ISSN:2335-8653

18th International Scientific Conference

THE VITAL NATURE SIGN

May 16th-17th, 2024

Kaunas, Lithuania

ABSTRACT BOOK

ISSN:2335-8653
ONLINE ISSN: 2335-8718

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

18th International Scientific Conference "The Vital Nature Sign" Program

Thursday, 16th of May 2024

9.00-9.15 (Time zone GMT+3) **Opening of the conference** ***Welcome speech***

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. Audrius Maruška; Dr. Giedrė Samuolienė***

9.15-9.30 **The Mechanisms of Action of Chromium (III), Iron (III), Nickel (II) and Molybdenum (VI) in Cells**
Sylwia Terpilowska

9.30-9.45 **Highly Efficient Verbascoside Production from Olive (*Olea europea* L. var. Cellina di Nardò) *in Vitro* Cell Cultures**
Enrica Donati, Giovanni Mita, Isabella Nicoletti, Federica Blando

9.45-10.00 **Complex Analysis of Volatiles in Human Breath and Urine as Metabolomics Tool for Non-Targeted Fingerprinting of Biological Samples**
Tomasz Ligor, Magdalena Ligor, Przemysław Adamczyk, Joanna Seifert, R.M. Gadzała-Kopciuch

10.00-10.15 **The Modulation of UVA-induced Phospholipid Profile Changes in Melanocytes and Melanoma Cells by Phytocannabinoids**
Wojciech Łuczaj, Ernest Gieniusz, Iwona Jarocka-Karpowicz, Elżbieta Skrzydlewska

10.15-10.30 **Anti-inflammatory Activity and Amino Acids Profile of Bee Pollen After Fermentation and Enzymatic Hydrolysis**
Vaida Damulienė, Donata Drapanauskaitė, Karolina Barčauskaitė, Vilma Kaškonienė, Paulius Kaškonas, Audrius Maruška

10.30-10.45 ***Coffee break***

10.45-11.00 **Assessment of Environmental Genotoxicity in the Šešupė River Using Mussels (*Unio tumidus*) in 2023**
Miglė Ragelytė, Grita Skujienė

11.00-11.15 **Selection of Animal for More Effective Animal-Assisted Therapy for Persons**
Elena Bartkiene, Vytaute Starkute, Dovile Klupsaite, Egle Zokaityte, Jovita Luksaite, Gintare Zokaityte

11.15-11.30 **Physical and Chemical Characteristics of the Droppings as Sensitive Markers of the Chicken Health Status**
Erika Mozuriene, Ernestas Mockus, Dovile Klupsaite, Vytaute Starkute, Ernesta Tolpeznikaite, Valentas Gruzauskas, Romas Gruzauskas, Agne Paulauskaite - Taraseviciene, Vidas Raudonis, Elena Bartkiene

11.30-11.45 **ESBL-Producing *Escherichia coli* in Poultry Farm: Effect of Vaccination on Bacteria Prevalence**
Beatrice Kasparaviciene, Jurgita Aksomaitiene, Neringa Kasetiene, Mindaugas Malakauskas, Aleksandr Novoslavskij

11.45-12.00 **Preliminary Indications on the Suitability of *Cannabis sativa* L. Plants to Tolerate and Phytoaccumulate Lithium: Implications for Phytotechnologies**
Fabrizio Pietrini, Gianluca D'Onofrio, Laura Passatore, Davide Marzi, Lorenzo Massimi, Maria Luisa Astolfi, Valentina Iannilli, Massimo Zacchini

12.00-12.15 (time zone GMT+3) **The Optimization of Sugar Beet Pulp Degradation by Edible Microscopic Fungi**
Zydrune Gaizauskaite, Renata Zvirdauskiene, Mantas Svazas, Daiva Zadeike

12.15-12.30 **The Evaluation of Fatty Acids Composition in Roe of Freshwater Fishes**
Zhanna Kazangeldina, **Raushan Uazhanova,** Gintarė Zaborskienė, Artūras Stimbirys, Vaida Andrulevičiūtė, Ingrida Sinkevičienė, Ernestas Mockus

12.30-13.00 ***Lunch break***

Flash presentations I. ***Chairpersons: Prof. Dr. Elena Bartkienė; Dr. Nicola Tiso***

13.00 **Modeling Oil-in-Water Dispersed System Utilizing *Sorbus aucuparia L.* Extract: A Study on Physical Properties Assessment**
Augustė Morta Vaitkutė, Agnė Mazurkevičiūtė, Kristina Zymonė

13.07 **Determination of Phenolic Compounds Composition of Water Extracts of *Equisetum arvense* (L.)**
Agnė Siaurusevičiūtė, Kristina Zymonė, Agnė Mazurkevičiūtė

13.14 **New Bipolar Derivatives with Diphenylsulfone or Dibenzophenone as TADF Based Emitters for OLEDs**
Gintare Krucaite, Raminta Beresneviciute, Daiva Tavgeniene, Saulius Grigalevicius, Yi-Ting Chen, Yu-Hsuan Chen, Chih-Hao Chang

13.21 **New Triphenylethylene Fragments Containing Compounds for the Realization of Blue and White Aggregation-induced Emission OLEDs with High Luminance**
Gintare Krucaite, Cheng-Yung Ho, Raminta Beresneviciute, Dovydas Blazevicius, Wei-Han Lin, Jhao-Cheng Lu, Chang-Yu Lin, Saulius Grigalevicius, Chih-Hao Chang

13.28 **Micromycete Contamination Analysis of Peanut (*Juglans regia*), Hazelnut (*Corylus avellana*) and Walnut (*Arachis hypogaea*)**
Greta Tverskytė, Rūta Mickienė, Audrius Maruška

13.35 **Antiplatelet Properties of Fractions Isolated from Various Organs of Sea Buckthorn (*Hippophae rhamnoides*) Measured by Flow Cytometry**
Bartosz Skalski, Joanna Rywaniak, Jerzy Żuchowski, Anna Stochmal, Beata Olas

13.42 **New Bipolar Derivatives Using as Potential TADF Emitters for OLEDs**
Daiva Tavgenienė, Raminta Beresnevičiūtė, Gintare Kručaitė, Sujith Sudheendran Swayamprabha, Jwo-Huei Jou, Saulius Grigalevičius

13.49 **Research of Preparation Techniques for Medical Plant Raw Materials to Optimize Extraction of Biologically Active Compounds**
Domantas Armonavičius, Audrius Maruška

13.56 **Investigation of the Antimicrobial Activity of Fresh and Dried Extracts of Rosehip (*Rosae pseudo-fructus*) and Guelder Rose (*Viburni fructus*) and Rosehip Seed Oil (*Rosae semen oleum*)**
Paula Batutytė, Rūta Mickienė, Audrius Sigitas Maruška

14.03 **OLEDs Achieving High Efficiency Through Naphtalimide-Based Bipolar Derivatives**
Raminta Beresneviciute, Prakalp Gautam, Mangey Ram Nagar, Gintare Krucaite, Daiva Tavgeniene, Jwo-Huei Jou, Saulius Grigalevicius

