, Merike Vaher

**14.03** **Bisphenol A – Potential Factor of Miscarriage**
Wioleta J. Omeljaniuk, Wojciech Miltyk

***14.10-14.25 Coffee break***

**Flash presentations.** ***Chairpersons: Dr. Mantas Stankevičius, MSc. Kristina Bimbiraitė-Survilienė***

**14.25** **Biological and Phytochemical Analyzes of 5 Plants Used Against Verminosis in Goats in DR Congo: Anthelminthic Screening, Acetylcholinesterase Inhibition, Cytotoxicity and Phenolic Compound Profiling**
Gaël Nzuzi Mavungu, Salvius Bakari Amuri, Amandine Nachtergael, Sandrina Vandenput, Sujogya Kumar Panda, Victor Okombe Embeya, Walter Luyten, Pierre Duez

**14.32** (time zone GMT+3) **Concentration-Dependent Effects of 2,3,5-Triiodobenzoic Acid on Spruce and Pine Seed Germination and Seedling Development In Vitro**
Guoda Kolkatovaitė, Jonas Žiauka

**14.39** **Training of Future Health Professionals on Zoonotic Diseases for Poor Communities in Lubumbashi Through the One Health Approach**
Ngona I. Abdallah, Maryabo K. Ghislaine, Bukasa K. Jean-Adelard, Ndibualonji B.B. Victor, Bakari A. Salvius

**14.46** **Cow's Milk Bacteria Identification and Differentiation by LDI-MS Approaches**
Jagoda Pałczyńska, Oleksandra Pryshchepa, Aleksandra Radtke, Piotr Piszczek, Aleksandra Florkiewicz, Ewelina Maślak, Paweł Pomastowski

**14.53** **Quantitative and Qualitative Analysis of Volatile Compounds in Different Varieties of Fibrous Hemp Inflorescences**
Algimanta Kundrotaitė, Karolina Barčauskaitė

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**15.00** **Towards Side Effect Free Anticancer Therapy**
Reda Rulinskaitė, Rūta Palepšienė, Saulius Šatkauskas, Renaldas Raišutis, Martynas Maciulevičius

**15.07** **Dosage Form Influence on Transungual Delivery of Amorolfine HCl**
Sigita Ramaškaitė, Indrė Šveikauskaitė-Radučienė

**15.14** **Pullulan as Film-Forming Polymer for Hydrophilic Nail Lacquer Formulations**
Kamilia Kanso, Indrė Šveikauskaitė-Radučienė

**15.21** **High Concentrations of Tetracycline Reduce Light Use Efficiency in *Brassica napus* Leaves**
Irena Januškaitienė, Austra Dikšaitytė, Giedrė Kacienė, Diana Miškelytė, Gintarė Sujetovienė, Renata Dagiliūtė, Jūratė Žaltauskaitė

## Closing of the Conference

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# ORAL PRESENTATIONS

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# FLASH PRESENTATIONS

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# ORAL PRESENTATIONS

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# An Overview of Chitosan Extraction from Shrimp Waste through Chemical Methods and the Use of Artificial Neural Networks for Predicting Factors Influencing Chitosan Yield

**Ahmed Hosney, Sana Ullah and Karolina Barčauskaitė**

*Lithuanian Research Center for Agriculture and Forestry, Instituto av. 1, Akademija, Kedainiai dist., Lithuania*
*Corresponding author: ahmed.hosney@lammc.lt*

**Abstract**

In shrimp aquaculture, there are two feasible options to obtain shrimp shells as a by-product waste: during the production phases of shrimp or after peeling by the end-user prior to cooking. This waste can be either a source of environmental pollution through improper disposal or a promising source of chitosan, a biodegradable and biocompatible biopolymer that has various applications in agriculture, industry, and biomedicine. Chitosan is a deacetylated form of chitin, which can be chemically extracted from shrimp shells through demineralization, deproteinization, and deacetylation. The aim of this research is to summarize recent literature on the chemical extraction of chitosan from shrimp shells and to present the physicochemical properties of chitosan extracted from shrimp shells, including yield, moisture content, solubility, ash content, and degree of deacetylation. Additionally, this review aims to analyze the impact of the main predictors of the chemical extraction process (demineralization, deproteinization, and deacetylation) on chitosan yield percentage using a multilayer perceptron artificial neural network. The current research involved a multilayer perceptron neural network on the data reported in reviewed articles regarding demineralization, deproteinization, and deacetylation parameters. These parameters were considered as input independent variables, while the chitosan yield percentage was the dependent variable. The purpose was to predict the primary influencing factors on chitosan yield. The study found that the concentration of alkali during deacetylation is the most critical parameter, followed by the concentrations of acid and alkali during demineralization and deproteinization, respectively. The outcomes of this review are intended to be applied in future studies for optimizing the chemical extraction of chitosan from shrimp waste.

**Keywords:** shrimp shells, chitosan; extraction, neural networks

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# Data Acquisition Strategies for Improved Proteome Profiling in Single Cell Proteomics

**Ákos Végvári**

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**Abstract**

A human cell has a 200-250 pg protein amount, which is a contribution of at least 10,000 proteins. However, the dynamic range of proteins present in cells is estimated to be $10^7$-$10^8$, which does not permit identification and quantification of a greater part of the proteome. Currently, the most successful mass spectrometry-based SCoPE-MS approach has overcome the sensitivity issue by using tandem mass tags reagents. Multiplexing single cell (SC) proteomes and adding a carrier proteome (CP) of multiple cells to increase not only the identifications of proteins obtained from a SC but also their quantification. Typically, a CP is formulated by proteins extracted from identical types of cells analyzed in the experiments to represent their expression profile, which exhibits the same dynamic range and thus effectively boosts peptide signals of high abundant proteins.

To overcome this shortcoming and increase the depth of SCP analysis this dynamic range needs to be narrowed that can be achieved by using a synthetic peptide mixture or normalized level of proteins (natural or recombinant). The preliminary results of a simplified CP with peptides at equalized levels could elevate the peptide signals in the single cell tandem mass tag (TMT) channels and afforded detection of middle and lower abundant proteins. This approach has the potential to cover either a broad range or selected subsets of proteins in single cell proteomes affording their deep profiling and their absolute quantification.

**Keywords:** single cell proteomics, mass spectrometry

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# Influence of Salting Method on Chemical Parameters and Sensory Indices of Cold Smoked Atlantic Salmon (*Salmo salar)*

**Artūras Stimbirys, Diana Vasiliauskaitė, Gintarė Zaborskienė, Ingrida Sinkevičienė, Ignė Juknienė**

*Veterinary Faculty of Lithuanian University of Health Sciences, Tilžės st. 18, Kaunas, LT-47181, Lithuania*
*Corresponding author: arturas.stimbirys@lsmu.lt*

## Abstract

The aim of this study was to investigate the influence of different salting methods on the chemical composition and sensory indices of cold smoked Atlantic salmon *(Salmo salar).* Ten fillets for each salting method of Atlantic salmon were investigated. Selected fish fillets were salted in three ways: dry, combination, injection [1]. After salting they were matured in boxes and on special purpose frames. The matured fillets were smoked using standard industrial cold smoking technologies [2]. All prepared products were tested according to standard methods in order to evaluate their chemical composition (protein, fat, moisture, dry matter, salt content), colour intensity. The sensory evaluation was performed also.

The moisture content of dry-salted salmon was the lowest compared to other tested samples ($P < 0.001$). Protein and fat content was highest in dry-salted salmon ($P < 0.001$). Protein content in dry salted salmon was 10% higher ($P < 0.001$) than in the injection-salting and 4.5% higher ($P < 0.001$) than in fish salted by combination method. The fat content of dry-salted salmon is 20% higher ($P < 0.001$) than in the injection-salting and 6.5% higher ($P < 0.001$) than in the combination salted fish.

Fillet colour intensity (C) did not depend on the salting method. Sensory evaluation has shown that the most acceptable product for consumers was cold smoked (dry salted) salmon, which has a firm consistency and a smoky aroma.

**Keywords:** salting, chemical composition, sensory evaluation, Atlantic salmon (*Salmo salar*), cold smoking

**References:**

[1] M. Bjørnevik, et al. Learning with Technologies. 2018; 91:431-438.
[2] I. Arvanitoyannis, et al. Food Bioprocess Technology. 2012; (3):831-853.

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# Bioaccumulation of n-Perfluorooctanoic Acid (PFOA) in Basil (*Ocimum basilicum* L.) Leaves

**Enrica Donati[1], Fabrizio Pietrini[2], Laura Passatore[2], Isabel Nogues[2], Anna Wyrwicka-Drewniak[3], Massimo Zacchini[2]**

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*[2]Research Institute on Terrestrial Ecosystems (IRET), National Research Council of Italy (CNR), via Salaria km 29.300, 00015 Monterotondo Scalo (Roma) –ITALY*
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## Abstract

Per- and polyfluoroalkyl substances (PFASs) are a large and complex group of synthetic chemicals containing at least one perfluorinated carbon moiety. Owing to their chemical structure, PFASs show unique properties, such as high thermal and chemical stability along with hydrophobicity and oleophobicity, compared to their non-fluorinated analogues [1]. These features have led to the extensive use of PFASs in a wide variety of industrial, commercial and domestic products. Nevertheless, their physicochemical characteristics also make them highly resistant to thermal, chemical and microbial degradation. PFASs have been found in several environmental compartments, including humans, precisely because of their extensive use and persistence. For this reason, they are deemed environmental pollutants and have been associated with adverse health effects [2]. Due to their occurrence in soil, PFASs are taken up by plants and transferred to different levels of the trophic chain, posing a risk to human health [3]. Therefore, the development of an efficient approach to evaluate the PFASs uptake in vegetables is needed [4]. PFASs analysis is a challenging task due to the low concentration levels of these compounds in plant samples and also due to the complex matrix constituents as some co-extracted components such as sugar, chlorophyll, protein or cuticular waxes can interfere in the reliability and stability of the analytical method [5]. Consequently, efficient sample preparation procedures and very sensitive determination techniques are necessary to achieve a better assessment of the PFASs accumulation in plants. This study aims at developing a reliable extraction and clean-up process followed by UPLC – MS analysis for the determination of n-perfluorooctanoic acid (PFOA) in basil (*Ocimum basilicum* L.), whose leaves are used as a culinary herb. The effects of PFOA exposure on basil plants were also evaluated at morpho-physiological level.

**Keywords:** per- and polyfluoroalkyl substances (PFASs), UPLC-MS, plant physiology, antioxidants, xenobiotics

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# Determination of Radical Scavenging Activity in Postharvest Microgreens *Brassica juncea* and *Amaranthus tricolor* Grown under Different Light Spectrum

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## Abstract

Lighting conditions are one of the most important factors which can influence nutritional value or shelf-life of leafy vegetables. It is beneficial to know the changes in antioxidant activity, during the postharvest stage dependent on light spectrum quality during growth. Thus, the aim was to determine how supplemental light quality during growth can influence the antioxidant activity of microgreens *Brassica juncea* and *Amaranthus tricolor* and how it changes during the postharvest storage.

The experiments were conducted in a greenhouse (Lat 55°), microgreens were grown in peat substrate with supplemental light-emitting diodes (LEDs) lighting with total PPFD of 250 µmol $m^{-2}s^{-1}$. 16 h photoperiod and 20±3°C temperature was maintained. For supplemental light different blue 450 nm (B), red 660 nm (R) and white 4000K (W) light compositions were used ($B_{75.6\%}$:$R_{24.2\%}$:$W_{0.02\%}$; $R_{88.9\%}$:$B_{11.1\%}$; R $_{77.6\%}$:$W_{9.9\%}$:$B_{3.5\%}$). Samples were taken on harvest day, 1, 3 and 5 days after harvest. Microgreens were held in the light (10 µmol $m^{-2}s^{-1}$), and dark during postharvest storage, 76% air humidity and 4±1 °C temperature was maintained. The highest FRAP antioxidant activity on harvest and during postharvest was determined in mustard microgreens grown under $B_{75.6\%}$:$R_{24.2\%}$:$W_{0.02\%}$ LEDs. Compared with the microgreens held in the dark and light, higher antioxidant activity was determined in mustard held in the dark. The highest antioxidant activity was determined in the amaranth microgreens grown under the same ($B_{75.6\%}$: $R_{24.2\%}$ :$W_{0.02\%}$) lighting as for mustard, however, there was no significant differences in antioxidant response between amaranth held in the dark or light, as it was demonstrated in mustard. Our findings suggest, that supplemental $B_{75.6\%}$: $R_{24.2\%}$ :$W_{0.02\%}$ LEDs can significantly increase the FRAP antioxidant activity in mustard and amaranth microgreens during harvest and postharvest stages, compared to other used lighting.

**Keywords:** lighting, postharvest storage, mustard, amaranth, microgreens.

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# Polyphenolic Compound Analysis in Alfalfa Using Supercritical Fluid Extraction and Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry

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**Abstract**

Alfalfa (*Medicago sativa* L.), a legume plant, is popular worldwide for its nutritional and health benefits. Alfalfa is rich in protein, vitamins, minerals, flavonoids, saponins, and isoflavones, and is traditionally used for medicinal purposes. Alfalfa's high fiber content helps maintain digestive health, preventing gastric ulcer, constipation, and intestinal diseases. Flavonoids have anti-inflammatory, antioxidant, anti-microbial, and anti-cancer activities and can lower cholesterol levels and prevent heart disease. Due to its high biomass production and phenolic compounds, *M. sativa* is considered a promising source of health-promoting compounds, making it a raw material for nutraceuticals and functional food ingredients. Modern extraction techniques, including ultrasound-assisted, microwave-assisted, supercritical fluid, and subcritical water extraction, are used to isolate and fractionate biologically active compounds. High-performance liquid chromatography, gas chromatography, and mass spectrometry are used for analysis. Supercritical fluid extraction with carbon dioxide was used in this study to analyze polyphenolic compounds, including flavonoids and phenolic acids, from alfalfa extracts obtained from various parts of the plant. The analysis revealed the presence of 23 active compounds, including 13 flavonoids and 10 phenolic acids. The study offers valuable insights into the potential health benefits of alfalfa and the development of new therapeutic agents and dietary supplements.

**Keywords:** alfalfa, biological active compounds, SFE, HPLC-MS/MS

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# Lactic Acid Bacteria Isolation, Identification, Characterization and Applications in the Food/Feed Industry

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## Abstract

The aim of this extensive scientific research focused on the isolation, identification, and characterization of lactic acid bacteria (LAB). These were then to be chosen for desirable properties such as antimicrobial, and antifungal properties, gas production and acid tolerance, and used in the food and/or feed industries. Thirteen different LAB strains belonging to four species were distinguished at the species-level using polymerase chain reaction (PCR). Most of the sourdough isolates showed versatile abilities in metabolizing carbohydrates. Gas production is a very important indicator for the selection of future LAB application areas, from the ones that were tested two strains distinguished themselves in this field: *Leu. mesenteroides* (No. 242) and *L. brevis* (No. 173). Viable counts higher than 7.0 $\log_{10}$ (CFU/ml) were observed for *L. paracasei* (No. 244), *L. casei* (No. 210), *L. brevis* (No. 173), *L. farraginis* (No. 206), *P. pentosaceus* (No. 183), *L. uvarum* (No. 245), and *L. plantarum* (No. 135) after 2 h exposure at pH 2.5. In addition, *L. plantarum* (No. 122), *L. casei* (No. 210), *L. curvatus* (No. 51), *L. paracasei* (No. 244), and *L. coryniformins* (No. 71) showed growth inhibitory properties against all fifteen pathogenic strains tested. Finally, all LAB isolates showed antifungal activities against *Aspergillus nidulans, Penicillium funiculosum,* and *Fusarium poae.* Therefore, the identification of extremely important indicators, such as antimicrobial and antifungal properties, among others, is highly desirable and important in the agri-food industry, leading to a very wide range of applications. Food industry by-products are processed to achieve sustainable waste management. It enables the development of feeds for animals and extends to the food industry.