14.10 **PFOA *vs* PFOS: the Different Responses of Selected Plants to Perfluoroalkyl Compounds (PFAS)**
Lidia Błażałek, Angelika Łacwik, Monika Olczyk, Massimo Zacchini, Fabrizio. Pietrini, Anna Wyrwicka-Drewniak

14.17 time zone (GMT+3) **Bicarbazole-Substituted Benzophenone Derivatives as Blue-Emitting Materials for Organic Light-Emitting Diodes**
Dovydas Blazevicius, Saulius Grigalevicius, Daiva Tavgeniene, Iram Siddiqui, Prakalp Gautam, Jayakumar Jayachandran, Sushanta Lenka, Jwo-Huei Jou

14.25-14.40 Coffee break

Flash presentations II. ***Chairpersons: Dr. Paulius Ruzgys; Dr. Rūta Mickienė***

14.40 **Evaluation of Total Phenolic Content, Total Flavonoid Content and Radical Scavenging Activity in Fractions of *Epilobium angustifolium* L. Extracts and Their i*n Vitro* Anticancer Activity**
Jūratė Bliumaitė, Audrius Sigitas Maruška, Domantas Armonavičius, Mantas Stankevičius, Arvydas Kanopka, Ona Ragažinskienė

14.47 **Investigation of the Electroporation-induced Bystander Effect using Nanosecond and Microsecond Pulses**
Ugnė Borinskytė, Neringa Barauskaitė Šarkinienė, Paulius Ruzgys, Saulius Šatkauskas

14.54 **The Total Phenolic Content and Antioxidant Activity in Buckwheat Honey from Lithuania**
Ernesta Zaksaitė, Mindaugas Liaudanskas, Sonata Trumbeckaitė

15.00 **Enhancing Oral Chemotherapy: Synthesis and Analysis of Lactoferrin-Cisplatin Complexes**
Paweł Fijałkowski, Oleksandra Pryshchepa, Paweł Pomastowski, Katarzyna Rafińska

15.07 **Concentration and Diversity of Micromycete Species in Raw Material of *Aegopodium podagraria* L.**
Ugnė Gabrytė, Rūta Mickienė, Audrius Sigitas Maruška

15.14 **Determination of Volatile Organic Compounds in Refill Fluids of Electronic Cigarettes by Headspace Solid-Phase Micro Extraction and Gas Chromatography–Mass Spectrometry**
Radvilė Gaučaitė, Kristina Bimbiraitė-Survilienė, Mantas Stankevičius

15.22 **Unravelling the Potential of Phenothiazine and Tetraphenylethylene as Emitters and HTMs for Efficient Doping Free Fluorescent and Multiple-Resonance TADF OLEDs**
Melika Ghasemi, Ramakant Gavale, Faizal Khan, Dmytro Volyniuk, Juozas Vidas Grazulevicius, Rajneesh Misra

15.30 **Nutritional Composition of *Tenebrio molitor* Larvae Reared on Substrates Derived from By-products**
Agnė Jankauskienė, Aistė Kabašinskienė

15.37 **The Antioxidant Effect of Silver Nanoparticles on Mouse Hepatoma *MH-22A* Cells and Their Electroporation**
Laura Kairytė, Raminta Rodaitė

15.44 **New Carbazole-Substituted Diphenyl Sulfones as Host Materials for Solution Processed TADF OLEDs**
Mantas Kirstukas, Sujith Sudheendran Swyamprabha, Kiran Kishore Kesavan, Iram Siddiqui, Dovydas Blazevicius, Jayakumar Jayachandran, Marius Eiordimtas, Sandhya Rani Nayak, Mangey Ram Nagar, Rohit Ashok Kumar Yadav, Gintare Krucaite, Sivakumar Vaidyanathan, Saulius Grigalevicius, Jwo-Huei Jou

15.50 **The Influence of Lighting Conditions and Soil Supplementation with *Trichoderma* fungi and Polymers Used in the Food Industry on the Growth of Herbs and the Profile of Biologically Active Compounds in *Thymus vulgaris* and *Thymus serpyllum***
Liliana Kozlowska, Kamila Kulbat-Warycha, Justyna Nawrocka, Dorota Żyżelewicz

15.57 **Evaluation of Antioxidant Activity and Antifungal Potential of Bee Product Extracts**
Aušrinė Venckaitytė, Vilma Kaškonienė, Rūta Mickienė, Audrius Maruška

16.04 **The Microsatellite Analysis of the Genome of *Tripolium pannonicum* (Jacq.) Dobrocz.**
Miłosz Wasicki, Agnieszka Ludwiczak, Małgorzata Szultka-Młyńska

time zone (GMT+3)

16.11 **Effect of Phthalates on Physical Activity in Rat Model**
Rokas Zalūba, Edita Paulikaitė, Justina Alčauskaitė, Violeta Žalgevičienė, Rokas Buišas, Grita Skujienė

16.18 **Evaluation of biodegradation of Plastics by White Rot Fungi**
Cemre Sahin, Nicola Tiso

16.25 **Evaluation of Irreversible Electroporation in Suspension and Monolayer Cell Cultures**
Aras Rafanavičius, Ingrida Šatkauskienė, Saulius Šatkauskas

16.30 **Closing of the Session**

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Friday, 17th of May 2024

Oral presentations. ***Chairpersons: Dr. Nicola Tiso; MSc. Kristina Bimbiraitė-Survilienė***

9.00-9.15 (time zone GMT+3)
Electrochemotherapy: Combining Bleomycin with Conductive Nanoparticles and Nano/Microsecond Pulse Bursts
Barbora Lekešytė, Paulina Malakauskaitė, Eglė Mickevičiūtė, Eivina Radzevičiūtė-Valčiukė, Veronika Malyško-Ptašinskė, Anna Szewczyka, Natalija German, Almira Ramanavičienė, Julita Kulbacka, Vitalij Novickij

9.15-9.30
The Impact of Bipolar Cancellation Phenomenon on Calcium Nano-Electrochemotherapy for B16 F10 Tumor Treatment
Eglė Mickevičiūtė, Eivina Radzevičiūtė-Valčiukė, Veronika Malyško-Ptašinskė, Paulina Malakauskaitė, Barbora Lekešytė, Nina Rembialkowska, Julita Kulbacka, Vitalij Novickij

9.30-9.45
Mitochondrial Depolarization, ATP Depletion and ROS Generation During High Frequency Nanosecond Electroporation
Paulina Malakauskaitė, Augustinas Želvys, Auksė Zinkevičienė, Eglė Mickevičiūtė, Eivina Radzevičiūtė-Valčiukė, Veronika Malyško-Ptašinskė, Barbora Lekešytė, Jurij Novickij, Vytautas Kašėta, Vitalij Novickij

9.45-10.00
Synthesis and Properties of Thianthrene Derivatives for Oxygen Sensing Applications
Lukas Dvylys, Rasa Keruckienė, Matas Gužauskas, Melika Ghasemi, Juozas Vidas Gražulevičius

10.00-10.15
Enhancing Agricultural Productivity: Nanotechnology's Impact on Cereal Crops and Economic Growth
Yanal Ahmad Alkuddsi, Areej Al-Khader, Ibrahim Al-Ghoraibi, Mohammad Almogdad, Saleh Hadi Al-Salem, Muhammad Ali Ali, Adel Al-Manoufi

10.15-10.30
The Effect of CBG and CBD on the Metabolic Response of Fibroblasts Exposed to UVA Radiation
Iwona Jarocka-Karpowicz, Adam Wroński, Arkadiusz Surażyński, Neven Zarkovic, Elżbieta Skrzydlewska

10.30-10.45 ***Coffee break***

10.45-11.00
Functionality and Sensory Properties of Dumplings with Meat Enriched with Omega-3 Fatty Acids Plant Sources
Vaida Andrulevičiūtė, Aistė Šerkšnaitė, Ingrida Sinkevičienė, Artūras Kašauskas, Artūras Stimbirys, Ignė Juknienė, Gintarė Zaborskienė

11.00-11.15
The Possibilities of Utilizing By-Products of Plant-Based Drink Production to Improve the Quality of Wheat Bread
Vita Lele, Vadims Bartkevics, Iveta Pugajeva, Anastasija Borisova, Egle Zokaityte, Vytaute Starkute, Paulina Zavistanaviciute, Dovile Klupsaite, Daiva Zadeike, Grazina Juodeikiene, Elena Bartkiene

11.15-11.30
Characteristics of Wheat Bread Enriched with Non-treated, Milled, and Fermented with *Lactiplantibacillus plantarum* Red Lentils (*Lens culinaris* L.)
Vytaute Starkute, Dovile Klupsaite, Ernestas Mockus, Egle Zokaityte, Emilis Radvila, Daiva Matuzevičiūtė, Kamilė Balynaitė, Arvydas Brėdikis, Gabrielė Ilgūnaitė, Akvilė Juškaitė, Vaneck Cho, Darius Cernauskas, Elena Bartkiene

11.30-11.45 (time zone GMT+3) **The Effect of Light Treatment During Pre- and Post-harvest Storage on Mustard and Kale Microgreens**
Ieva Gudžinskaitė, Kristina Laužikė, Giedrė Samuolienė

11.45-12.00 **Optimization of Antioxidant Extraction and Determination of the Antioxidant Effect of a Variety of Dates with Low Market Value (Mech-Degla)**
Brahmi Fatiha, Guerfi Fatiha, Djouadi Safia, Djoudi Souhila, Boulekbache-Makhlouf Lila

12.00-12.15 **Evaluating Pyrrolizidine Alkaloids in Latvia's Predominant Weeds and their Transfer to Medicinal Plants**
Emīls Francis, Arta Kronberga, Ieva Mežaka, Gundars Skudriņš, Ilva Nakurte

12.15-12.30 **Multifunctional Nutraceutical Combinations Based on Bioconverted Milk Permeate, Apple Juice, Bovine Colostrum, Grapefruit Essential Oil and Berry By-Products**
Emilis Radvila, Vita Lele, Domantas Medonas, Deimante Ivanauskaite, Edikas Vysniauskas, Paulina Sakaite, Greta Kotinskaite, Laurynas Marciulionis, Viktorija Klein, Ieva Tatarunaite, Paulina Gliaubiciute, Monika Zimkaite, Evaldas Kentra, Egle Zokaityte, Vytaute Starkute, Paulina Zavistanaviciute, Dovile Klupsaite, Ernestas Mockus, Ernesta Tolpeznikaite, Vadims Bartkevics, Modestas Ruzauskas, Pranas Viskelis, Elena Bartkiene

12.00-13.00 ***Lunch break***

Flash presentations I. ***Chairpersons: Prof. dr. Arvydas Kanopka; Prof. Dr. Massimo Zacchini***

13.00 **An Evaluation of Food Waste Treated with Physical and Biotechnological Methods**
Ieva Krivaitė, Vilma Kaškonienė, Audrius Maruška

13.07 **Exploring the Physicochemical and Sensory Profiles of Hybrid Spreadable Cheese Analogues Incorporating White Kidney Beans and Cow's Milk**
Ibaratkan Kurbanova, Lina Lauciene, Loreta Serniene, Mukarama Musulmanova

13.14 **Comparison of Antioxidant Activity in Flowers, Leaves and Stems of *Lythrum salicaria* L. by ABTS and FRAP Assays**
Goda Lukoševičiūtė, Audronis Lukošius

13.21 **Comparative Analysis of Physicochemical and Sensory Characteristics of Kurut Drink Based on Different Types of Dairy Whey**
Askar M. Mametjanov, Loreta Serniene, Lina Lauciene, Agne Vasiliauskaite, Kristina Kondrotiene, Mukarama Musulmanova

13.28 **Effect Of Newly Synthesized Compounds on Gene Expression in Cancer Cells**
Aidan Marmion, Inga Pečiulienė

13.35 **Auxin Effect on *in Vitro* Growth of Larch Endophytic Fungus**
Yana Martynova, Jonas Žiauka

13.42 **Sensitivity of *Fusarium* spp. Isolated from Weeds and Crops to Metconazole, Prothioconazole and Tebuconazole**
Neringa Matelionienė, Skaidrė Supronienė, Renata Žvirdauskienė, Gražina Kadžienė, Evelina Zavtrikovienė

13.49 **Sucrose Effect on Shoot and Root Development in the *in Vitro* Culture of Black Pine (*Pinus nigra*) Embryos**
Jokūbas Nagulevičius, Jonas Žiauka

13.56 **Toxic Effects of Di(2-ethylhexyl)phthalate and Dibuthylphthalate on Hippocampus Neuron Density and Region Thickness**
Laurynas Orla, Edita Paulikaitė, Rasa Aukštikalnienė, Urtė Neniškytė, Violeta Žalgevičienė, Grita Skujienė

14.03 time zone GMT+3) **Influence of Room Temperature on the Volatile Compounds of Tea Tree Oil (*Melaleuca alternifolia* Cheel) Studied by Gas Chromatography**
Miglė Kazlauskaitė, Kristina Bimbiraitė-Survilienė, Audrius Maruška

14.10-14.25 Coffee break

Flash presentations. *Chairpersons: Dr. Benas G. Urbonavičius; Dr. Vilma Kaškonienė*

14.25 **Chitosan as a Seed Treatment: Investigating its Antifungal Properties**
Aurelija Ramanauskaitė, Povilas Mulerčikas, Sonata Kazlauskaitė, Vykintas Baublys

14.32 **Honey - a Natural Medicine; Its Antimicrobial Activity**
Dominika Błońska, Bogusław Buszewski

14.39 **Investigating the Effect of Pulse Number on Cell Survival and the Efficiency of Concurrent *vs* Separate Electroporation of Proteins and Small Molecules *in Vitro***
Evaldas Ramanciuškas, Justinas Venckus, Saulius Šatkauskas