**Keywords:** lactic acid bacteria, fermentation, isolation, characterization, identification

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# The Method Development for the Separation and Determination of Cyclitols and Carbohydrates from Biological Samples - As a Proposal for a New Diagnostic Method

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## Abstract

Carbohydrates and cyclitols are important groups of compounds widely found in plant and animal tissues. Carbohydrates are organic compounds that provide living cells with energy necessary for the proper functioning. Whereas, cyclitols play an important role in the processes of cellular regulation, signal transduction and osmoregulation, they are also components of cell membranes [1,2]. The major source of cyclitols namely myo-inositol in human body is through dietary uptake, or to a lesser extent through a de novo multistep conversion from glucose-6-phosphate [3]. However, it should also pay attention to the problem the presence of glucose in urine, which is referred to as glucosuria. Under normal circumstances, sugars should not be detected in human urine. A statement of sugars in urine may indicate diabetes, abnormal kidney function or insulin secretion disorders. If glucose is found in urine, then its concentration in blood is also high. Considering the mentioned above, in this study methods of isolation and determination of cyclitols and carbohydrates from biological samples have been discussed and compared. Our investigations concern the determination of carbohydrates in extracts obtained from urine samples, firstly by the spectroscopic metod requiring use of anthrone and second by use of liquid chromatography with Corona-CAD detector (HPLC-Corona-CAD), which was also used for the determination of myo-inositol. Before HPLC analyses each urine sample has been subjected to the cleaning procedure with acetonitrile (method I) and magnetite nanoparticles (method II). During this study samples obtained from both healthy volunteers and patients with urological diseases have been analysed. Obtained results indicate that there is some relationship between the presence of carbohydrates and cyclitols in urine sample depending on the health status.

**Keywords:** urine samples, carbohydrates, cyclitols, extraction, HPLC

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# Green Synthesis of Natural Colchinoids Using Electrochemistry

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## Abstract

Colchicine is a natural pseudo-alkaloid which in clinical trials back in 1935 showed some potential to inhibit cancer cell growth,[1] but its high toxicity precluded further therapeutical use. More recently, an investigation of colchicine analogues revealed that allocolchicinoids derivatives, where the 7-membered tropolone ring was replaced with a benzene ring showed promising biological activity and less toxicity compared to the parent colchicine.[2] *N*-Acetylcolchinol, a tubulin polymerisation inhibitor, was even developed as the prodrug,[3] but in clinical trials, it was found to be toxic for use in humans and therefore was discontinued. Nonetheless, the pronounced biological activity of colchicine alkaloids continues to attract the attention of researchers to this class of compounds, thus maintaining the need for a robust methodology to access their synthetic analogues. Herein we report a green and highly efficient route to colchicine and *N*-acetylcolchinol[4] starting from inexpensive commercially available starting materials. Importantly, the redox steps that require the use of toxic stoichiometric reagents or transition metals were replaced by electrochemical methods. The asymmetric reduction step was carried out organocatalytically, thus avoiding the use of transition metal catalysts. The target compounds were obtained with good chemical yield and high stereoselectivity.

**Keywords:** asymmetric synthesis, electrochemistry, green chemistry, organocatalysis

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# The Influence of New Breeds Purple Wheat Wholemeal Flour Fermented with *Lactiplantibacillus plantarum* LUHS135 for the Quality Parameters and Acrylamide Formation in Wheat Bread

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## Abstract

The purpose of this work was to evaluate the impact of colored (purple) wheat (CW) new breed varieties 8526-2 and 8529-1 wholemeal flour ($CW_{WF}$) untreated or fermented with *Lactiplantibacillus plantarum* (No. 135) on the quality parameters and acrylamide formation in wheat bread (WB). To implement the aim, different quantities (5, 10, 15, and 20%) of untreated and fermented $CW_{WF}$ were tested for WB preparation. The acidity parameters, color, hardness characteristics, amylolytic/proteolytic enzymes activities, and antioxidant properties of $CW_{WF}$, as well as WB quality, overall acceptability, and acrylamide concentration (AC), were analysed. Fermentation technology with No. 135 of $CW_{WF}$ increased free radical ($DPPH^-$) scavenging activity ($p=0.001$) and L* (lightness) color, however reduced hardness parameters of both $CW_{WF}$ varieties. Incorporation of $CW_{WF}$ could have potential advantages for WB quality and AC reduction. In every case, fermentation technology increased total phenolic compounds, on average by 52%. Fermentation technology and CW variety significantly affected bread crumb a* colour coordinates. The addition of fermented $CW_{WF}$ significantly decreased AC formation in WB samples ($p \leq 0.0001$). The lowest AC was found in WB samples prepared with 5% of $CW_{WF}$ for variety 8526-1. Finally, the addition of new varieties of $CW_{WF}$ to bread improved several quality parameters, such as bread specific volume (on average 6.25% higher), while $CW_{WF}$ fermented with *Lp. plantarum* (No. 135) possessed $DPPH^-$ free radical scavenging activity and could be suggested for acrylamide reduction in WB.

**Keywords:** purple wheat, lactic acid bacteria, fermentation, bread quality, acrylamide

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# Nutraceutical Gummy Candies Formulations with Biomodified *Arthrospira platensis* as a Source of L-Glutamic and Gamma-Aminobutyric Acids

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## Abstract

The goal of this study was to evaluate for quality parameters of the biomodified *Arthrospira platensis* (Spirulina) powder with *Lactiplantibacillus plantarum* (No.122) strain, and in the following to apply in gummy candies preparation, which would be characterized as a rich in L-Glutamic ($L_{Glu}$) and Gamma-Aminobutyric (GABA) acids and as sucrose-free. Spirulina samples biomodification was carried out with No.122 strain and was evaluate their pH, colour coordinates, $L_{Glu}$ and GABA contents, as well as fatty acid profile (FA). Subsequently, the most optimal biomodification conditions of Spirulina were selected (24 and 48 hours), and different amounts of Spirulina (from 0.5 to 5.0 g) were tested during nutraceutical gummy candies preparations. Additionally, different sweet tasting, texture forming, sour tasting, and Spirulina odour masking ingredients for the formulations were tested. For the gummy candies were subjected to analyses of colour coordinates, texture hardness, and overall acceptability. Additionally, samples showing the highest acceptability were tested by using the „*FaceReader*" technique. The highest intensity (0.052) of "happy", which is a positive emotion, was produced by XyAgAa chewing candy with 3 g of Spirulina and 0.1 µl of *Mentha spicata* essential oil. Biomodification showed a tendencies of omega-6 reduction, however increasing of omega-3 in Spirulina. The dominants ones of FA in Spirulina were palmitic (C16:0), linoleic (C18:2), and gamma linolenic (C18:3ɣ) acids. Additionally, in 48 h-fermented samples, alfa-linolenic acid (C18:3α) was established (0.605% of total fat content). It can be stated that biomodified *Arthrospira platensis* with No.122 strain in tandem with *Citrus paradise* and *Mentha spicata* essential oils could be used to develop nutraceutical gummy candies with high overall acceptability ($p \leq 0.05$) and improved their functional value.

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# Scalded and Scalded Fermented with *Lactiplantibacillus paracasei* Strain Rye Wholemeal Flour Influence on the Quality and Acrylamide Formation in Bread

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## Abstract

The goal of this investigation was to evaluate the impact of scalded (Sc) and scalded and fermented (ScF) with *Lactiplantibacillus paracasei* (LUHS244) strain rye wholemeal flour ($RW_{MF}$) on the quality and acrylamide content in semi-wheat rye bread ($SW_{RB}$). To achieve this goal different quantities of Sc and ScF for the $SW_{RB}$ preparation were tested. For Sc and ScF quality and for $RW_{MF}$ quality parameters and safety characteristics were evaluated. Based on the obtained results, sugars content was increased in $RW_{MF}$. In comparing with rye wholemeal, lower concentrations of free amino acids were established in Sc, however fermentation technology with LUHS244 increased the concentrations of several amino acids, as well as γ-aminobutyric acid. Supplement of Sc and ScF had a significant influence ($p \leq 0.05$) on bread shape coefficient, mass loss and colour coordinates. Fermentation with LUHS244 improved flavour and overall acceptability of $SW_{RB}$. Acrylamide content in $SW_{RB}$ samples prepared with ScF was on average – 236.3 μg/kg. It can be stated that fermentation technology and optimal quantity of Sc could significantly affected the quality of the $SW_{RB}$ samples. Bread prepared with ScF improved sensory properties and overall acceptability, as well as γ-aminobutyric acid in $SW_{RB}$, while the amount of acrylamide can be controlled by using 5 and 10% quantity of scalded rye whole-meal flour.

**Keywords:** lactic acid bacteria, fermentation, bread quality, scalding, acrylamide

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# The Mechanisms of Molybdenum Toxicity

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**Abstract**

Molybdenum is an essential metal for animals and humans. It plays a particular role in the active sites of metalloenzymes: xanthine oxidoreductase, aldehyde oxidase, mitochondrial amidoxime reductase component, and nitrate reductase. The most important oxidation states for molybdenum are +2, +3, +4 and +6. However, molybdenum compounds can be toxic.

In our study cytotoxicity and genotoxicity were assessed. Molybdenum was non-toxic when assessed by MTT reduction assay and LDH release assay and NRU assay after molybdenum treatment. Genotoxicity was assessed both by analyzing induction of micronuclei formation and DNA damage - formation of single- and double-strand breaks by comet assay. In both assays increase in the percentage of tail DNA and the number of micronuclei in comet and micronucleus assays, respectively, were observed. The treated cells displayed characteristic apoptosis in comparison to control cells. Nuclear convulsion and fragmentation and cytoplasmic blebbing were observed. Moreover, the giant or multinuclear cells and cytoplasmic vacuolation were observed. Molybdenum evokes frameshift mutation more often than base–pair substitution mutation. The result of this activity is the destruction of DNA structure. DNA destruction should be removed by repair enzymes before replication. The study shows that DNA decreases in G0/G1 and S phases, while it increases in the G2/M phase after incubation with molybdenum. It suggests that molybdenum activates the G2/M cell cycle checkpoint. G2/M DNA damage checkpoint serves to prevent cells from entering mitosis with genomic DNA damage. Molybdenum induced intracellular ROS generation in a concentration-dependent manner. The concentration of intracellular ROS and MDA increased after molybdenum treatment. However, the activity of SOD, CAT and GPx decreased. These observations confirm the study in which a decrease in MTP (Mitochondrial Transmembrane Potential) was observed. The decrease of MTP is induced by reactive oxygen species. Moreover, the increase of caspases-3, 6 and 9 were observed. The presented results show that the intrinsic pathway of apoptosis can be induced by the overproduction of ROS. However, molybdenum also activates the concentration of caspase-12. This caspase is a specific marker of ER-induced apoptosis. The data obtained from Raman Spectroscopy analysis showed a stronger lipid signal and lipid droplets in cells treated with molybdenum. Detailed analysis of these Raman bands showed that the main components of these lipidic structures are oleic acid derivatives, i.e. triolein and cholesteryl oleate. Based on these observations it can be concluded that the ER-mediated apoptosis pathway is activated in cells treated with molybdenum.

**Keywords:** molybdenum, apoptosis, intrinsic pathway, ER-mediated apoptosis

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# Formation of Bioactive Compounds in Fermented Microalgae Spirulina

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## Abstract

Spirulina (*Arthrospira platensis*) is cultivated worldwide, because this microalgae shows beneficial activities in humans and animals [1,2]. The aim of this study was to investigate the changes in bioactive compounds (biogenic amines (BA), gamma-aminobutyric acid (GABA) and L-glutamic acid (L-Glu)) during the different types of fermentation (submerged (SMF) and solid-state (SSF) fermentation) of Spirulina with lactobacilli strains (*Lacticaseibacillus paracasei* No. 244; *Leuconostoc mesenteroides* No. 225; *Levilactobacillus brevis* No. 173 and *Liquorilactobacillus uvarum* No. 245). The highest concentrations of GABA (2396 mg/kg) and L-Glu (3841 mg/kg) were found after 48 h of SSF with No.173 and No.244 strains, respectively. The LAB strain used for bio-treatment, process conditions, and the interaction of these factors, were significant on GABA concentration in Spirulina samples ($p \leq 0.05$). The ratios of BA/L-Glu and BA/GABA in Spirulina samples were ranged from 0.31 to 10.7 and from 0.5 to 62.0, respectively. Also, correlations between GABA concentration and BA (putrescine, cadaverine, histamine, tyramine, spermidine, and spermine) contents were found. Additionally, correlations between L-Glu concentration and putrescine, tryptamine, spermine and spermidine were established. Finally, it can be stated, that despite that high concentration of desirable compounds during the fermentation process can be obtained, non-desirable compounds (e.g., BA) also are formed, due to the similar mechanism of their synthesis. For this reason, it is strongly recommended to control the final product.

**Keywords:** spirulina, L-glutamic acid, gamma-aminobutyric acid, biogenic amines

**Acknowledgments:** This work is based on work from COST Action 18,101 SOURDOMICS—Sourdough biotech-nology network towards novel, sustainable and healthier food and bioprocesses (https://sourdomics.com/; https://www.cost.eu/actions/CA18101/, accessed on 16 December 2022) and is supported by COST (European Cooperation in Science and Technology) (https://www.cost.eu/, accessed on 16 December 2022).

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# Application of Bio-preservatives in the Production of Hot-smoked Pork Sausages, Evaluation of the Effect on the Formation of Carcinogenic Substances

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## Abstract

Synthetic preservatives and antioxidants are still used in the meat industry, but the findings of some scientists point to the potential carcinogenicity of synthetic food additives [1] making their further use in food production controversial. The purpose of our research was to apply selected bio-preservatives in the production of Hot-smoked pork sausages and to evaluate the impact on the formation of carcinogenic substances. Hot-smoked pork sausages were produced in a small meat processing company. The minced meat was mixed with spices, bacterial culture (BC) mixture S-10 (*S. carnosus* and *S. vitulinus*) (0.25 g/kg) and selected natural commercial plant additives. Six different groups of sausages were supplemented with the same mixture of CB, five groups of them - with different tested agents: (1) a mixture of rosemary and pomegranate (0.6 g/kg); (2) Garlic essence (0.15 g/kg); (3) onion extract (0.06 g/kg); (4) green tea extract (0.15 g/kg); (5) rosemary extract (0.08 g/kg); (6) a control group - without the extracts. Physico-chemical and enzymatic studies, sensory analysis of the samples was performed in this study. The content of polycyclic aromatic hydrocarbons (PAH) was determined in the laboratory of the National Food and Veterinary Risk Assessment Institute according to validated method by liquid chromatography. The samples for N-nitrosamines analysis were prepared according Yuan et al. [2]. A gas chromatograph with a mass spectrometer was used to determine the amount of volatile N-nitrosamines; an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm, PerkinElmer Elite TM 1, Linealibera, USA) - to optimize the methodology. A standard solutions and calibration curves for each nitrosamine were used for identification and quantification.

Added biopreservatives significantly reduced nitrite and nitrate content in the sausages, compared to the control sample ($p<0.05$, in all cases). The formation of N-nitrosamines was significantly depended on the amount of nitrites ($r=0.692$, $p<0.05$). The biopreservatives reduced the amount of N-nitrosodimethylamine, but N-nitrosopyrrolidine was detected in almost all samples, except the sample with rosemary extract, the highest total amount of N-nitrosamines was in the sample with garlic essence (2.814±0.387 μg/kg). The amount of all detected PAH and the concentration of benzo(a)pyrene exceeded the maximum limited levels. PAH levels in the samples ranged from 35.9±4.5 μg/kg to 37.5±4.3 μg/kg, and the average concentration of benzo(a)pyrene was 6.1±1.1 μg/kg, there were no significant differences in the samples. It must be recognized that the control of the technological process is a necessary factor that can ensure the safety of hot smoked sausages and partially reduce the formation of carcinogenic substances.

**Keywords:** hot-smoked sausages, biopreservatives, volatile N-nitrosamines, PAH

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# Treatment of Freshwater Organisms with Polyethylene Microparticles: Toxicity and Trophic Transfer Evaluation in *Spirodela polyrhiza* (L.) Schleid. and *Echinogammarus veneris* (Heller, 1865)

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## Abstract

Microplastics (MPs) are widely recognised as an emerging threat to the biota. In fact, their widespread diffusion in aquatic and terrestrial habitats, even extreme [1], has inevitably led to interact with living organisms. In this regard, fragmentary information is present in the literature about the toxicity on the main physiological processes in plants and animals. MPs are also supposed to serve as a carrier for other contaminants, in particular heavy metals, allowing them to enter the organism by a process called the "Trojan horse effect" [2]. Another concern regards the possible transfer of MPs along the food chain leading to humans. In this context, the knowledge about the mechanisms involved in the MP transfer at the base of the freshwater food web, involving primary producer and primary consumer organisms, is still lacking [3]. To address both the challenging issues, *i.e.* the toxicity of MPs to biota and the MP trophic transfer in the freshwater food chain, two model organisms for ecotoxicological studies were targeted, namely the duckweed plant *Spirodela polyrhiza* (L.) Schleid. and the amphipod *Echinogammarus veneris* (Heller, 1865). Different lab assays were then arranged to evaluate the effects of polyethylene microparticles on both the aquatic plant (7-day assay) and the gammarid (24-h assay). Further, MP-treated *Spirodela* plants was used to feed gammarid individuals. After 24h, the digestive tracts of the gammarids were removed and the presence of MP particles was analysed. Morpho-physiological analyses (growth parameters, pigment content and photosynthetic performances) highlighted the lack of toxic effects of MPs in duckweed. In contrast, a notable genotoxic effect (evaluated by the Comet assay) was observed in the haemocytes of gammarid individuals exposed to MPs, with a 3-time higher DNA damage to control. Finally, the gut content of the gammarids fed with *Spirodela* MP-treated plants revealed the presence of 7.6 MP particles per individual, highlighting the occurrence of the trophic transfer of MPs between the two organisms. Overall, the obtained results put in evidence that MP presence in the aquatic ecosystem represents a potential threat to the biota and a serious concern for human health given the involvement of the studied organisms in the food web leading to the human diet, directly or through intermediate steps.

**Keywords:** amphipods, aquatic ecosystem, aquatic toxicology, comet assay, duckweeds, plant physiology

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# Functionalised Rice Bran as a Promissing Substrate Supplement to Enhance Horticulture Plant Growth and Resistance Against Stem and Root Rot-Associated Pathogens

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## Abstract

The regulation of horticultural plant growth and the facilitation of the environmental stresses are important factors characterising the productivity of cultivated plants. In this study, the bioactive components of fermented (F), also ultrasonicated (US), and enzyme hydrolysed (E) rice bran (RB) was used to plant growth substrate (PGS) in a greenhouse experiment on beans and tomatoes to enhance their growth promotion ability and resistance against stem and root rot-associated pathogens. Functionalised RB was analysed for the organic acid, phenolic acid, sugar, and amino acid profiles, and antifungal activity against seventeen plant phytopathogens associated with stem and root rot. The efficiency of the applied tools was evaluated according to the germination rate of seeds, shoot height and root length of seedlings, as well as chlorophyll concentration in plants. Fermented RB showed the most growth inhibitory effect against soil-born plant pathogens such as *Fusariu*m, *Pythium*, *Sclerotinia*, *Aspergillus*, *Pseudomonas,* and *Verticillium* spp. Beans and tomatoes have grown in with $RB_{US+E}$ and $RB_F$ supplemented PGS significantly ($P < 0.05$) increased germination rate (up to 75%), root length (26–36%), and shoot height (25–47%) compared to seedlings grown in PGS. RB additives increased up to 32.5% of the chlorophyll content in both plants grown under greenhouse conditions. The present study suggests that the biological potential of rice processing waste can be increased using solid-state fermentation and US technologies. Supplementation of PGS with functionalised RB additives positively affected the growth performance of beans and tomatoes.

**Keywords:** rice bran, solid state fermentation, ultrasound, bioactive components, tomatoes and beans, resistance, growth performance

**Acknowledgments:** This research has received funding from European Regional Development Fund (project No. 01.2.2-LMT-K-718-01-0062) under grant agreement with the Research Council of Lithuania (LMTLT).

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# The Influence of Different Treatment Time and Marinades Based on Valorized Food Industry By-Products on the Quality and Safety Parameters of Lamb Meat

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## Abstract

The purpose of this study was to create marinades recipes based on valorized food industry by-products and adjust them for lamb meat (LM) treatments to ameliorate the meat's biosafety and quality. For this, different marinade (M) recipes were developed using fermented *Lacticaseibacillus casei* (Lc) and *Liquorilactobacillus uvarum* (Lu) acid whey (AW) alone or in combination with lyophilsed apple (AP) or blackcurrant (BC) by-products. In the first stage of the reserch, the best fermentation time for the created marinades were chosen, according acidity results and LAB counts in the samples and in addition to this, for latter marinades antimicrobial activity was tested. In the second stage of the reserch, created marinade formulations were applied for LM treatment and quality and biosafety parameters evaluation after 24 and 48 h of treatment were performed. It was found that, appropriate marinades fermentation time was 48 h, because in all tested samples, excluding M-$AW_{Lu}$BC, the higher than 7.99 log10 CFU/ml LAB count, and lower than 4.00 pH values, were determined. M-$AW_{Lu}$AP and M-$AW_{Lu}$BC inhibited the growth of all tested pathogenic bacteria strains, as well as had positive influence on water holding capacity and overall acceptability of the LM. Evaluating biogenic amines content, histamine, cadaverine, putrescine, tryptamine and phenylethylamine were not determined in all LM samples treated for 24 and 48 h. In conclusion, M-$AW_{Lc}$AP and M-$AW_{Lu}$AP marinades formulations can be recommended for LM treatment due to higher overall acceptability, meat tenderness, and water capacity. However, to achieve these results, LM treatment with selected marinades should be carried out for 48 h.

**Keywords:** dairy industry by-products; juice industry by-products; lactic acid bacteria; fermentation; lamb meat; valorization

**Acknowledgments:** The authors gratefully acknowledge the COST Action 18101 SOURDOMICS - Sourdough biotechnology network towards novel, healthier, and sustainable food and bioprocesses (https://sourdomics.com/; https://www.cost.eu/actions/CA18101/ (accessed on 8 March 2021)), where the author E.B. is the leader of the working group entitled “Project design and development of innovative prototypes of products and small-scale processing technologies” and is supported by COST (European Cooperation in Science and Technology; https://www.cost.eu). COST is a funding agency for research and innovation networks.

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# FLAH PRESENTATION

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# Effect of ZnO Nanoparticles of Different Sizes on Growth Parameters of Swiss Chard (*Beta vulgaris* ssp. *cicla* L.)

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**Abstract**

Zinc (Zn) is classified as a trace element, although this element is necessary to ensure optimal conditions for plant growth. The aim of this study was to evaluate the effect of zinc oxide nanoparticles (ZnO NPs) sprayed onto leaves on the shoot and root biomass and leaf area of baby leaf Swiss chard (*Beta vulgaris* ssp. *cicla* L. cv. Barese). Plants were grown in hydroponic Ebb systems in controlled environment chambers. Swiss chard were sprayed with suspensions of ZnO NPs of various sizes (18, 35–45, and 80–200 nm) at a concentration of 200 ppm. Plants that were sprayed with distilled water are considered as control. The results show that the most effective size of ZnO NPs was 35–45 nm. A suspension of nanoparticles of 35–45 nm size improved fresh and dry shoot and root biomass by 2 times. In addition, leaf area increased 1.8 times when Swiss chard was sprayed with a 35-45 nm ZnO NP suspension. The leaf chlorophyll index was statistically reliably increased by suspensions of ZnO NPs of all sizes, but the smallest 18 nm nanoparticle suspension had a negative effect on flavonol index. In conclusion, these results revealed that ZnO NPs could be used in Swiss chard production to improve yield and quality.

**Keywords:** swiss chard, nanoparticles, zinc oxide, growth

**Acknowledgments:** This project has received funding from the Research Council of Lithuania (LMTLT), agreement No. S-MIP-21-27.

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# Nutritional Value and Functionality of Beef Sausage Enriched with Omega-3 Fatty Acid Sources in Tandem with Fruit, or Vegetable Additives

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**Abstract**

Dietary supplements in tandem with vegetables and fruits could improve the taste of meat products, enrich the nutritional value, and increase the consumption of recommended food raw materials. The aim of this study was to evaluate the nutritional and energy values, and sensory properties of cooked beef sausages by adding of different omega-3 fatty acids (FA) sources (linseed oil, Spanish sage seeds and fish oil) in tandem with beetroots, carrots or zucchini and apples.

The nutritional and energy values, content of fatty acids, sensory properties were evaluated of newly developed beef sausages. The increased protein content (from 20.70 ± 0.14% in sausages with linseed oil and carrots to 23.60 ± 0.11% in sausages with Spanish sage seeds and beetroots) compared with control (20.60 ± 0.12%) was characteristic for newly produced sausages. The amount of fats increased by adding linseed oil (from 19.80 ± 0.31%, in control group, till 34.30 ± 0.41%) while the amount of carbohydrates was increased by adding beetroots to sausages.

The addition of linseed oil, Spanish sage seeds and fish oil increased omega-3 FA amount in products, especially with fish oil (15.00 ± 1.5% of total fatty acids content (TFA)) and linseed oil and beetroots (29.43 ± 1.9% of TFA), while only 0.07 ± 0.03% of omega-3 FA was evaluated in the control sample. The lowest increase of omega-3 FA in the range of 3.6 ± 0.21% - 5.03 ± 0.53% of TFA was noticed in samples with Spanish sage seeds. A ratio of omega-6 to omega-3 FA was lower compared with control group in all newly produced sausages. Nevertheless, the lowest value of the ratio in the range of 0.5-1.5 is observed in sausages with the addition of fish oil, but the taste did not reach the desired level. The addition of Spanish sage seeds or linseed oil diversify the nutrition and addition of vegetable or fruit could increase the amount of fibre.

**Key words:** beef, omega-3 fatty acids, functional food, linseed oil, Spanish sage seeds, fish oil

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# Spectrometric and Chromatographic Assessment of Radical Scavenging and Bioactive Compound Accumulation in Medical Plants

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## Abstract

Cancer is a significant global health challenge that profoundly impacts millions of individuals worldwide and remains one of the leading causes of death. Despite recent advancements in cancer treatments, the search for more effective and less toxic therapies remains crucial. Natural substances such as those derived from medicinal plants offer promising avenues for cancer prevention and treatment, and their potential remains under investigation. As such, global research on natural antioxidants, including polyphenols such as ellagitannins, found in plants, is highly relevant and holds promise for providing alternative solutions to the ongoing challenges of cancer treatment. Vytautas Magnus University scientist has been conducting research on the isolation of phyto-compounds from herbal medicinal raw materials and their antioxidant properties for many years [1, 2].

In this study, a total of 14 medicinal plants were collected from the Botanical Garden of Vytautas Magnus University at various stages of growth, resulting in 26 sample specimens. Methanolic extracts were prepared for spectrometric and chromatographic analyses. Total phenolic and flavonoid contents were determined, along with radical-scavenging activity, using a UV-VIS spectrometer. Specifically, the Folin-Ciocalteu colorimetric method was employed to determine total phenolic content, while the $AlCl_3$ colorimetric method was used to assess total flavonoid content. The radical scavenging activity of all medical plant extracts was evaluated using the DPPH radical scavenging method [3].

The separation and identification of phenolic compounds were performed using a modular high-performance liquid chromatography system. The compounds were determined through an external standards method based on their retention times [4].

Future studies will include the extraction and fractionation of anticancer ellagitannins accumulating plants using different strategies. The research will also develop and adapt solid-phase anticancer plant separation equipment and raw materials for quality assessment [5]. Anticancer studies of the extract fractions will also be performed using *in vitro* cancer cell lines.

**Keywords:** medical plants, anticancer compounds, ellagitannins

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# Assessment of the Effect of Agrotechnologies on Total Respiration in Agroecosystems

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## Abstract

Crop lands cover about 12% of the Earth's surface and one third of the land surface in Europe [1]; therefore, it plays a significant role in generation of anthropogenic emissions. Globally, agriculture accounts for 10–12% of total anthropogenic emissions of greenhouse gas (GHG), in the EU – 9.78%, out of which 4.94% are emissions from the soil. Agriculture produces near 21.4% of total emissions in Lithuania [2]. Soil is an important and the largest carbon reservoir, which accumulates about 53% of terrestrial carbon [3]. The main source of carbon is plant biomass (and residues) in the soil. Soil carbon may be lost not only by leaching, but uptake by plants and removed with the harvest as well. The aim was to investigate and compare farming impact on total respiration rate (Ra+s) of ley and wheat agroecosystems.

Different total respiration of assessed agroecosystems was subjected on seasonal meteorological conditions (specifically, precipitation and temperature in summer season). The analysis of carbon respirational emissions exhibited that ley and winter wheat agroecosystems of organic farming emitted nearly 12% and 14% less than those of convenient farming. Summarizing, the crop and farming type can modify the $CO_2$ emissions to atmosphere. Nonetheless, the difference between carbon emissions for OF and CF agroecosystems was insignificant, and thus crops might be a more important component than farming type in planned rotation. Specifically, grassland is preferred over wheat (irrespective of farming type) in curbing the atmosphere C in biomass.