14.46 **Optimizing Intracellular Delivery of Proteins *in Vitro* Through Electroporation**
Aditya Rout, Justinas Venckus, Saulius Šatkauskas

14.53 **Calcium Delivery to Breast Cancer Cells via Sonoporation**
Reda Rulinskaitė, Saulius Šatkauskas, Renaldas Raišutis, Martynas Maciulevičius

15.00 **Multi-cropping Influence on Biomass Productivity and Energy-Environmental Indicates**
Jovita Balandaitė, Kęstutis Romaneckas, Rasa Kimbirauskienė, Aušra Sinkevičienė, Austėja Švereikaitė, Ugnius Ginelevičius

15.07 **Quantitative Estimation and Comparison of Vitamin C in *Actinidia* (*Actinidia Arguta* (Siebold & Zucc.) Planch. ex Miq. and *Actinidia Kolomikta* (Rupr. & Maxim.) Maxim.) Berries by HPLC-DAD-MS Method**
Livija Skėrytė, Kristina Zymonė, Mindaugas Marksa, Laima Česonienė

15.14 ***In Vitro* Studies on Plasmid DNA Transfer into Cells**
Gerda Skindaraitė, Ernestas Urbanskas, Saulius Šatkauskas

15.21 **Tonic Compositions Based on Mineral-rich Waters from Ciechocinek**
M. Sugajski, M. Buszewska-Forajta, B. Buszewski

15.28 **Development of an Open-Source Ozonometer for Citizen Scientists**
Vladas Šatas, Benas Gabrielis Urbonavičius

15.35 **Determination of Volatile Compounds in Food Supplements by HS-SPME Coupled with GC-MS**
Laura Šimaitytė, Kristina Bimbiraitė-Survilienė, Juozas Šiurkus, Audrius Maruška

15.42 **The Effect of DNMT1 Inhibitors on Cell Viability**
Arnas Treimakas, Eglė Jakubauskienė

15.49 **The Total Phenolic Content and Antioxidant Activity of Invasive *Amelanchier* × *Spicata* (Lam.) Koch Leaves: Variation from Different Habitats Lithuanian**
Sandra Saunoriūtė, Lina Raudonė

15.55 **Closing of the Conference**

ISSN:2335-8653
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ORAL PRESENTATIONS

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FLASH PRESENTATIONS

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ORAL PRESENTATIONS

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Functionality and Sensory Properties of Dumplings with Meat Enriched with Omega-3 Fatty Acids Plant Sources

Vaida Andrulevičiūtė, Aistė Šerkšnaitė, Ingrida Sinkevičienė, Artūras Kašauskas, Artūras Stimbirys, Ignė Juknienė, Gintarė Zaborskienė

Lithuanian University of Health Sciences, Tilžės g. 18, Kaunas, Lithuania

Corresponding author: vaida.andruleviciute@lsmu.lt

Abstract

Cardiovascular disease remains the leading cause of mortality worldwide. The consumption of functional foods as an integral part of prevention is positively recognized. Specifically, Functional food product development is one of the main tendencies in the present food industry. The aim of this study was to develop recipes for dumplings with meat filling, enriched with omega 3 fatty acid additives (flaxseed, hemp, and chia seeds) and to evaluate the sensory properties and the functionality (the ratio of omega 6 and omega 3 fatty acids, atherogenicity (AI) and thrombogenicity (TI) indices) of the developed products.

The original recipes of dumplings with meat filing enriched with ground and unground additives of flaxseed, hemp and chia seeds were prepared. According to the sensory properties results, the fatty acids (FA) profiles of 5 samples were determined by gas chromatography, and the ratio of omega 6, and omega 3 fatty acids, the AI and TI indices were calculated. Data were analysed using the SPSS 26.0 statistical package.

The most acceptable dumplings were found to be supplemented with whole hemp seeds, while the dumplings with the addition of chia seeds were declared unacceptable and unsuitable for production and further research. The saturated fatty acids content in products varies from 34.21±0.01% of total fatty acids content (TFA) enriched with grounded ham seeds to 44.08±0.01% of TFA enriched with flaxseed, prevailing palmitic (C16:0) and stearic (C18:0) acids. From monounsaturated FA Oleic acid (C18:1) was the most predominant in the products ($p<0.05$) with levels ranging from 23.20±0.01% of TFA with flaxseeds to 33.78±0.01% of TFA with hemp seeds. Among the polyunsaturated FA, linoleic acid (C18:2) was predominant in the dumplings, the levels ranged from 11.46±0.01% of TFA with hemp seeds to 22.08±0.01 % with grounded hemp seeds. The samples supplemented with flaxseed were with the best ratio of omega 6 and omega 3 fatty acids (1.51:1). The lowest AI index is in dumplings with ground hemp (0.28), while TI in products with ground flaxseed (0.26). These dumplings are recognized as the healthiest. Comparing the addition of ground and unground flaxseed samples, ground seeds significantly ($p<0.05$) reduced the TI indices by 50%.

Keywords: functional food, dumplings, flaxseed, hemp, chia seeds.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

Selection of Animal for More Effective Animal-Assisted Therapy for Persons

Elena Bartkiene[1,2], Vytaute Starkute[1,2], Dovile Klupsaite[1], Egle Zokaityte[1], Jovita Luksaite[1], Gintare Zokaityte[1]

[1]Institute of Animal Rearing Technologies, Faculty of Animal Sciences, Lithuanian University of Health Sciences, Tilzes Str. 18, LT-47181, LT-44307 Kaunas, Lithuania
[2]Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Tilzes Str. 18, LT-47181 Kaunas, Lithuania

Corresponding author: elena.bartkiene@lsmu.lt

Abstract

It is known that human (HU) and animal (AN) interaction can have a positive influence on HU wellness. However, only recently have these interactions started to be investigated on a scientific level [1,2]. Despite that, animal-assisted therapy (AAT) has become very popular nowadays, and its purpose is the tendential inclusion of an assisted AN in a HU treatment plan to realize specific aims [3]. We hypothesized that AAT effectiveness could be improved by the selection of AN according to the emotions by different AN for HU. To analyze the emotions induced for HU, additionally to the Likert scale, the FaceReader (FR) technique was applied (Noldus Information Technology, Wageningen, The Netherlands). The pilot study was conducted with 18–64 years old adult HU participants. This was the 1st study, where the FR was used to the emotions induced in HU by AN evaluation, with the aim of improving the AAT methodology. The obtained results could be the 1st step in lowering stressful situations for HU as well as for AN during AAT. During the experiment, typical images of different AN species (pigs, sheep, horses, dogs, cats) and different breeds of those species were used. Also, the main traits of character of the AN for the tested HU were presented. It was established that the AN species is significant on the intensity of the 'neutral' and 'happy' emotions induced in HU. Also, the AN breed was significant on 'happy' intensity. It was found that some of the experiment participants' the highest intensity of 'happy' emotion and the highest valence were different for different AN species and breeds. Finally, the applied strategy could lead to personalized AN selection for AAT for HU, by using both their highest expression of the 'happy' as well as valence, as both are related with a positive emotional state. This study showed that the FR and applied methodology could be used as a personalized strategy for the improvement of AAT. Additionally, this methodology can be suggested for HU to select a pet. However, it must be pointed out, that this methodology should be more standardized, e.g., the cards of AN species and breeds should be standardized, and more HU from different groups (age, persons with different disorders, etc.) should be involved.

Keywords: animal-assisted therapy, emotions, personalized strategy.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Optimization of Antioxidant Extraction and Determination of the Antioxidant Effect of a Variety of Dates with Low Market Value (Mech-Degla)

Brahmi Fatiha, Guerfi Fatiha, Djouadi Safia, Djoudi Souhila, Boulekbache-Makhlouf Lila

Laboratory of biomathematics, biochemistry, biophysics and science, Department of Food Sciences, Faculty of Nature and Life Sciences, University of Bejaia, 06000, Bejaia, Algeria

Corresponding author: fatiha.brahmi@univ-bejaia.dz

Abstract

The date fruit of the date palm (*Phoenix dactylifera* L.) has always been since immemorial time an important part of the diet for both humans and animals [1]. Mech Degla is one of the varieties that have the ability to be turned into powder by drying. This study is conducted with the aim of maximizing the extraction of antioxidants (phenolic compounds) from the pulp of a variety of this date with low market value (Mech-Degla). Therefore, an optimization of the conditions for the extraction of total polyphenols (TPP) from powders of this variety by maceration is carried out. Then, the contents of TPP, flavonoids, flavonols, tannins and antioxidant activity were evaluated by spectrophotometric methods according to our previous study [2, 3]. for the extract obtained by adopting the optimal parameters. the maximum TPP content of the Mech-Degla pulp is 343.8 ± 37.33 mg Gallic Acid Equivalent/100 g dry matter is obtained with 40% (*v/v*) ethanol, a time of 120 min and a ratio of 1/50 g/mL and the contents of flavonoids, flavonols and tannins are also higher in the extract of this date variety. From the antioxidant tests we deduced that the Mech Degla date pulp also has a high antioxidant power using the DPPH and phosphomolybdate tests, respectively.

Keywords: Mech-Degla, extraction, optimization, phenolic compounds, antioxidant activity.

Acknowledgments: The authors would like to thank everyone who contributed to the completion of this work.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Highly Efficient Verbascoside Production from Olive (*Olea europea* L. var. Cellina di Nardò) *In Vitro* Cell Cultures

Enrica Donati[1], Giovanni Mita[2], Isabella Nicoletti[1], Federica Blando[2]

[1]Institute for Biological System (ISB), National Research Council (CNR), Territorial Research Area Rome 1, Strada Provinciale 35d, 9 – 00010 Montelibretti (Rome), Italy
[2]Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Lecce Territorial unit, Via Provinciale Lecce-Monteroni 73100 Lecce, Italy
Corresponding author: enrica.donati@cnr.it

Abstract

Olive (*Olea europea* L.) is a typical tree of the Mediterranean vegetation and one of the most important trees for the agricultural economy of the Mediterranean region. It has attracted considerable attention as a natural source of health-promoting compounds, useful for the food and pharmaceutical industries [1]. Among them is verbascoside, a water-soluble phenylethanoid compound, known for its antioxidant, antibacterial, antiviral, anti-inflammatory as well as antitumor activities [2]. *In vitro* plant cell culture is a well-established and sustainable technology to produce plant bioactive compounds [3]. Studies on olive *in vitro* cultures are limited because this specie is considered recalcitrant to the biotechnological techniques. In this regard, the results of establishing "Cellina di Nardò" ("CdN") olive *in vitro* cell culture, characterized for its growth and metabolic features, are reported. Polyphenolic profile of the *in vitro* "CdN" olive cells extract was analysed by UPLC-ESI-MS. Chromatographic analysis revealed that the extract contained almost exclusively verbascoside and this notable verbascoside production was in accordance with a very high antioxidant capacity of the extract. This ability of olive cell cultures to produce high amount of verbascoside is a very promising feature that could be exploited for scaling up and producing the metabolite of interest, often found in plants in very limited quantities.

Keywords: plant cell suspension culture, olive, verbascoside, UPLC-ESI-MS.

Acknowledgments: This work was funded in part by CNR project FOE-2021 DBA.AD005.225, the NUTRAGE project.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Effect of CBG and CBD on the Metabolic Response of Fibroblasts Exposed to UVA Radiation

Iwona Jarocka-Karpowicz[1], Adam Wroński[2], Arkadiusz Surażyński[3], Neven Zarkovic[4], Elżbieta Skrzydlewska[1]

[1]Department of Analytical Chemistry, Medical University of Bialystok, A. Mickiewicza 2D, 15-222 Bialystok, Poland
[2]Dermatological Specialized Center "DERMAL" NZOZ in Bialystok, Nowy Swiat 17/5, 15-453 Bialystok, Poland
[3]Department of Medicinal Chemistry, Medical University of Bialystok, Kilinskiego 1, 15-069 Bialystok, Poland
[4]Laboratory for Oxidative Stress, Rudjer Boskovic Institute, Bijenicka 54, HR-10000, Zagreb, Croatia

Corresponding author: iwona.jarocka-karpowicz@umb.edu.pl

Abstract

The skin, as the most external organ, is exposed every day to sunlight, the main component of which is UVA radiation, which disrupts the metabolism of both epidermal and dermal cells, causing, among others, oxidative stress and inflammation. Therefore, there is still a need for bioactive compounds that would limit the effects of excessive exposure to UVA radiation without causing undesirable side effects. The group of such compounds includes phytocannabinoids found in *Cannabis Sativa* L, mainly CBG and CBD, which, as confirmed by literature data, can be used in relation to various metabolic disorders, including those affecting the skin. The aim of this study was to analyze *in vitro* effects of the CBG and CBD on fibroblasts, which share physicochemical similarities but differ in biological effects. Furthermore, combined use of both compounds (CBG+CBD) was analyzed aiming to increase their effectiveness in counteracting altered redox balance/inflammatory signaling and metabolism of UVA-irradiated human skin fibroblasts. The obtained results indicate that the effects of CBG and CBD modulating redox balance might indeed be enhanced when both phytocannabinoids are used simultaneously. These effects include reduction of NOX activity, ROS levels and modification of antioxidant elements protecting phospholipids, especially the Trx-dependent system. The reduction of UVA-induced lipid peroxidation and protein modifications was confirmed by lower levels of 4-HNE-protein adducts, of protein carbonyl groups and by recovery of collagen expression. Moreover, the use of CBG and CBG, especially together, also leads to the reduction in the activity of enzymes involved in the synthesis of eicosanoids. Modification of antioxidant signaling (Nrf2/HO-1) by CBG+CBD was associated with reduced pro-inflammatory signaling (NFκB/TNFα). The metabolic responses of fibroblasts to the action of phytocannabinoids, applied both to control cells as well as after exposure to UVA radiation, indicate the beneficial protective and regenerative effects of these natural compounds, which suggests their possible use, especially to limit the harmful effects of UVA on skin cells.