**Keywords:** $CO_2$, farming, crops

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# Potential of Agrotechnologies to Reduce Carbon Footprint Facility in Agroecosystems

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## Abstract

Rising anthropogenic $CO_2$ emissions into atmosphere force the choice of agro technologies and cultivated crops aimed at mitigating climate change [1]. For this reason, carbon footprint of organic and conventional agrosystems was assessed for estimation of their life cycle inventory (LCI) [2]. It should be noted that life cycle inventory is a principal tool to assess the environmental sustainability of agroecosystems. This aggregated impact's method also might be important for determining the main impact categories, and then searching techniques how to reduce their negative influence on agroecosystem sustainability. Results implied that proper crop choice within the crop rotation schemes may lead to reduction of $CO_2$ emissions. The major sources of the increased $CO_2$ emission in conventional farming were intensive agrotechnologies (machinery), mineral fertilizers and pesticides. However, elimination of chemical pesticides, herbicides and fertilizers in organic cultivation led to lower environmental impacts. Therefore, extensive agro technologies combined with elimination of chemical pesticides, herbicides and fertilizers in organic farming led to lower environmental impacts.

**Keywords:** $CO_2$ emissions, LCI, farming, crops

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# Assessment of Invasive Species' Photosynthetic and Transpiration Response to Invaded Habitat

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**Abstract**
Investigated invasive species colonized grassland and bushes habitats with different moisture and radiation conditions. *R. confertus* prefer fully insolated ecotones of damp grassland where mean soil moisture and temperature were observed the highest. *H. sosnowskyi* and *F. japonica* grown in scrubs habitats characterized with medium soil moisture. The insolation conditions were measured as photosynthetically active radiation (PAR) and varied between 36.00 and 1083.67 μmol $m^{-2}$ $s^{-1}$ concerning meteorological conditions and habitat. Substantial mean PAR was available for invasive species in invaded habitats. *F. japonica* and *H. sosnowskyi* settled in shadow shrubs habitats with the lowest mean PAR rates. *R. confertus* invaded open grassland habitats where the mean PAR assessed 529.35 μmol $m^{-2}$ $s^{-1}$. Therefore significantly (p=0.000) the highest mean photosynthesis rate 11.60 μmol $m^{-2}$ $s^{-1}$ was observed for *R. confertus*. The lowest mean photosynthesis rate 3.02 and 4.52 μmol $m^{-2}$ $s^{-1}$ exhibited *F. japonica* and *H. sosnowskyi* in shadow habitats. Moreover, the drivers of carbon gain resulted photosynthesis values in relation to water loss during transpiration (T) at leaf level and depended on many environment' impacts, that must be subjected to complex analyses. These physiological data of assessed invasive species confirmed their positive adaptation to the environment. They also developed enhanced photosynthetic activity during their vegetative period in the new environment of invaded habitat.

**Keywords:** insolation, photosynthesis, transpiration, invasive species

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# The Study of Bystander Effect After Combining Calcium Electroporation, Electrotransfer of Anticancer Drug Bleomycin or Irreversible Electroporation

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## Abstract

The technique of electroporation (EP) is used to improve exogenous molecule transfer thru the cell membrane. When cells are exposed to a high-intensity electric field, EP occurs and this process causes the creation of hydrophilic pores, as a result of increased transmembrane potential. Reversible EP is used in anticancer therapies, such as calcium electroporation or bleomycin electrotransfer. If the transmembrane potential is significantly higher than the EP threshold, irreversible electroporation occurs, and this process could be used as a method for cancer tissue ablation.

Both therapies share similarities with another anticancer therapy – radiotherapy. It is known that at lethal doses irradiated cells secrete signaling molecules that affect adjacent unirradiated cells. The "Bystander effect" is the term given to this phenomenon. "Bystander effect" is well-known in radiotherapy, but it has not been explored in the process of electrochemotherapy. According to our recent publication, this effect could occur during Bleomycin electrotransfer and irreversible electroporation [1]. Further research into the Bystander Effect led to the use of the combination of different anticancer therapies. The aim of this research is to assess the "Bystander effect" after combination of CaCl2 electrotransfer. electrotransfer of anticancer drug bleomycin or irreversible electroporation.

In this study, we used 4T1 (mouse breast cancer) cells. The BTX electroporator was used to create electric fields. One 100 ms long and 1400 V/cm amplitude pulse was used to electrotransfer CaCl2 and bleomycin molecules and for irreversible electroporation, we used the amplitude of 2800 V/cm. After electroporation, the cells were incubated for 10 minutes on a 24-well plate, then 0.2 ml RPMI growth medium was added and incubated for 48 hours. After that time, the media was withdrawn and put on untreated cells and after 6 days the viability of cells was measured by colony formation assay.

We obtain, that the Bystander effect occurs after a different combination of calcium electroporation, irreversible electroporation, and bleomycin electrotransfer. Then the media after treatment is diluted twice the Bystander effect did not occur. However, combining two different bystander effects by adding half of the media from cells after bleomycin electrotransfer and half of media from cells after irreversible electroporation resulted in a significant reduction of cell viability. Also, we can obtain similar results if we combine bleomycin electrotransfer and calcium electroporation.

**Keywords:** electroporation, Bystander effect, bleomycin electrotransfer, irreversible electroporation, calcium electroporation

**Acknowledgments:** This work was supported by grant Nr. S-MIP-19-13 from the Research Council of Lithuania.

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# Composition of Volatile Compounds of Roman Chamomile at Different Vegetation Phases

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## Abstract

Medicinal, aromatic and spicy plants are among the most important sources of bioactive compounds with various health benefits. Roman chamomile (C*hamaemelum nobile* L.) is a perennial herb found in wild habitats and cultivated in Western Europe and some other regions as a medicinal and ornamental plant. Some Roman chamomile products have been used as a flavour and fragrance ingredients; while due to antimicrobial, antioxidant, anti-diabetes, anticancer, and anti-inflammatory and other effects it has been used in medicinal and cosmetic products [1-4]. These effects are attributed to the secondary metabolites present in Roman chamomile, mainly essential oil and polyphenolic antioxidants [3, 4]. C*hamaemelum nobile* herb was cultivated in Kaunas Botanical Garden at Vytautas Magnus University and collected at six growth phases in the period of June 6 – August 16, 2022. The objective of this study was to compare the changes of aroma profile. Dried herb was ground using a 0.5 mm hole size sieve. The released volatile compounds were collected by headspace solid phase microextraction and analysed by gas chromatography with time-of-flight mass spectrometry detector (HS-SPME-GC-TOFMS). In total, >70 compounds were detected, the major constituents being angelic, isobutyric, butyric, methacrylic, tiglic acid esters and several monoterpenes, that is in agreement with our previous work [5]. However, the composition of volatiles was highly dependent on the harvesting time. 3-Methylpentyl angelate was dominant at all growing phases; it constituted from 26.4% ($A_3$) to 58.4% ($Z_2$). Thus, it may be recognized as a specific chemotype feature of the studied plant; many previously performed studies reported isobutyl angelate as a major volatile constituent. Other quantitatively important compounds were angelic acid esters, such as methallyl angelate (5.3-14.3%), isoamyl angelate (3.2-8.4%), 2-methylbutyl angelate (1.4-2.4%). Other important esters were 3-methylamyl isobutyrate (4.0-11.4%), 3-methylamyl methacrylate (3.0-6.0%), prenyl acetate (0.6-2.9%), hexyl acetate (0.8-4.9%), and etc. Pinocarvone, *(E)*-pinocarveol and α-pinene were dominant terpenes. This study confirmed that Roman chamomile is a good source of characteristic volatile aroma compounds, and could be used as fragrance ingredients in perfumery and flavourings in foods.

**Keywords:** C*hamaemelum nobile* L., growth phases, essential oil, volatile compounds

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# Mathematical Modelling of Drying Kinetics of Forced Convection Drying Method of Potato Peels Waste

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## Abstract

The potato peel (PP) is rich of active compounds that could be employed as a constituent in functional food formulation. Nevertheless, because of their elevated moisture amount, fresh PP is a perishable by-product, so, the initial stage could be the dryness to improve and extend the storage time. In this direction, this study deals with the drying properties, and focuses on the prediction of the drying kinetics of potato peels by convection drying (CD). The points to be emphasized are the illustrations of drying kinetics by showing the variability of moisture level and drying rate (DR) depending on time and moisture amount, respectively, the presentation of drying kinetics modelling, the determination of the effective moisture diffusivity ($D_{eff}$), and the calculation of the specific energy consumption (SECe) and the energy efficiency (EE).

The necessary times during CD to have weight stability at different temperatures were of 440, 290, 220, 160, and 90 min for 40, 60, 80, 100, and 120 °C, respectively. The variation of the drying rate (DR) against moisture content revealed that using CD resulted in an elevated moisture loss at the first step of drying compared to the final stage for all the adopted temperatures. 22 mathematical models were undertaken to predict the drying kinetics and the best model was chosen based on the $R^2$, $\chi^2$, and RMSE values. Sledz model was the more appropriate with values of $0.9995 \leq R^2 \leq 0.9999$, $\chi^2 = 0.0000$, and $0.0054 \leq RMSE \leq 0.0030$. It was recorded that the SECe values are inversely proportional while those of EE were proportional to the used temperatures. So, the best SECe value of $245 \times 10^{+07}$ MJ/kg was obtained at 40 °C, and the lowest one of $62.7 \times 10^{+07}$ MJ/kg at 120 °C. For EE values, they varied from $0.097 \times 10^{-03}$ to $0.380 \times 10^{-03}$% when temperatures varied from 40 to 120 °C**.** Hence, it is suggested to use this process for drying potato peels for their expected use in various fields including the food processing industries.

**Keywords:** potato peels, drying kinetics, forced convection drying, specific energy consumption, energy efficiency

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# Examining the Impact of Stainless Steel and Titanium Electrodes on Cell Transfection, Permeability, Pore Resealing and Viability

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## Abstract

Electroporation (EP) is a technique that increases cell permeability through short electric pulses, allowing for enhanced transfer of molecules into cells or extraction out of cells. During EP, golden standard stainless-steel electrode anode releases metal iron ions, which can cause changes in medium pH, cell viability and fluorescence quenching of fluorophores [1]. Research have shown that aluminum and copper electrodes contaminate samples with ions more significantly than stainless steel or more inert electrodes [2]. Moreover, the release of metal ions also depends on the electric pulse parameters [3]. However, inert titanium alloy electrodes have not been used for EP. In this research, the impact of titanium and stainless-steel electrodes on cell permeabilization, gene transfer, viability and irreversible EP was investigated, while keeping the same pulse power by changing pulse duration and intensity accordingly.

The research object was Chinese hamster ovarian (CHO) cells. Cells were electroporated with high voltage electric pulse parameters as follows: duration 0.1, 0.5, and 1 ms, strength varied from 0,445 kV/cm to 1,8 kV/cm accordingly. Flow cytometry was used to quantify the number of cells that were permeabilized or irreversibly electroporated by measuring the number of propidium iodide ($PI^+$) positive cells and their fluorescence intensity. To evaluate transfection efficiency the pEGFP-N1 plasmid was used. Transfected (pEGFP-$N1^+$) or pEGFP positive cells were counted using flow cytometry 24 hours after EP. MTS assay was used to evaluate CHO cell viability 24 hours after treatments.

The results showed that stainless-steel and titanium electrodes yielded almost identical numbers of permeable CHO cells after EP, similar results were obtained concerning cell viability, transfection and pore resealing. Regarding cell viability after transfection, it was discovered that cell viability was higher after EP without plasmid than after transfection. Therefore, the results imply that transfecting cells with plasmid using EP, the plasmid itself holds a negative effect on cell viability, when transferred into the cell.

Overall, our data suggest that using stainless-steel and titanium electrodes for EP would produce similar results in terms of electropermeabilization, gene electrotransfer, amount of irreversible EP cell and cell viability.

**Keywords:** electroporation, titanium, stainless-steel, electrodes, transfection

**Acknowledgments:** This work was funded by Research Council of Lithuania Project P-ST-22-235.

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# Biological and Phytochemical Analyzes of 5 Plants Used Against Verminosis in Goats in DR Congo: Anthelminthic Screening, Acetylcholinesterase Inhibition, Cytotoxicity and Phenolic Compound Profiling

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## Abstract

Our previous research indicated that 5 medicinal plants belonging to the genera *Oldfieldia* and *Vitex* are widely used by farmers to treat symptoms related to internal parasitosis in goats in Lubumbashi (DR Congo). The aim of this study was to analyze the biological and phytochemical aspects of these plants.

Sequential n-heptane, dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) extracts of these plants (leaves, root bark and stem bark) were screened against *Caenorhabditis elegans* (N2), acetylcholinesterase (AChE) inhibition, cytotoxicity on mammalian cells (FHs-74 Int) and phenolic profiling by the HPTLC and HPLC methods.

The results indicate that 16 of the 84 tested extracts, mainly root bark, were very active on nematode motility with $IC_{50}$ values ranged between 63.0 ± 2.5 µg/ml (*O. dactylophylla* MeOH, root bark) and 174.7 ± 2.6 µg/ml (*V. doniana* n-heptane, stem bark) versus 53.9 ± 1.6 µg/ml for levamisole (50 µM; 10.2 µg/ml). These extracts exhibited moderate AChE inhibitory activity (dose and plant organ dependent) with $IC_{50}$ ranged from 37.8 ± 7.2 µg/ml (*O. dactylophylla* MeOH, root bark) to >200 µg/ml compared to galantamine (2.9 µg/ml). The leaf extracts showed high levels of cellular toxicity, with $IC_{50}$ values ranged from 10.4 and 14.7 µg/ml for respectively the DCM extract of *V. madiensis* 3-5_ and n-heptane extract of *V. madiensis* 5. All stem bark extracts showed toxicity with $IC_{50}$ values < 40 µg/ml. In general, the root barks have low toxicity ($IC_{50}$ >100 µg/ml). The extracts tested contain polyphenols, among which vitexin and chlorogenic acid have been clearly identified.

This study laid scientific foundations on the biological properties of the plants most used in ethnoveterinary practices. The identified phenolic compounds could be partially responsible for the observed activities. Further studies should focus on bioguided activities from promoter extracts for the purposes of identification and isolation of these compounds.

**Keywords:** plant extracts, *Vitex*, *Oldfieldia*, nematode motility, toxicity, polyphenols

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# *In Vitro* Studies on Drug and Gene Transfer into Cells

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## Abstract

Currently, the use of viral and chemical vectors is the most widespread in clinical gene therapy trials, but the side effects of these vectors, such as immunogenicity, toxicity, non-specificity or low efficacy, remain an unresolved problem. Compared to viral or chemical vector methods, electroporation of foreign genes is significantly simpler, cheaper and safer, but due to the lack of efficacy of tissue transfection, electroporation therapy has not yet reached clinical trials. Therefore, the delivery of foreign genes into cells or tissues by electropermeabilisation is a relatively new and extensively studied problem in medicine and biotechnology, and recent research in this field has focused on maximising the efficiency of gene electroporation [1,2].

The aim of study was theoretical analysis of gene transfer, evaluation of the properties and applicability of viral and non-viral vector types, and analysis of the electroporation methodology and its application in genetic engineering practice.

Different electroporation parameters used to measure the viability of Chinese hamster ovary cells (CHO-K1) had different effects under different conditions (current strength, voltage and pulse duration), while maintaining the same energy content. The most effective cell viability in both plasmid and cell-free environments was found at 1800 V/cm; 61 μs. while stronger and shorter impulses had a greater negative impact on cell viability. Transfection during the electroporation process is relatively dependent on the parameters of the electrical pulse used - the most efficient gene transfer was recorded at 1400 V/cm; 100 μs, while delivery did not occur at other parameters. The total cell fluorescence data are regular to the transfection data, with a peak value obtained at 1400 V/cm and a pulse duration of 100 μs.

**Keywords:** gene transfer, electroporation, vector, electropermeabilisation

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# Concentration-Dependent Effects of 2,3,5-Triiodobenzoic Acid on Spruce and Pine Seed Germination and Seedling Development In Vitro

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## Abstract

2,3,5-Triiodobenzoic acid (TIBA) is a chemical, known for its ability to inhibit the polar transport of plant hormone auxin [1]. Various effects of TIBA on different plant species have been reported [2-4]; however, the data about TIBA effects on ecologically and economically important conifer tree species from the genera *Picea* (spruce) and *Pinus* (pine) are scarce. In this context, the present study was designed to test the effects of different TIBA concentrations on seed germination and seedling development of two *Picea* species – Norway spruce (*Picea abies*) and Serbian spruce (*Picea omorika*) – and two pine species – Scots pine (*Pinus sylvestris*) and black pine (*Pinus nigra*). For the experiments, the seeds of these four species were surface-disinfected and planted on the nutrient medium in vitro. The medium was supplemented with different concentrations of TIBA – 1, 3, or 5 μmol/l. The TIBA-free medium was used as control in all the experiments.

The results showed that the effects of TIBA on seed germination and seedling development were concentration-dependent, but not in a linear manner. Regarding seed germination, the effect of 1 μmol/l was either insignificant or positive as in *Picea abies* and *Pinus nigra*. In the latter species, for instance, the germination rate on the medium containing 1 μmol/l TIBA was more than doubled, in comparison to the control. In contrast, 3 μmol/l TIBA had a significant negative effect on seed germination rate, that was observed in all four studied conifer species. Surprisingly, an even higher concentration of TIBA, 5 μmol/l, was largely ineffective in this respect. Regarding seedling development, the positive effect of 1 μmol/l TIBA was observed for three of the four studied species; in both *Pinus sylvestris* and *Pinus nigra*, it increased the average shoot length and, in *Picea abies*, it increased the average root length. Thus, 1 μmol/l, but not the other tested TIBA concentrations, was shown as a potentially useful supplement for in vitro-planted conifer seed cultures.

**Keywords:** auxin transport inhibitor, conifer, *Picea*, *Pinus*

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# Training of Future Health Professionals on Zoonotic Diseases for Poor Communities in Lubumbashi through One Health Approach

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**Abstract**

Objectives: The study aims at setting up human resources for the appropriation of the One Health Approach. The specific objectives of the study are to (1) determine the quality of clinical services provided to communities, (2) determine the risk of exposure of communities living in promiscuity with goats and poultry destined for sale in the public market, (3) identify the need for good environmental management, and (4) clarify the impact of the local area on medicines and the occurrence of antimicrobial resistance. Methods: The methods consist of (1) training on the One Health Approach, (2) data collection in the health area, (3) poor neighbourhoods, (4) the public market, and (5) pharmaceutical depots in poor neighbourhoods of Lubumbashi. Results: The training of health professionals was focused on the observation of (1) the health area that presented cases (14%) of loss of life recorded following a lack of reframing of the diagnosis made, (2) the inadequacy of the place of storage of drugs by the ambient temperature, a factor of alteration of the quality of the drug, the therapeutic power factors likely to be the etiological agent of antimicrobial resistance; (3) the absence of toilets and poor waste management favouring the proliferation of biological agents likely to cause diseases with unfavourable outcomes for poor populations; (4) promiscuity with animals favouring exposure to brucellosis in caprines and salmonellosis in poultry; (5) conditions of sale of perishable products favouring the spread of biological agents of diseases likely to create a debilitating clinical picture. Conclusion: The training allows for the appropriation of skills to mitigate the occurrence of zoonotic diseases through the public market as well as the absence of toilets and poor waste management. Also, sick individuals do not have the opportunity to access a good diagnosis or good quality medicines.

**Keywords:** One Health approach, zoonosis, communities

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# The Impact of Enzymatic Hydrolysis to Ovine Meat By-Products Antioxidant and Antimicrobic Properties

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## Abstract

Meat by-products are rich in proteins, which are valuable sources of biologically active compounds [1]. The recycling and reuse of animal wastes allows for a more sustainable industry and reduce utilization costs [2]. The purpose of the study was to evaluate the antioxidant and antimicrobial properties of lyophilized ovine meat by-products hydrolysates after different fermentation period (3 h, 6 h, 24 h) with pepsin and papain. The antioxidant activity was determined by measuring the absorbance of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ of bovine hearts and livers hydrolysates. The antimicrobial activity of lyophilized meat by-products hydrolysates was evaluated by agar diffusion method, using cultures of reference strains (*Listeria monocytogenes* ATCC 13932, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* subsp. *aureus* ATCC 25923, *Salmonella enterica* subsp. *enterica serovar Typhimurium* ATCC 14028, *Escherichia coli* ATCC 25922). The highest antimicrobial activity was determined using hydrolysates of ovine hearts after 24 hours with papain enzyme against bacteria *L. monocytogenes* ATCC 13932 (21.00 ± 0.10 mm) and less - against *E. coli* ATCC 25922 (10.00 ± 0.10 mm.). The highest values of antioxidant properties of hydrolysates were determined in hydrolysates of ovine liver with papain for 24 h: $ABTS^{\bullet+}$ (92.1± 0.88%) and $DPPH^{\bullet}$ (89.06 ± 2.02%). Ovine livers and hearts is a suitable protein source that can be easily hydrolyzed by enzymes to obtain hydrolysates with antioxidant and antimicrobial properties for development of new functional ingredients.

**Keywords:** enzymatic hydrolysis, ovine by-products, antioxidant and antimicrobial activity

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# Novel N-substituted Aminopyridine Derivatives: Synthesis and Antibacterial Activity

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## Abstract

It is well known that new resistant bacterial species are constantly evolving, which poses a threat to the effectiveness of antibiotics [1]. Computational methods are increasingly used to model and predict potential drug candidates. Unfortunately, there is a lack of accuracy in predicting precise structures of active compounds. New molecules can be and still are being created by trying to combine several fragments that have previously shown similar activity. The prepared set of compounds is then tested, and further modifications are planned according to the preliminary results of the biotesting. One of the directions in the development of substances with antibacterial effects is the synthesis of *N*-heteroaryl-2-arylacetamides [2]. The pyridine fragment is also found in substances possessing antibacterial effects [3].

Preliminary antibacterial properties studies of novel *N*-heteroaryl-2-arylacetamides have been carried out in collaboration between chemists and microbiologists. In this study, 2-(3-chlorophenyl)-*N*-(pyridin-2-yl)acetamide and 2-(3-chlorophenyl)-*N*-(pyridin-4-yl)acetamide were observed to exhibit antibacterial activity against *E. coli dh5a* bacterial line. Therefore, a new goal was set: to expand the library of investigated *N*-heteroaryl-2-arylacetamides by adding new analog derivatives aiming to identify correlation of antibacterial properties to the position of the chlorine atom in the benzene ring, the position of the pyridine connection and additional substituents in pyridine ring.

To reliably prove the structure of the obtained novel compounds, spectral analysis (NMR, IR) and mass spectrometric analysis (MS) methods were used. All compounds prepared for biological studies were purified to at least 97% purity (LC-MS) and the results of their antibacterial activity against *E.coli dh5a* bacteria line were evaluated. During the presentation, the synthesis of compounds and the results of biological tests will be discussed.

**Keywords:** antibacterial activity, *N*-acylation reaction, *N*-pyridinyl-2-arylacetamides

**Acknowledgments:** This project has received funding from the Research Council of Lithuania (LMTLT), agreement No S-ST-22-8.

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# Analysis of Biologically Active Compounds of Medicinal Plants of the Notreli Family

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**Abstract**

Medicinal, aromatic plants are known from ancient times to the present day. often a medicinal plant raw material with a wide spectrum of action and causes fewer side effects on the human body. The pharmacological effect of herbal preparations depends on the biologically active compounds present in the raw material of the medicinal plant. The aim of this study was to evaluate differences of the total amount of phenolic compounds flavonoids and antioxidant activity with DPPH reaction of sage (*Salvia officinalis* L.), hyssop (*Hyssopus officinalis* L.), lemon balm (*Melissa officinalis* L.) by means of spectrophotometric methods.

After carrying out the general analysis of phenolic compounds of sage (*Salvia officinalis* L.), hyssop (*Hyssopus officinalis* L.), and lemon balm (*Melissa officinalis* L.) herb by spectrophotometric method, it was determined that they contain, respectively: 80.56 mg/g RE, 120.68 mg/g RE, 102.32 mg/g RE. The relative standard deviation is 21.6%, 8.54%, 7%, respectively. After evaluating the total amount of flavonoids in the methanolic extracts of sage (*Salvia officinalis* L.), hyssop (*Hyssopus officinalis* L.), lemon balm *(Melissa officinalis* L.) herb, it was found - 37.64 mg/g RE, 13.08 mg/ g RE, 28.24 mg/g RE. Relative standard deviation -5.9%, 5.8%, 4.8%, respectively. After analyzing the results of the radical scavenging activity of the methanolic extracts of sage (*Salvia officinalis* L.), hyssop (*Hyssopus officinalis* L.), lemon balm (*Melissa officinalis* L.) herb, the determined amount is 119 mg/g RE, 111.6 mg/g, respectively RE, 28.24 mg/g RE. The relative standard deviations are 8.4%, 3.4%, 13.4%, respectively.

**Keywords:** *Salvia officinalis* L., *Hyssopus officinalis* L., *Melissa officinalis* L., spectrophotometric methods

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# Determination of Radical Scavenging Activity in Postharvest Microgreens *Brassica oleracea* Grown under Different Light Spectrum and Intensity

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## Abstract

The postharvest quality of leafy vegetables is influenced by lighting conditions during growth. Propper lighting conditions may enhance nutritional value or prolong shelf-life of leafy vegetables. Thus, the aim was to determine how supplemental light quality during growth can influence the antioxidant activity of microgreens *Brassica oleracea*. The experiments were conducted in a greenhouse (Lat 55°), where microgreens were grown in peat substrate with supplemental light-emitting diodes (LEDs) lighting consisting of different blue 450 nm (B), red 660 nm (R) and white 4000K (W) light compositions ($B_{75.6\%}$:$R_{24.2\%}$:$W_{0.02\%}$; $R_{88.9\%}$:$B_{11.1\%}$; $R_{77.6\%}$:$W_{9.9\%}$:$B_{3.5\%}$). Total PPFD of 250 µmol $m^{-2}s^{-1}$, 16h photoperiod and 20±3 °C temperature was maintained. The lowest FRAP and ABTS antioxidant activity as well as DPPH radical scavenging activity was determined in microgreens grown under lighting that consisted of $B_{75.6\%}$:$R_{24.2\%}$:$W_{0.02\%}$ LEDs. However no significant differences was determined between microgreens grown under lighting consisting of $R_{88.9\%}$:$B_{11.1\%}$ and $R_{77.6\%}$:$W_{9.9\%}$:$B_{3.5\%}$. Our findings suggest, that supplemental $B_{75.6\%}$: $R_{24.2\%}$ :$W_{0.02\%}$ LEDs significantly decreased the FRAP, ABTS antioxidant activity and DPPH radical scavenging activity in kale microgreens during harvest stage, compared to other used lighting.

**Keywords:** lighting, postharvest storage, kale, microgreens

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# Quantitative and Qualitative Analysis of Volatile Compounds in Different Varieties of Fibrous Hemp Inflorescences

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## Abstract

Volatile organic compounds (VOCs) are among the most important secondary metabolites produced by plants. More than 1,700 different types of volatile organic compounds have been identified, which are isolated from plant flowers, fruits, leaves, stems and roots [1]. Fibre hemp contains volatile organic compounds that are part of essential oils and have anti-fungal, anti-viral, anti-cancer, anti-inflammatory, anti-hyperglycemic, anti-parasitic and anti-microbial effects [1,2]. It is believed that volatile organic compounds found in plants can at least partially replace synthetic pesticides, so it is important to study their chemical composition and determine which varieties of fibrous hemp have the greatest diversity of volatile organic compounds and which varieties have the highest amount of VOCs.

In this study 5 different varieties of fibre hemp inflorescences were examined: *Feno Joy*, *Feno Moon*, *Cherry White, Lemon Haze and Felina 32*. Dried plant materials were crushed and examined using headspace gas chromatography-mass spectrometry.

Results show that the quality and quantity of volatile compounds vary depending on the variety of fibre hemp. It was determined that the most predominant compounds were: α-pinene, trans-caryophyllene and α-humulene.

**Keywords:** *Cannabis sativa* L., volatile compounds, headspace gas chromatography-mass spectrometry

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# Estonian Honey as an Anti-*Borrelia* Agent

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## Abstract

Honey is a natural product that has been used in ethnomedicine since the early humans. The nectar collected from plants and processed by honeybees is a complex mixture containing carbohydrates (70-80% w/w), water (10-20% w/w) and a variety of other minor components [1]. Honey contains about 0.1–0.5% of polyphenols (flavonoids, phenolic acids, and phenolic acid derivatives), which grant honey its beneficial properties of antioxidant capacity and antibacterial activity [2]. As honey made up of different types of plant nectar results in varied polyphenolic contents, the antioxidant and antibacterial properties of different types of honey are also different. Whereas it has been shown that various honeys can inhibit up to 10 Gram-positive and Gram-negative bacteria [3], no such studies have been carried out using the diverse Estonian honey samples. In addition to the thorough characterization of a variety of Estonian honeys, our research is currently focused on the determination of their antibacterial properties, specifically on the different morphological forms of *Borrelia burgdorferi*. The tick-borne illness caused by these bacteria, Lyme disease, is a significant health risk not only in Estonia, but in several other European countries, and the United States. The development and endurance of the latent, antibiotic-resistant forms of *B. burgdorferi* is likely the reason for the persistence of chronic Lyme disease. Therefore, there is a need for alternative or combined treatments, one of which could be the utilization of different natural sources of antioxidants. Our research group has demonstrated that various types of Estonian honey are rich in polyphenolics and show anti-*Borrelia* activity. The presentation will cover the results of antibacterial activity using spectrophotometric measurements with two different dyes (propidium iodide and SYBR Green I), and discussion about the biofilm-specific effects seen on microscopic analyses.