Keywords: UVA radiation, skin fibroblasts, CBG, CBD, lipid modification, antioxidants, inflammation.

Acknowledgments: The authors are thankful to the COST Action EpiLipidNET, CA19105—Pan-European Network in Lipidomics and EpiLipidomics for possible authors international cooperation.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Possibilities of Utilizing By-Products of Plant-Based Drink Production to Improve the Quality of Wheat Bread

Vita Lele[1,2], Vadims Bartkevics[3,4], Iveta Pugajeva[4], Anastasija Borisova[4], Egle Zokaityte[1,2], Vytaute Starkute[1,2], Paulina Zavistanaviciute[1,2], Dovile Klupsaite[2], Daiva Zadeike[5], Grazina Juodeikiene[5], Elena Bartkiene[1,2]

[1]Department of Food Safety and Quality, Faculty of Veterinary, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Faculty of Animal Sciences, Institute of Animal Rearing Technologies, Lithuanian University of Health Sciences, Kaunas, Lithuania
[3]Centre of Food Chemistry, University of Latvia, Riga, Latvia,
[4]Institute of Food Safety, Animal Health and Environment "BIOR," Riga, Latvia
[5]Department of Food Science and Technology, Kaunas University of Technology, Kaunas, Lithuania
Corresponding author: vita.lele@lsmu.lt

Abstract

By-products that remain after plant-based beverages' production is a global issue. In pursuit of the principle of sustainable production and/or waste-free production, the processing and utilization of by-products of food industry products in technology of other food industry production is an aim. Before carrying out the experiment it was expected that adding in fermented or treated with ultrasound by-products of plant-based beverages production would improve the overall quality and the end-product's added value would also increase. Therefore, in this study preparation by-products of almond, oat and coconut drinks that were treated with ultrasound (37 kHz) or fermented with *Lacticaseibacillus casei* LBCLUHS210 were employed in varying quantities (5, 10, 15, and 20%) in wheat bread recipe. Acrylamide content and other quality parameters as well as bread texture, specific volume, hardness during the storage, overall acceptability and microbiological parameters in the wheat bread were evaluated. Wheat bread which was prepared with oat drink preparation by-product contained the least acrylamide. On average it was found 14.7±0.9 μg/kg. Among the fermented samples, 12-h-fermented almond and oat, as well as 24-h-fermented coconut drinks preparation by - products (pH values of 2.94±0.3, 2.41±0.1, and 4.50±0.4, respectively; total enterobacteria and mold/yeast were not found) were selected for wheat bread production. By-products used, the quality of the by-products and also the treatment of by-products determined/had an effect on the dough and bread quality parameters in most cases. The highest overall acceptability of the wheat bread prepared with 20% fermented almond drink preparation by-product, 15% fermented oat drink preparation by-product, and 15% ultrasonicated oat drink preparation by-product was established. After 96 h of storage, the lowest hardness (on average, 1.2±0.2 mJ) of the breads prepared with 5% fermented almond drink preparation by-product, coconut drink preparation by-product, and oat drink preparation by-product and ultrasonicated coconut drink preparation by-product was found. Finally, 15% fermented oat drink preparation by-product could be safely used for wheat bread preparation because the prepared bread showed high overall acceptability, as well as low acrylamide content.

Keywords: by-products, wheat bread quality, lactic acid bacteria, acrylamide.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

Complex Analysis of Volatiles in Human Breath and Urine as Metabolomics Tool for Non-Targeted Fingerprinting of Biological Samples

Tomasz Ligor[1], Magdalena Ligor[1], Przemysław Adamczyk[2], Joanna Seifert[3], R.M. Gadzała-Kopciuch[1]

[1]Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, 7 Gagarina Str., 87-100 Torun, Poland
[2]Department of General and Oncologic Urology, Nicolaus Copernicus Hospital in Torun, 87-100 Toruń, Poland
[3]Department of Nephrology, Diabetology and Internal Medicine, Nicolaus Copernicus University, Rydygier Hospital, 87-100 Toruń, Poland

Corresponding author: tligor@umk.pl

Abstract

Volatiles produced by human body cells can be an important source of information about health or possible disease stages. Some diseases contribute to the change of the individual component concentrations in exhaled breath. Some biomarkers of diseases may be volatile substances usually not found in the exhaled breath of a healthy person. Currently, the great interest are methods enabling for the early detection of respiratory diseases based on volatile organic compounds (VOCs). The analysis of VOCs in the breath is of great importance for detection of early stage of diseases and metabolic processes. Analysis of exhaled breath and urine samples for specific VOCs may allow for early detection of lung diseases. Collecting exhaled air and urine samples is painless, non-invasive and safe, which is particularly important for the popularization of such tests. The breath analysis might be useful as fast diagnostic method. It is a non-invasive and painlessness procedure, and its sampling does not require skilled medical staff. Exhaled gas analyses might be complementary method to classical method i.e. biopsy, blood**,** and urine sampling. However, breath analysis presents many problems such as: exhaled compounds are present at very low concentrations (typically ppb, ppt level) and are found in a matrix where interference from numerous exogenous compounds is expected. However, the complexity of sample preparation, extraction and identification of potential disease markers means that volatile biomarkers are not currently used. The project involved multi-directional research on VOCs identification, quantitative analysis and sample preparation methods. VOCs studies were conducted using gas chromatography and mass spectrometry. SPME (Solid Phase Microextraction) and NTD (Needle Trap Device) methods were used for the extraction. Gas chromatography and mass spectrometry (GC/MS) is the most useful technique for identification of VOCs in breath. In current study breath samples were collected from patients with lung cancer, chronic obstructive pulmonary disease and asthma. A control group was also recruited. Statistical methods were used to select potential biomarkers. An analytical procedure generally involved few steps: sampling, preconcentration, separation, detection and identification.

Keywords: human breath, urine samples, VOCs, extraction, GC/MS.

Acknowledgments: This work was financed by The National Centre for Research and Development (Warsaw, Poland) in frame of Polish–Turkish bilateral project “A comparative study of volatile organic compound biomarkers in breath and urine samples collected from Polish and Turkish communities for monitoring of various respiratory diseases” (POLTUR2/4/2018).
T. Ligor, M. Ligor and R.M. Gadzała-Kopciuch are members of Toruń Center of Excellence “Towards Personalized Medicine” operating under Excellence Initiative-Research University.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Modulation of UVA-induced Phospholipid Profile Changes in Melanocytes and Melanoma Cells by Phytocannabinoids

Wojciech Łuczaj, Ernest Gieniusz, Iwona Jarocka-Karpowicz, Elżbieta Skrzydlewska

Department of Inorganic and Analytical Chemistry, Medical University of Bialystok, Poland
Corresponding author: wojciech.luczaj@umb.edu.pl