**Keywords:** honey, antioxidants, antibacterial activity, Lyme disease, *Borrelia burgdorferi*, biofilm

**Acknowledgments:** This research was supported by the Estonian Center of Analytical Chemistry (ECAC) funded by the Estonian Research Council (TT4) and the R&D project SS220044 “Evaluation of antioxidant and antibacterial activity of plant extracts” funded by Tallinn University of Technology.

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# Evaluation of the Effect of Enzymatic Hydrolysis on Bee Bread and Bee Pollen

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## Abstract

Bee products such as bee bread, propolis, royal jelly, venom, and pollen are multi-component natural substances prepared by bees (*Apis mellifera*). These products can be used for human well-being. Pollen and bee bread positively affect the human body, reducing the incidence of heart disease and inhibiting the development of many diseases, thanks to the presence of biologically active substances such as phenolic compounds and flavonoids, that can scavenge free radicals. Enzymatic hydrolysis involves breaking down large molecules into smaller ones using enzymes. In the case of bee bread and pollen, this process can increase the release of nutrients from the pollen by breaking down complex carbohydrates and proteins into simpler forms that can be more easily digested by humans. This can increase the nutritional value of bee bread and pollen, making them more beneficial for the human body [1]. Enzymes act as catalysts, thus mentioned action can be understood as the ability of enzymes to break down essential components of the cell wall [2]. To our knowledge, there is insufficient scientific data on the influence of enzyme mixture (Viscozyme® L) and pure cellulase on the levels of bioactive compounds in bee bread and pollen.

This study aimed to evaluate the impact of enzyme mixture (Viscozyme® L) and cellulase on the quantities of bioactive compounds in bee bread and pollen. The total amount of phenolic compounds, flavonoids, and antiradical activity was evaluated in bee bread and bee pollen samples before and after enzymatic hydrolysis using spectrophotometric methods [3]. The results were expressed in mg of rutin equivalents (RE) in 1 g of the sample.

The results showed different behavior of enzymes and tested parameters. For example, the application of enzyme mixture Viscozyme® allowed to significantly ($p \leq 0.05$) increased the total phenolic compounds content both in bee bread and pollen by 1.1 times, while cellulase had an impact only on pollen phenolic compounds (a significant ($p \leq 0.05$) increase by 1.1 times was obtained). Unfortunately, the tested enzymes did not have a significant effect on the enhancement of the recovery of flavonoids and antiradical activity both in bee bread and in pollen.

**Keywords:** bee bread, pollen, enzymatic hydrolysis, Viscozyme®, cellulase, biologically active compounds

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# Bisphenol A – Potential Factor of Miscarriage

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## Abstract

Miscarriage is the spontaneous end of a pregnancy at a stage where the embryo or fetus is incapable of surviving independently [1]. Factors that cause the first miscarriage may also contribute to the next one miscarriage. The immune dysfunctions in the etiology of miscarriage have been widely investigated for a few years [2,3]. Humans, especially pregnant women, are exposed to environmental pollutants every day, eg. endocrine disruptors. Bisphenol A is a xenoestrogen that was indicated to disrupt the endocrine system and may cause reproductive problems. In these studies, we aimed to verify the potential relationship between BPA and other parameters in the miscarriage process.

All samples were collected from pregnant women (control group) and women after their first miscarriage. The levels of anti-PR3, anti-MPO, TNF-a, MCP-1, and NADPH oxidase subunits NOX1 and NCF2 were measured. TAS and GSH-Px activity was also determined. The concentration of BPA was determined using gas chromatography.

The findings of our previous study qualified two different groups of women who had miscarriages [4,5]. The first group of women with miscarriage (who did not have NET structures in the placental) we referred to as “NETs-negative”. The second group included women with miscarriages (who had NETs identified within the placental) we referred to as “NETs-positive”. Our results showed that the concentrations of BPA were significantly higher in the serum of women in the “NETs-positive” group. The same situation we noticed in the levels of MCP-1 and TNF-a were significantly higher in the “NETs-positive” group compared to the other both groups (“NETs-negative" and control group). The levels of NOX1 and NCF2 were also higher in the serum of women in the “NETs-positive” group. The TAS activity was significantly lower in women with miscarriages compared to healthy pregnant women, but the GSH-Px activity was significantly higher.

The study showed that BPA could play a role in the course of miscarriage, especially with significantly lower antioxidative status in women with miscarriages. Our results indicate the need to limit the exposure of all women planning pregnancy to some of the estrogens, especially BPA.

**Keywords:** BPA, miscarriage, antioxidant status

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# Cow's Milk Bacteria Identification and Differentiation By LDI-MS Approaches

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## Abstract

Mastitis, an inflammation of the mammary gland is a very common disease found in dairy cow herds. It is present in two forms: clinical giving visible symptoms, and subclinical, difficult to detect. The consequences of the prevailing inflammation are mainly a decrease in the quality and quantity of produced milk and huge financial costs. The main factors causing mastitis are both pathogenic and environmental bacteria. Identification of the bacteria responsible for mastitis, especially the subclinical form, is essential in order to select an appropriate treatment. Environmental bacteria are often not taken into consideration during diagnosis despite being one of the causes for severe mastitis. Because environmental bacteria often are not taken into account in clinical studies, it is necessary to develop a methodology for their identification.

In the study MALDI-TOF MS (Matrix-assisted laser desorption/ionization time of flight mass spectrometry) technique was used to identify bacteria isolated from milk samples. Lately, the technique became more popular in clinical diagnosis. Two various systems were used for the identification of bacteria from the samples of cow's milk. Bacteria were analyzed using two instruments, namely Microflex LRF by Bruker and Zybio EXS2600 Mass Spectrometry System that mainly differ in their reference spectra bases.

There were differences in identification between the instruments, which made impossible to distinguish and correctly identify some closely related bacterial strains. This is caused, for example, by the insufficient number of spectra in the reference databases. Thus, NALDI-TOF MS (Nano-assisted laser desorption/ionization time of flight mass spectrometry) method was used to distinguish selected bacteria. It is a matrix-free technique that enables the analysis of low-molecular-weight compounds. It can be used to distinguish closely related bacterial strains based on their lipid fingerprint. In the study bacterial lipid extracts were analyzed using a prepared plate with silver thin layers deposited via chemical vapor deposition (CVD) process. Utilized approach allowed more precise differentiation of microorganisms.

**Keywords:** mastitis, bacteria differentiation, MALDI-TOF MS, NALDI-TOF MS, CVD

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# Independent and Simultaneous Electrotransfer of Large Charged Molecules

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**Abstract**

After the discovery that the gene is the fundamental component of inheritance, the objective of medicine shifted towards gene therapy, which is based on editing the genome to produce therapeutic benefits. However, the applicability of gene therapy is complicated by the plasma membrane that acts as a barrier between the inside and outside of the cell. Thus, the low efficiency of the transfer of molecules, used in gene therapy, is one of the biggest limiting factors of the technology. One potential method to bypass this barrier of the membrane and deliver therapeutic molecules into cells is electroporation. Electroporation is a technique used to increase the permeability of plasma membrane by applying external electric fields. The electrotransfer mechanism of large charged molecules depends on the type of molecule. pDNA molecules undergo a multi-step process involving electrophoresis, interaction with the membrane at the cathode-facing side of the cell, translocation to the cytoplasm, and migration toward the nucleus. Smaller nucleic acids, such as antisense oligonucleotides or siRNA also enter the cell via electrophoresis, only on the cathode-facing side and during pulse application. Because pDNA and siRNA molecules enter the cytoplasm at the cathode-facing side, the study aimed to examine the effect of simultaneous electrotransfer utilizing confocal fluorescence microscopy. Results demonstrated the novel transfer mechanism of oligonucleotides/siRNA molecules that is driven by both electrophoresis and post-pulsation diffusion. What is more, the study also discovered that the efficiency of siRNA electrotransfer is negatively affected by pDNA presence. Overall, this research provides new insights into the electrotransfer mechanism of large charged molecules that could potentially contribute to the development of applicability, efficiency, and safety of the systems of gene therapy.

**Keywords:** gene therapy, electrotransfer, pDNA, siRNA, antisense oligonucleotides

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# MALDI/TOF MS Technique as a Method for Identifying Microorganisms of Forensic Significance with Simultaneous Indication of Differences in Protein Profiles of Strains from Different Sources

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## Abstract

Microorganisms occur naturally in all environments, such as soil, water and the interiors of living organisms - animals and humans. Microbes are an important aspect in environmental protection, as well as human health or life. Their presence can have beneficial effects - especially those that naturally occur in the intestinal bacterial flora. Some microorganisms are pathogenic and danger to ourselves, but also to the nature around us. Not only the microorganisms, but also the products they produce and their ability to immunosuppress the host can be harmful to human.

Detection and identification of pathogens is a challenge for microbiology laboratories. In some cases - rapid diagnosis - is a key element in the fight against the disease caused by a particular microorganism. Mutations, increased drug resistance - as well as manufacturers' failure to comply with food safety laws - are forcing the use of ever-improving methods to identify microorganisms. In the forensic aspect - it is important not only to identify the pathogen itself, which led to a threat to health or life, but also to identify the specific source - from which this microorganism came.

MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization-Time Of Flight Mass Spectrometry) technique was used to identify bacteria isolated from dairy products (milk, cream, buttermilk whey) and dairy wastewater. A series of spectra were obtained showing the unique protein profiles of the bacteria studied. The same bacterial strains from different sources were used for comparison. The differences found in their protein profiles were indicated. A series of repetitions were performed to obtain more spectra. This made it possible to check the reproducibility of the results obtained. Identification was performed using Bruker's Microflex LRF.

**Keywords:** MALDI-TOF MS, patogens, bacteria identification, protein profile

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# Analysis of High- and Low-Energy Electroporation Effect on Molecule Transfer

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## Abstract

The method of Electroporation (EP) utilizes short electric pulses to permeate cell plasma membrane (PM) to facilitate movement of intra- and extracellular molecules across the PM [1]. The primary function of this technique is to deliver hydrophilic molecules, such as drugs, oligo- and polynucleotides into cells. Researchers are exploring its potential in gene therapy for treating neurological and congenital diseases, cancer electrochemotherapy, and the development of cancer vaccines through oligonucleotide electrotransfection. However, the mass, charge, and shape of the compound influence the nature of electrotransfer, and different EP parameters must be used to determine the electrotransfer properties of molecules of different sizes such as plasmid DNA (pDNA), oligonucleotides and propidium iodide (PI) to evaluate the influence of treatment conditions on electrotransfer efficiency [2].

In the experiments, electroporation was conducted on Chinese hamster ovary cells suspended in a low conductivity medium and placed within a 2 mm gap between stainless steel electrodes. PI, oligonucleotide and pDNA electrotransfer were carried out using low-energy (1.57 mJ) and high-energy (28.8 mJ) pulses. The efficiency of electrotransfer was determined using flow cytometry, and GFP pDNA expression was evaluated 24 h after the EP procedure. PI electrotransfer was visualised and quantitatively analysed using fluorescence microscopy and coplanarly arranged copper electrodes that delivered the pulsation.

The results demonstrated that the electrophoretic force from electrical pulses had a significant effect on oligonucleotide electrotransfer but did not impact PI transfer. On the contrary, PI transfer benefited from increased electric field strength rather that pulse duration, and molecule drift continued after the external electric pulsation ceased. Increasing pulse voltage additionally led to growing asymmetry of permeabilized cell membrane area on each of its poles. After treatment with 10 high-energy pulses, most cells remained permeable up to 15 minutes after the treatment was conducted, with a viability decrease to as low as 10% of control cell viability. Additionally, the maximum pDNA expression was observed when applying one high voltage low-energy pulse, and longer pulses had an adverse effect on cell viability and negligible transfection rates. Comparing this to oligonucleotide electrotransfer, it is evident that the mechanism behind the uptake of nucleic acids of varying size into electropermeabilized cells varies, as well as shedding light on the applicability of electroporation of different pulsation energy.

**Keywords:** electroporation, electrotransfection.

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# The Impact of Fermentation using Symbiotic Cultures Combined with Ultrasound Treatment on Antioxidant and Antibacterial Activity of Common Oak (*Quercus robur*) Bark

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## Abstract

The biological properties of the plant depend not only on the quantity and quality of accumulated biologically active substances but also on the part of the plant. Literature data show that the biological activity of plant raw materials can be increased during fermentation and/or ultrasound extraction [1]. The aim of this study was to evaluate the impact of fermentation using symbiotic kombucha (*Medusomyces gisevii*) and tibico cultures combined with ultrasonication treatment on the bark of common oak (*Quercus robur*). To our knowledge, there are no literature data about the impact of combined treatment (ultrasound and symbiotic cultures such as kombucha and water kefir tibico) on the common oak grown in Lithuania bark.

Firstly, the plant material was treated with ultrasound and after fermented with kombucha or tibico beverage. After 48 h fermentation, the liquid part was collected while the residues were re-extracted with methanol. Control samples were prepared in a similar way, just distilled water was used instead of symbiotic cultures. The total amount of phenolic compounds and antiradical activity were evaluated in obtained extracts by spectrophotometric methods [2-3]. Results were expressed as rutin equivalents (mg RE/g). The antimicrobial activity of the extracts was evaluated by an agar diffusion test against *Lactobacillus plantarum* and *Candida albicans* cultures [4]. The mean radius of the clear zone around each common oak sample was measured in mm.

The combination of ultrasound treatment and fermentation of the plant material, allowed to increase in the total phenolic content and antiradical activity of the common oak bark. The total phenolic content increased by 4.5 and 4.0 times in combined extract with kombucha and tibico culture, respectively. The antiradical activity significantly ($p \leq 0.05$) increased by 5.5 and 5.8 times, respectively. Antimicrobial activity of common oak bark was established in all methanolic extracts obtained after ultrasound treatment and fermentation against the tested *Lactobacillus plantarum* and *Candida albicans* cultures. Antibacterial activity against *Lactobacillus plantarum* significantly ($p \leq 0.05$) increased by 2.4 and 2.7 times in the extracts obtained after kombucha and tibico fermentation, respectively. Antifungal activity of *Candida albicans* significantly ($p \leq 0.05$) increased by 1.2 and 1.1 times in the extracts with kombucha and tibico culture, respectively.

**Keywords:** common oak, ultrasound, fermentation, antioxidant activity, antibacterial activity

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# White Rot Fungus Enzymes Laccase and Manganese Peroxidase Influence on Bioremediation

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## Abstract

Bioremediation has recently gained a lot of attention as an alternative to a traditional degradation technique of the pollutant [1]. Bioremediation processes require less energy resources and costs, while being environmentally friendly. Fungi are organisms that are gaining increasing attention due to their wide potential for bioremediation applications. Due to their intracellular and intracellular enzymatic mechanisms and their ability to release acids, fungi are able to attack and metabolize a wide range of compound classes, including organic and inorganic pollutants. The aim of the research is to identify how effective white – rot fungi, *Pleurotus ostreatus* can bioremediate polyphenol flavonoid rutin as a substrate.

Two white rot fungi, *Pleurotus ostreatus* and *Pleurotus eryngii* were grown in malt extract liquid medium. The enzymatic activity was measured using UV spectrophotometry [2,3]. After inoculating *P. ostreatus* in liquid medium with rutin, the flavonoids concentration was analyzed using solid phase extraction [4] and later determined using HPLC-UV detection. Extracts from the fungi were prepared in 75 % methanol, extracted using PVDF 0.45 μm membrane filters and rutin concentration was determined with said method.