Abstract

Genetic conditions, along with exposure to harmful external elements such as physical and chemical stressors like UV radiation, are thought to induce structural and functional alterations in skin cell components, particularly epidermal cells. These changes may ultimately contribute to the development of cancerous transformations. UV radiation has been demonstrated to induce mutations in melanocytes, which can result in the formation of melanoma, known as the most aggressive type of skin cancer. Phospholipids as key structural components of cell membranes are involved in carcinogenesis since they play an important role in signal transduction. Given the association between alterations in lipid composition and various cancers, including skin cancer, our objective was to assess how two phytocannabinoids, cannabidiol (CBD) and cannabigerol (CBG), influence changes in phospholipid and ceramide (CER) profiles induced by UVA irradiation in human melanocytes and melanoma. Our findings revealed that UVA radiation led to a notable increase in phosphatidylcholine (PC), phosphatidylinositol (PI) and sphingomyelin (SM) species and decrease of CERs content in both types of cells, while the increase in ether-linked phosphatidylethanolamines (PEo) was specifically noted in melanocytes. When UVA-irradiated melanocytes or melanoma cells were exposed to CBD and/or CBG, there was a notable decrease in the relative content of PC, PI, and SM species. However, this effect was more prominent in melanoma cells. Interestingly, CBD treatment resulted in a reduction of PEo content solely in UVA-irradiated melanocytes, not in melanoma cells, whereas CBG led to additional upregulation of PEo species. Furthermore, the combination of CBD and CBG resulted in distinct alterations in the relative contents of CER and SM species in both irradiated and non-irradiated melanoma cells. These findings highlight the divergent responses of melanocytes and melanoma cells to phytocannabinoid action, indicating their influence on distinct metabolic pathways in both cancer and physiological conditions. Our findings also indicated that the potential of the investigated phytocannabinoids to counteract alterations in the phospholipid profile of UVA-irradiated melanoma cells, hich could potentially contribute to tumor progression, is restricted.

Keywords: cannabidiol, cannabigerol, melanocytes, melanoma, phospholipids.

Acknowledgments: This study was financed by the Ministry of Science and Education in Poland.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

Physical and Chemical Characteristics of the Droppings as Sensitive Markers of the Chicken Health Status

Erika Mozuriene[1], Ernestas Mockus[1], Dovile Klupsaite[1], Vytaute Starkute[1,2], Ernesta Tolpeznikaite[1], Valentas Gruzauskas[3], Romas Gruzauskas[3], Agne Paulauskaite - Taraseviciene[3], Vidas Raudonis[3], Elena Bartkiene [1,2]

[1]Institute of Animal Rearing Technologies, Faculty of Animal Sciences, Lithuanian University of Health Sciences, Mickeviciaus Str. 9, LT-44307 Kaunas, Lithuania
[2]Department of Food Safety and Quality, Faculty of Veterinary Medicine, Lithuanian University of Health Sciences, Mickeviciaus Str. 9, LT-44307 Kaunas, Lithuania
[3]Artificial Intelligence Centre, Kaunas University of Technology, K. Barsausko 59, 51423 Kaunas, Lithuania
Corresponding author: erika.mozuriene@lsmuni.lt

Abstract

Broiler production is an important part of the poultry industry and accounts for a large proportion of the meat products in the global market [1]. At the same time, with the increase in demand for livestock products, broiler production is intensified which makes disease control more challenging [2]. The outbreak of chicken disease has been a major concern around the world, as the poultry industry supplies a significant portion of the global protein needs [3]. Characteristics of poultry droppings can reflect the health status of chickens. However, the existing literature lacks information on physical chemical parameters of droppings, which could be useful in development of practical and reliable diagnostic tools for monitoring of chickens' welfare status.

The aim of this study was to analyse the physical and chemical characteristics of chicken droppings, which were collected during different age periods and classified by visual inspection into normal (ND) and abnormal (AD). Significant differences were found in the texture, pH, and dry matter (DM), fatty acids (FAs), short-chain fatty acid (SCFA), and volatile compounds (VC) between tested dropping groups ($p \leq 0.05$). The age period of chicken had a significant influence on color coordinates, texture, pH, DM, and SCFA contents in ND and AD as well as on all FAs content in ND ($p \leq 0.05$). ND had harder texture, lower values of a* and b* color coordinates, higher DM content, higher level of linoleic FA, and lower level of α-linolenic FA than AD in each age period ($p \leq 0.05$). The predominant SCFA was acetic acid, which content was significantly lower in ND compared to AD. The content of alcohols and organic acids were the highest in most of ND in different age periods, while ketones dominated in ND and AD.

In conclusion, the majority of the tested dropping characteristics were influenced by age period. While certain characteristics demonstrate differences between ND and AD, a likely broader range of droppings is required to provide more distinct trends regarding the distribution of characteristics across different droppings.

Keywords: broilers, droppings, color parameters, short-chain fatty acids, volatile compounds.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Characteristics of Wheat Bread Enriched with Non-Treated, Milled, and Fermented with *Lactiplantibacillus plantarum* Red Lentils (*Lens culinaris* L.)

Vytaute Starkute[1,2], Dovile Klupsaite[1], Ernestas Mockus[1], Egle Zokaityte[1], Emilis Radvila[2], Daiva Matuzevičiūtė[2], Kamilė Balynaitė[2], Arvydas Brėdikis[2], Gabrielė Ilgūnaitė[2], Akvilė Juškaitė[2], Vaneck Cho[2], Darius Cernauskas[3], Elena Bartkiene[1,2]

[1]Institute of Animal Rearing Technologies, Faculty of Animal Sciences, Lithuanian University of Health Sciences, Tilzes Str. 18, LT-47181, LT-44307 Kaunas, Lithuania

[2]Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Tilzes Str. 18, LT-47181 Kaunas, Lithuania

[3]Food Institute, Kaunas University of Technology, Radvilenu Road 19, LT-50254 Kaunas, Lithuania