The results of the enzymatic activity assay showed that *P. eryngii* produced more enzymes overall, with the laccase having stronger enzymatic activity, than laccase produced by *P. ostreatus*. *P. ostreatus* produced a lesser number of enzymes overall with its manganese peroxidase having stronger enzymatic activity, than the manganese peroxidase, produced by *P. eryngii*. After preparing extracts from the liquid medium with rutin via solid phase extraction and later analyzing them with HPLC – UV, after 4 days, the rutin concentration was significantly decreased, compared with the first day of the analysis and remained low in all of the liquid medium extracts from *P. ostreatus*, throughout the entire period of 14 days.

**Keywords:** bioremediation, white rot fungi, *Pleurotus ostreatus*, *Pleurotus eryngii*, laccase, manganese peroxidase

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# Size of Hypoxic Regions in Glioblastoma Spheroids Influence mRNA Isoform Formation

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## Abstract

Alternative splicing is a process which enables the formation of two or more mRNA isoforms out of one pre-mRNA. These transcripts may code proteins with different structure and functions. Alternative splicing is highly important because it increases proteomic diversity and contributes to the adaptation processes of the cells to different environmental conditions, for example, hypoxia. Hypoxia is defined as decreased oxygen level in cells and it is prevalent during illnesses like cancer.

Currently, a lot of eukaryotic cell lines are cultivated using monolayer, also known as 2D culture method, where cells are attached to the flat surface of the growing dish. However, this type of method does not mimic actual physiology of tissues because most cells in organisms grow in the three-dimensional microenvironment. Hence, there is a great need to use other alternative methods to cultivate cells in 3D structures *in vitro* [1,2].

In this study, we used glioblastoma U-87 cells to form three-dimensional structures – spheroids out of different number of cells and performed experiments to determine the relation between the size of the spheroid and the size of their hypoxic regions. Also, we demonstrate that an increase in the size of hypoxic regions in spheroids alters mRNA isoform formation.

**Keywords:** hypoxia, splicing, spheroid

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# Analysis of Biologically Active Compounds in Natural Food Supplements

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## Abstract

In recent decades, consumers' commitment to a healthier lifestyle and a more varied diet has grown rapidly, resulting in increased demand for quality and variety in the foods and supplements they consume. Thus, to improve or maintain health, consumers must purchase products such as dietary supplements to complement their diet [1]. Maughan et al. (2018) recently defined a dietary supplement as: a food, food component, nutrient, or non-food compound that is purposefully administered in conjunction with a regular diet to achieve specific health and/or performance benefits [2].

The aim of the work was to assess the amount of biologically active compounds in selected food supplements prepared with methanol and aqueous solution using spectrophotometric methods. Tasks: to determine the total amount of phenolic compounds, flavonoids and radical scavenging activity in food supplements using spectrophotometric methods.

Spectrophotometric analysis studies were performed and the obtained results corresponded to the results of the literature analysis articles. When determining the total amount of phenolic compounds, more compounds were detected using the methodology with the methanolic solution than with the aqueous one. The methanolic solution also dominated the determination of the total flavonoid content in relation to the aqueous solution. However, selected dietary supplement extracts exhibit strong radical scavenging activity in aqueous solution.

**Keywords:** biologically active compounds, natural food supplements, spectrophotometric methods, total phenolic content, flavonoid content, radical scavenging activity

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# Factors Determining the Severity of COVID-19 in Pakistani Population

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## Abstract

The main objective of the presented study was to determine the various risk factors associated with the severity of coronavirus disease 2019 (COVID-19) in the Pakistani population. This study had three major aims: To study the dispersal of COVID-19 symptoms among different age groups and gender groups and to elaborate on the manifestation of COVID-19 symptoms in patients with various comorbidities. The participants (n = 1353) of this study were the individuals who consulted various collection centers of Hormone Lab Lahore, Pakistan for COVID-19 testing. The SARS-CoV-2 real-time reverse-transcriptase– polymerase-chain-reaction (RT-PCR) testing was conducted from July to November 2020. A COVID-19-confirmed case was defined as having a positive result through an RT-PCR assay of nasopharyngeal swab specimens. We classified the study participants as asymptomatic and had severe or mild symptoms based on the guidelines provided by the WHO. Of the total individuals tested, 8% were RT-PCR positive for COVID-19 while 92% tested negative. The advanced age group (51-60 years) showed the most severe symptoms (19%). In terms of gender biases, only a slightly higher percentage (7%) of severe symptoms was observed in the male population compared to female individuals (3%). Comorbidities, such as hypertension, diabetes, cardiovascular diseases, kidney, and pulmonary diseases along with cancer, have been documented to worsen the COVID-19 condition in patients. According to this study, only a small percentage of the population developed severe symptoms, while most cases recorded, were asymptomatic. However, several risk factors, such as increased age and comorbidities were found to be worsening the COVID-19 condition.

**Keywords**: coronavirus disease 2019, COVID-19 risk factors, Pakistani population, comorbidities, RT-PCR testing, asymptomatic, severe symptoms, mild symptoms

**Acknowledgments:** University of the Punjab, Lahore, Pakistan is acknowledged for providing funding for this research work.

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# Abscisic Acid: Antioxdiant Response of Different Growth Strategy Crops

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## Abstract

Plant phytohormones participate in all aspects of plant development. One of stress-related phytohormone is abscisic acid, which mainly participates in water movement from root to leaf and results in whole plant adaptation processes. The impact of exogenous abscisic acid on antioxidant response of different growth strategy plants was evaluated. Lettuce (*Lactuca sativa* L.) cv. Green Cos Lobjoits was grown in a greenhouse with 16 hour photoperiod, 70% humidity and average temperature of 21-22 °C during the day and 15-17 °C at night was maintained. Pea (*Pisum sativum* L.) cv. Respect grew in a vegetation area with a shed during May-July season (lat. 55°, Lithuania). Experiment was split into two stages: 1) abscisic acid (ABA) were applied at rates of 0, 15, 30, 45, 60 mg/l for lettuce and 0, 30, 60, 90, 120 mg/l for pea; 2) abscisic acid were applied in combination with growth promoting phytohromones (kinetin (KIN); indole-3-acetic acid (IAA) and gibberellic acid (GA)) at rates of 30 mg/l. Exogenous phytohormones were applied at critical development stages: 3 leaves for lettuce and at 6-8 leaves and before flowering for pea. Measurements were performed after 5 days of each application and at technical maturity. Results showed that compared to single application of abscisic acid, the combination of stress and growth phythoromones resulted the increase of antioxidant response in both plants.

**Keywords:** exogenous phytohormones, abscisic acid, lettuce, pea, biostimulants, antioxidants, onthogenesis

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# Synthesis and Spatial Structure Determination of 2-amino-4,7-diaryl-3-cyano-5,6,7,8-tetrahydro-4*H*-chromenes

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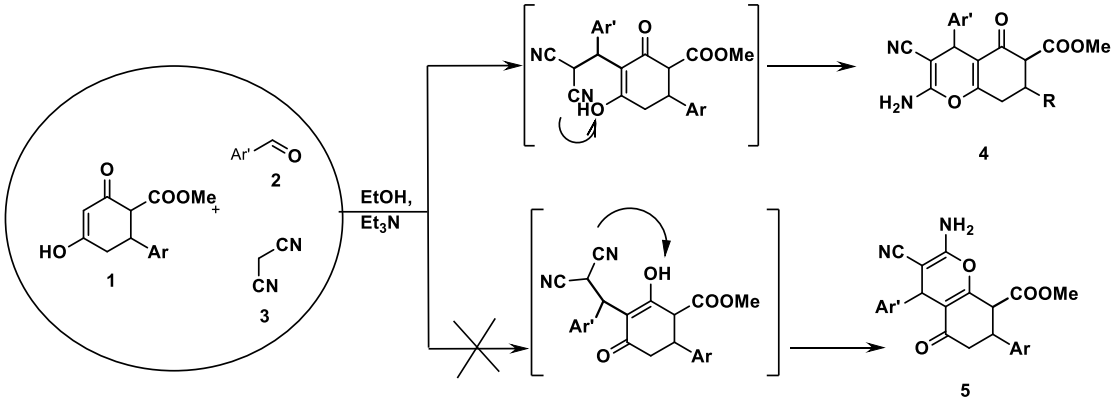
**Abstract**

Pyran derivatives exhibit a large variety of pharmacological activities (anti-inflammatory, antibacterial, anticoagulant, etc.). We show the synthesis of new derivatives of 2-amino-4H-pyran. The use of esters **1** as an enolnucleophile in a three-component reaction with aromatic aldehydes and malononitrile allowed us to develop a simple and efficient method for the synthesis of new carboanelated derivatives of 2-amino-4H-pyran, in particular, 2-amino-3-cyano-5,6,7,8-tetrahydro-4H-chromenes **4**with high yields from readily available reagents.




One of the most challenging tasks is to determine the composition and the spatial structure of the synthesized compounds, especially, if several stereoisomers can form during the synthesis.

Esters**1** contain two asymmetric carbons and, therefore, can exist as two pairs of enantiomers: a pair of enantiomers with a trans-arrangement of the ester group and an aryl residue and a pair of enantiomers with a cis-configuration. Analysis of $^1$H NMR spectra showed that only one of the two possible pairs of enantiomers is formed, which are in the trans-configuration. This was also confirmed by X-ray diffraction.

Theoretically, in this three-component interaction the formation of two isomers is possible depending on oxygen atom of the ester **1** with which heterocyclization proceeds: 6-substituted **4** or 8-substituted **5**. As our further research showed, a number of new 2-amino-4,7-diaryl-6-methoxycarbonyl-5-oxo-3-cyano-5,6,7,8-tetrahydro-7-R-4H-chromenes **4** were synthesized with high yields.

**Keywords:** 2-amino-4H-pyran, 4H-chromene, three-component interaction, enolnucleophile, X-ray diffraction analysis

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# Comparison of Phenolic Content and Antioxidant Activity of *Bjerkandera adusta* Extracts Before and After Heat-Shock Treatment

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## Abstract

Nutraceuticals are various biological molecules that provide health benefits to humans [1]. These compounds come in various forms and from various sources. For example, phenols, which have antioxidative properties which are useful in medicine, can be found in plants, fungi, animals, and their extracts [2]. Most cells grow in environments that suit their needs, however, sometimes environments may change and create various stresses on the cells. Thermal stress is a process where living organisms that are exposed to higher or lower than preferable temperatures try to protect themselves by producing various metabolic compounds, primarily proteins. This organism response can be used to induce the production of a variety of molecules useful in medicine or industry. It is known that heat shock increases the antioxidative effects of plants and fungi [3-5]. However, the relationship between phenolic content and antioxidant activity after heat shock in fungi is still scarcely investigated [5]. Therefore, multiple species of fungi *Bjerkandera adusta, Pleurotus ostreatus, Lentinula edodes* were screened for antioxidant activity and total phenolic content. After preliminary experiments, the highest amount of phenolic compounds and antioxidant activity was observed in *Bjerkandera adusta* extracts (data not shown). This was done using Folin–Ciocalteu method for quantitative phenolic compound analysis, while DPPH was used to evaluate the antioxidant activity. Further analysis of the extracts of *Bjerkandera adusta* treated by heat shock revealed an increase in the antioxidant activity but not in the phenolic content.

**Keywords:** nutraceuticals, *Bjerkandera adusta*, phenolics, antioxidant activity, heat shock

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# Pullulan as Film-Forming Polymer for Hydrophilic Nail Lacquer Formulations

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## Abstract

Nail lacquers are topical solutions intended for use on nails and have been found to be useful in the treatment of onychomycosis [1]. The film acts a drug reservoir from which drug releases slowly and penetrates into and through the nail [2]. The type of the polymer affects the reservoir properties and releasing properties of the film [3].

The aim of this study was to evaluate pullulan as the film-forming polymer for hydrophilic nail lacquer formulations. To achieve this goal, experimental planning and optimization of hydrophilic nail lacquer compositions were performed based on physical properties of nail lacquers and *in vitro* release results. For *ex vivo* permeation and penetration study, human nail clippings were used as nail model and "wetted cotton ball" to mimic nail bed system. Quantitative analysis of antifungal agent was done by UPLC.

Two optimized hydrophilic nail lacquers were formulated with the solvent system (purified water: ethanol: butyl acetate), Transcutol P and Pullulan. Hydrophilic nail lacquer OPT 1 with Transcutol P as plasticizer had the highest released amount of antifungal agent in *in vitro* release testing; and the hydrophilic nail lacquer OPT 2 with Triacetin as plasticizer had the highest accumulated amount of antifungal agent in the nail clippings and acceptor medium in *ex vivo* transungual permeation study.

Pullulan was found to be a suitable polymer for hydrophilic nail lacquer formulation. It was determined that the plasticizer nature has a statistically significant ($p < 0.05$) influence on the penetration of active substance to the acceptor media from hydrophilic nail lacquer formulations; and that hydrophilic film-forming polymer exhibit high penetration properties of antifungal drug into the nail model.

**Keywords:** transungual delivery, onychomycosis, nail lacquer, hydrophilic film-forming polymer, plasticizer, drug release, drug accumulation, drug penetration

**Acknowledgments:** The authors are thankful to the department of Clinical Pharmacy for the opportunity to carry out this research.

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# Dosage Form Influence on Transungual Delivery of Amorolfine HCl

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## Abstract

Onychomycosis is a fungal nail infection caused by dermatophytes, non-dermatophyte molds and yeasts [1]. The main challenge for topical drug delivery is the structure of the nail. The keratinized nail plate, which contains a stable network of disulfide bonds, limits the penetration of the drug into the nail [2]. Therefore, the effectiveness of topical medications in the treatment of fungal nail infection can be determined by the different dosage form usage.

The aim of this study was to evaluate pharmaceutical factor such as dosage form influence on the transungual delivery of amorolfine HCl. For the study, human nail clippings were used as a model membrane of a human nail, and "wetted cotton ball" simulated the nail bed. Three different pharmaceutical forms were prepared to evaluate delivery of amorolfine HCl to nail. Quantitative analysis of penetrated amorolfine HCl was performed by UPLC.

It was determined that dosage form had influence on accumulation of amorolfine HCl. Nail lacquers, film-forming solutions and creams were made to compare dosage form impact on amorolfine HCl accumulation. Statistically significantly highest amount of amorolfine HCl accumulated in the nails was determined when nail lacquers were used. Furthermore, it was investigated, that longer dosage form contact time with nail surface statistically significantly increases accumulation rates of amorolfine HCl in the nail layers.

The current study demonstrated that different dosage forms have significant impact on amorolfine HCl accumulation in nails.

**Keywords:** onychomycosis, amorolfine HCl, dosage form, transungual delivery

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# Towards Side-Effect Free Anticancer Therapy

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## Abstract

The eventual goal of cancer treatment is to reduce the size of a malignant tumor while minimizing the associated side-effects. The cytotoxic properties of bleomycin make it a commonly used as a chemotherapeutic agent in the treatment of cancer [1]. Although bleomycin can inhibit or suppress tumor growth, it can also cause serious or fatal side-effects, including lung damage that can lead to pulmonary fibrosis [2]. The numerous side-effects of chemotherapy can prolong the duration required for recovery.

The employment of $Ca2^{+}$ in cancer therapy is currently under attention: scientific evidence supports the effectiveness of calcium electroporation as a treatment method for cancer [3].

The potential of SP in cancer treatment could surpass electroporation; however, the delivery of $Ca^{2+}$ and the potential uses of sonotherapy have not yet been thoroughly researched. The use of $Ca^{2+}$ in cancer treatment has the potential to substantially decrease the adverse effects resulting from conventional cytotoxic drugs.

Our initial findings suggest that Chinese hamster ovary cells (CHO) undergo rapid cell death within 20 minutes when treated with $Ca^{2+}$ at a concentration of 5 mM. This is supported by similar results obtained from cell viability assays conducted at various time intervals. The rapid cellular reaction triggered by $Ca^{2+}$ delivery through SP can create novel possibilities for initiating quick (< 20 min) cell death via intrinsic $Ca^{2+}$ activated mechanisms [4]. This stands in contrast to the slow death (taking 24 – 72 hours) induced by chemotherapy or gene therapy.

**Keywords:** calcium, microbubbles, sonoporation, ultrasound

**Acknowledgments:** This work was supported by the grant: “The development of controlled cancer treatment modality based on $Ca^{2+}$ delivery via ultrasound (S-PD-22-51)” from the Research Council of Lithuania.

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# Antimicrobial Activity and Phytochemical Composition of Oregano, Peppermint, Thyme, and Lavender Essential Oils

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**Abstract:**

This summary of research results describes three important experiments in which the antibacterial properties and composition of essential oils of four different plants (oregano, peppermint, thyme, and lavender) were studied. First, it was found that the essential oil of oregano has significant antibacterial effects on all the tested bacterial cultures, despite its concentration being only 5%. It was also found that the essential oils of thyme and peppermint are more effective against certain Gram- bacterial cultures. However, the true essential oil of lavender, used at a concentration of 10%, was only able to suppress the growth of certain Gram+ bacteria. Secondly, using gas chromatography-mass spectrometry, components that contribute to the antibacterial effect of each plant's essential oil were identified. Carvacrol was found in oregano, linalool in thyme, menthol in peppermint, and linalool in lavender. Thirdly, the dominant components in each plant's essential oils were quantitatively evaluated during the study. In common thyme (*Thymus serpyllum* L.), linalool was found to be 0.541 ± 0.09 mg/ml, in common oregano (*Origanum vulgare* L.), carvacrol was 0.742 ± 0.05 mg/ml, in peppermint (*Mentha piperita* L.), menthol was 0.725 ± 0.11 mg/ml, and in true lavender (*Lavandula angustifolia* L.), linalool was found to be 0.581 ± 0.05 mg/ml. These research results can be useful for further studies on the mechanisms of plant antibacterial activity and their potential application in medicine or other industrial sectors.

**Keywords:** microorganisms, antibacterial properties, oregano, peppermint, thyme, lavender

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# Distribution of Micromicetus in Fresh Medicinal Material of Rosehip (*Rosa canina* L.) and Guelder-rose (*Viburnum opulus* L.)

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## Abstract

Molds are very common - there are thousands of species, and they can be found year-round in a wide variety of environments, both outdoors and indoors. Contamination of food products, berries, fruits with micromicetus is one of the most urgent problems related to human and animal safety. For this reason, in many countries of the world, more and more attention is paid to the microbiological and mycotoxicological safety of products. It is important to be able to identify molds to prevent further problems. The aim of this research is to analyze what type of micromicetus grow on rosehip (*Rosa canina* L.) and guelder-rose (*Viburnum opulus* L.) medicinal plant material. Individual parts of the plant, berries, leaves and twigs, were studied. A medium for the growth of molds with the antibiotic chloramphenicol was prepared. Rosehip (*Rosa canina* L.) and guelder-rose (*Viburnum Opulus* L.) and rosehip leaves are placed symmetrically in 5-6 parts on Petri dishes with medium. Rosehip (*Rosa canina* L.) twigs, guelder-rose (*Viburnum opulus* L.) leaves and twigs are cut with sterile scissors into pieces up to two cm long, which are placed at a distance of 1-2 cm from each other on nutrient agar. 5-6 pieces are placed in one petri dish with a medium. Individual plant parts were incubated in a thermostat at a temperature of 26-28 ℃. Cultivated molds are identified through a microscope. Fungi grew in 5 days. Different types of fungi grew: *Circinella circinans, Acremonium fusidioides, Aspergillus flavus, Mucor hiemalis, Trichoderma hamatum, Dipodascus albidus, Candida guilliermondii, Trichoderma harzianum.*

**Keywords:** rosehip (*Rosa canina* L.), guelder-rose (*Viburnum opulus* L.), fungi, molds, micromicetus, fresh medicinal plant material.

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# Evaluation of the Impact of Various Treatment Methods on Bee-Collected Pollen Biological Activity

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## Abstract

Bee pollen is natural bee's (*Apis mellifera*) product which is mainly composed of pollen collected from various wild or agricultural plants. Pollen goes through a few stages to become a final product: firstly, treatment with bee saliva enzymes and secondly, agglutination with nectar where pollen is stuck together into small chunks then small granules are received which are called bee pollen. Bee pollen are rich in nutrients, like proteins, glucose, vitamins, like B and C, minerals, like potassium and calcium, and phenolic compounds which antioxidant properties can reduce the reactive oxygen species damage to the cells and improve human health [1]. Unfortunately, the bioavailability of bee pollen is limited, due to the strong pollen wall, which is called exine. Sporopollenin is the primary component of exine wall, and it makes it elastic, sturdy, and resilient to chemical degradation, due to that, humans digest around 10-15% of bee pollen [2].

The aim of the study – evaluate the biological activity of bee pollen after treating them with various methods. Bee pollen was treated with tibico and kombucha mixture, pure *Bifidobacterium infantis* bacteria, hydrolytic enzymes, ultrasound, and ultrasound treatment followed by *B. infantis* fermentation or enzymatic hydrolysis. Phenolic compounds, flavonoids, and radical scavenging activity were determined in water fractions and methanolic extracts of fermented material using spectrophotometric tests [3, 4].

Studies have shown that all treatments had a positive effect on the increase of biologically active compounds. The level of increase depended on both the region of bee pollen collection and the treatment method. The most promising treatments would be ultrasound treatment, ultrasound combined with hydrolytic enzymes, fermentation with a mixture of tibico and kombucha, and fermentation with *B. infantis* lactic acid bacteria.

**Keywords:** bee pollen, tibico, kombucha, *B. infantis*, ultrasound, hydrolysis.

**Acknowledgments:** Financial support from the Research Council of Lithuania project No. S-ST-22-62 is acknowledged.

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# Monitoring Research of Genotype to Scrapie in Lithuanian Sheep Breeds

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## Abstract

Scrapie is a naturally occurring and fatal neurologic disease of sheep that is caused by a misfolded protein called a prion. Shedding of the scrapie agent occurs before the onset of clinical signs [1]. Disease characterized a long incubation period, a slow course, degenerative changes in the central nervous system, disorder of coordination of movements (ataxia), body trembling (tremor), itching, loss of fur [2].

The main principles of prevention and control of this disease is monitor the genotype of animals in the flock. This makes it possible to detect genetic resistance and selective breeding of sheep with scrapie-resistant genotypes. Up to now the five alleles $A_{136}R_{154}R_{171}$ (ARR) and accordingly ARQ, ARH, AHQ and VRQ are known to be linked to scrapie susceptibility and have been found in breed specific frequencies. The VRQ allele in breeds are associated with highest risk, whereas allele ARR confers genetic resistance to scrapie [3].

Monitoring studies of Lithuanian sheep breeds have shown a predominance of scrapie-resistant groups. The most number of sheep were found with genotype G2, which was 42%. This group shows that sheep are genetically resistant and that these animals need careful selection when used for breeding. 34% of the total flock were sheep with the genotype ARR/ARR group G1. It is thought to be among the most resistant to scrapie disease and such animals are suitable for further breeding and selection. Group G3 AHQ/ARQ was 20% of the total number and the animals are classified as resistant to scrapie and can be used for breeding in small numbers. Groups G4 ARR/VRQ and G5 ARQ/VRQ were 1.4 – 2.6 % and these animals can't use for further breeding and selection.

Analysis of genotype was made on different sheep breeds. The most resistant animals with the ARR/ARR genotype were found to be Lithuanian Blackhead and Suffolk sheep. Therefore, according to the results of the study, the highest number of Suffolk sheep of risk group G1 was 62 %, and sheep of Lithuanian Blackhead breed group G2 was 49%.

**Key words:** scrapie, genotypes, Lithuanian sheep breeds

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# High Concentrations of Tetracycline Reduce Light Use Efficiency in *Brassica napus* Leaves

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## Abstract

The potential risk of antibiotics to environmental quality is becoming an urgent issue. Recently, antibiotics have been recognized as a new class of environmental pollutants. Antibiotics used in veterinary medicine or as growth promoters often appear in manure that becomes fertilizer in agriculture. One of the most commonly used antibiotics in veterinary medicine and agriculture is tetracycline (TC), which, due to poor absorption in the gastrointestinal tract of animals, is eliminated with excrement in the form of unmetabolized residues and thus reaches the fields. The aim of this work was to investigate changes in chlorophyll *a* fluorescence parameters of spring oilseed rape (*Brassica napus* L.) under the effect of different concentrations of tetracycline in a controlled environment. Plants were grown in pots filled with a mixture of field soil, perlite and fine sand (volume ratio 5:3:2). The investigated TC concentration in the soil was obtained with the prepared solution of tetracycline hydrochloride in the soil: 0, 250, and 500 mg/kg. Seeds of the *B. napus* were sown in plastic pots filled with prepared soil mixtures with different TC concentrations. 48 days after sowing chlorophyll *a* (*chl a*) fluorescence parameters measurements were taken with the Plant Efficiency Analyser, PEA (Hansatech Instruments, Ltd., King's Lynn, Norfolk, England) with randomly selected youngest fully expanded leaves.

The tested concentrations of TC had a negative effect on the *chl a* fluorescence indicators of rapeseed. The effect of 250 mg/kg concentration on maximum II photosystem quantum efficiency rate (Fv/Fm) was insignificant, while higher concentration significantly reduced this indicator by 13%. Photosynthetic efficiency (PIabs) index at 250 and 500 mg/kg TC exposure decreased by 54% and 83%, respectively, compared to control plants ($p<0.05$). These changes in the viability of the photosystem may have been due to an increase in the amounts of dissociated energy DIo/RC in plants. For instance, at at 250 and 500 mg/kg TC exposure, DIo/RC of *B. napus* increased by 64% and 263%, respectively, compared to control plants ($p<0.05$). Antibiotic pollution also increased the part of energy transferred in the form of electron transport (ETo/RC) or used for the regeneration of reaction centers (REo/RC). Again, statistically significant changes were obtained only at 500 mg/kg TC exposure, when ETo/RC and REo/RC increased by 44% and 43%, respectively. The above-mentioned changes in energy use resulted in 22% ($p<0.05$) and 35% ($p<0.05$) losses of dry aboveground biomass at 250 and 500 mg/kg TC exposure, respectively. This study highlights that high TC concentrations have a negative impact on energy use efficiency and the growth of *B. napus*.

**Keywords:** tetracycline, *Brassica napus*, chlorophyll fluorescence

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# Application of Highly Ordered π-electron Materials Incapsulating in Monolithic Columns

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## Abstract

Recent studies have revealed problems related to the separation and analysis of phytochemical compounds. Physicochemically "similar" compounds are difficult to separate using conventional separation methods. Current chromatographic methods are incapable of separating compounds with antiradical activity, and anticancer properties, such as amphiphilic polyphenols. In order to solve this problem, new generations of sorptive materials of unique selectivity and new strategic analytical solutions have been investigated.

The most promising approach to this problem is the synthesis of organic compounds of highly ordered π-electron structure. However, these kinds of materials are UV/visible light impermeable, so the conventional optical detection methods used in the micro-analysis of the capillary format cannot be applied to the analytical systems and forces to search for new detection methods.

This work covers complex research related to the synthesis of monolithic capillary columns using actrylamide derivatives and integrating highly ordered π-electron chemical structures as stationary phases into capillary-format columns (internal diameter of 50-100 μm), and investigation of applicability of obtained systems for the high selectivity separations and analysis of anti-cancer, radical scavenging, antioxidant, antimicrobial and antiviral phytochemical compounds.

**Keywords:** phytochemical compounds, anticancer, antioxidant, highly ordered π-electron chemical structures, monolithic capillary columns

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# Comparative Efficacy of *Nicotiana tabacum* and *Azadirchta indica* Extracts Along with Their Green Synthesized Nano-ZnO Particles Against *Brevicoryne brassicae* L.

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## Abstract

*Brevicoryne brassicae* L. is the most destructive pest of brassica crop and causes yield losses upto 30-35%. Traditional insecticides are effective tools against brassica aphid but they cause environmental pollution, resistance, and damage to our biodiversity. Nanotechnology appears to be a new technique to combat insect infestations. ZnO nanoparticles are a better alternative to standard chemicals due to their green synthesis. This study was conducted to determine the toxicity of *Nicotiana tabacum* and *Azadirchta indica* extracts, as well as the ZnO nanoparticles mediated by these plants extract against aphid. Soxhlet apparatus was used for extraction of plant extracts. ZnO nanoparticles were synthesized by adding 0.082 g ZnO and 10 ml plant extracts to make 1 mM solution in 1000 ml distilled water at room temperature. Confirmation of nanoparticles was done by UV Spectrometry. Efficacy of plant extracts and ZnO nanoparticles was assessed by leaf dip method. The bioassay was performed by applying five concentrations of plant extracts (20, 10, 05, 2.5 and 1.25 percent) and ZnO nanoparticles (800, 400, 200, 100 and 50 ppm). Research trials were conducted under CRD with three replications of each concentration. In petri dishes 10 aphids were released on treated leaves. Dead and live insects were counted by hand lens. Data for mortality was noted after 24, 48, 72 and 96 hours. Results indicated that *A. indica* and *N. tabacum* extracts showed highest mortality 65 and 62% at 20% concentration respectively, after 96 hours. While ZnO nanoparticles of *A. indica* and N. *tabacum* showed 78 and 65% mortality at 800 ppm respectively, after 96 hours against *B. brassicae*. The $LC_{50}$ of *A. indica* and *N. tabacum* mediated ZnO nanoparticles against *B. brassicae* was 386.110 and 271.599 ppm after exposure time of 96 hours respectively. While the $LC_{50}$ of *A. indica* and *N. tabacum* extracts against *B. brassicae* was 5.48525 and 6.26495 ppm after exposure time of 96 hours respectively. It was evaluated that synthesized nanoparticles are more effective to control aphids and have no effect on environment as well as on human health.

**Keywords:** biopesticides, ZnO nanoparticles, plant extracts, pest management, toxicity, aphid