Corresponding author: elena.bartkiene@lsmu.lt

Abstract

The present study aimed to investigate the impact of non-treated, milled, and fermented with *Lactiplantibacillus plantarum* red lentils (*Lens culinaris* L.) on wheat bread (WB) quality and safety parameters. The WB formula consisted of 1.0 kg of wheat flour, 1.5% salt, 3% instant yeast, and 1000 mL water. The tested WBs were prepared by adding to the main recipe 5, 10, 15, 20, and 25% of non-treated and pre-treated lentils. In total, 21 group of WB were prepared and tested (control WB; 5 groups with non-fermented non-milled lentils; 5 groups with non-fermented milled lentils; 5 groups with fermented non-milled lentils; and 5 groups with fermented milled lentils). The latter parameters of the WB were analysed: specific volume, shape coefficient, porosity, moisture content, mass loss after baking, crust and crumb chromaticity characteristics, texture hardness, volatile compounds (VC), and acrylamide (AA) concentration. It was established, that the analysed factors 'quantity of lentils', 'fermentation of lentils', and 'milling of lentils' were significant on most of WB characteristics: on specific volume all three factors $p<0.001$; on shape coefficient $p<0.001$, $p=0.010$, $p<0.001$, respectively; 'quantity of lentils' and 'milling of lentils' on porosity $p=0.035$ and $p=0.003$, respectively; on moisture content all three factors $p<0.001$; on mass loss after baking $p=0.002$, $p<0.001$, $p<0.001$, respectively (except 'fermentation of lentils' was not significant on WB porosity). Moderate and strong positive correlations were found between WB specific volume and porosity ($r=0.447$, $p<0.001$) as well as between WB specific volume and mass loss after baking ($r=0.691$, $p<0.001$). The highest concentration of AA was found in WB prepared with 25% of non-milled lentils (44.0 µg/kg). Negative moderate correlation was found between AA and moisture content in WB ($r=-0.551$, $p<0.001$). Weak and moderate negative correlations were found between AA content and WB crust and crumb lightness (L*) ($r=-0.386$, $p=0.002$ and $r=-0.445$, $p<0.001$, respectively). Positive moderate correlations were found between WB VC 2-ethyl-6-methylpyrazine; 2-ethyl-3-methylpyrazine; acetic acid and AA concentration in WB ($r=0.407$, $p<0.001$; $r=0.449$, $p<0.001$; and $r=0.426$, $p<0.001$, respectively). Finally, this study showed that most analysed factors and their interactions were significant on most of the WB quality parameters as well as on AA concentration in WB (on AA concentration in WB $p<0.001$). This study showed, that not higher than 10% of lentils for WB enrichment can be recommended (regardless of how it is pre-treated), because higher content of lentils in WB formula leads to higher concentration of AA formation.

Keywords: lentils, wheat bread, acrylamide, fermentation.

Acknowledgments: This work is based upon the work from COST Action CA21149 ACRYRED—Reducing acrylamide exposure of consumers by a cereals supply-chain approach targeting asparagine.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Mechanisms of Action of Chromium(III), Iron(III), Nickel(II) and Molybdenum(VI) in Cells

Sylwia Terpilowska

Jan Kochanowski University, Collegium Medicum, Department of Surgical Medicine with the Laboratory of Medical Genetics, IX Wieków Kielc 19A Av., 25-317 Kielce, Poland

Corresponding author: sylter1@wp.pl

Abstract

Chromium, iron, nickel and molybdenum belong to the group of trace elements. The human and animal organisms need them only in small amounts. The levels of microelements (trace elements) in the organism are below 1 μg/g of wet tissue. Despite the low concentration of microelements in body fluids and tissues, trace elements are involved in many cellular pathways. The research presented in these studies aimed to investigate the metabolism of chromium(III), iron(III), nickel(II) and molybdenum(VI). In addition, the nature of the interaction between chromium(III) and other elements was investigated. This is a very important problem in modern medicine. In recent years, there has been an increase in the intake of vitamin and mineral supplements and dietary supplements. Biomaterials containing these micronutrients are also used on a wide scale in medicine. In human and animal organisms there may be exceeded safe concentrations of these elements leading to unknown interactions.

Investigations carried out demonstrated the possibility of apoptosis induction in cells via an extrinsic (nickel) and intrinsic (chromium, nickel, iron and molybdenum) pathway. The available literature pointed to the involvement of ROS in the induction of apoptosis. The present study showed that not only ROS are responsible for the induction of apoptosis, but also the impairment of antioxidant enzymes. ROS affect mitochondria, causing mitochondrial damage, release of cytochrome *c* and activation of caspases: 9, 3, 6 and 7. In addition, an increase in Mg^{2+} ion concentration causes a decrease in Bcl-2 expression and an increase in Bax expression, which affects cytochrome *c* release. In addition, apoptosis is induced by damage to RE, which leads to the release of Ca^{2+} ions into the cytoplasm. The excess of these ions negatively affects cell cycle regulation and its inhibition in the G1/S and G2/M phases and during division spindle formation. Damage to RE also causes the formation of lipid droplets in the cytoplasm and activation of caspase 12 leading to apoptosis. In addition, an imbalance of Na^{+}/K^{+} ions leads to disruption of the resting cell membrane potential and vesicle formation on the membrane surface. Regardless of their ability to induce apoptosis by altering the metabolism of the cell membrane, mitochondria and RE, the elements themselves and through oxygen free radicals interact with the cell's DNA to cause damage and mutation. Moreover, chromium(III) and iron(III) show synergistic effects. Chromium(III) and molybdenum(VI) or chromium(III) and nickel (II) show antagonistic effects – chromium(III) protects from nickel(II) or molybdenum (VI) toxicity

Keywords: chromium, iron, molybdenum, nickel.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

Preliminary Indications on the Suitability of *Cannabis sativa* L. Plants to Tolerate and Phytoaccumulate Lithium: Implications for Phytotechnologies

Fabrizio Pietrini[1], Gianluca D'Onofrio[1], Laura Passatore[1], Davide Marzi[1], Lorenzo Massimi[2], Maria Luisa Astolfi[3], Valentina Iannilli[4], Massimo Zacchini[1]

[1]Research Institute on Terrestrial Ecosystems (IRET), National Research Council of Italy (CNR), via Salaria km 29.300, 00015 Monterotondo Scalo (Rome), Italy
[2]Department of Environmental Biology, Sapienza University of Rome, P.le Aldo Moro 5, 00185 (Rome), Italy
[3]Department of Chemistry, Sapienza University of Rome, P.le Aldo Moro 5, 00185, Rome, Italy
[4]ENEA, Italian National Agency for New Technologies, Energy and Sustainable Economic Development C.R. Casaccia, Via Anguillarese, 301, 00123 Roma, Italy

Corresponding author: massimo.zacchini@cnr.it

Abstract

Lithium (Li), an alkali metal and the least dense metal (0.534 g/cm^3), has received particular attention due to its increasing demand for the production of Li-ion batteries. Concerns have been raised about the impact on biota of Li mining residues and improper disposal of Li containing materials [1]. Measures must be taken to mitigate these negative effects, and nature-based technologies could be of interest for their environmental and economic sustainability. Plant-lithium interactions have recently been studied to evaluate both the metal toxicity [2] and the plant ability for phytoaccumulation (phytomining). To get an insight on both these issues, a preliminary ecotoxicity assay on *Lepidium sativum* L. was carried out to choose the Li concentrations to be tested. Then, two proofs of concept under lab conditions were conducted, targeting the suitability of *Cannabis sativa* L. to tolerate and bioaccumulate Li added in the growth medium. For this purpose, *in vitro* and hydroponic trials were carried out. Regarding the former, micro-shoots of *C. sativa* were exposed for two weeks, under controlled microenvironmental conditions, to 0 (control), 50 mg/L, 150 mg/L, 300 mg/L LiCl supplied by the Murashige and Skoog growth medium. In the latter, three-week-old plantlets were grown for 10 days in growth chamber in nutrient solution containing 0 (control), 50 mg/L, 150 mg/L, 300 mg/L LiCl. At the end of the two experiments, plants were analysed for morpho-physiological and ionomic traits by evaluating biometric parameters, pigment content, photosynthetic performances (chlorophyll fluorescence imaging analysis), macro and micronutrients concentration and Li accumulation in the organs. Results highlighted the ability of hemp plants to absorb, accumulate and translocate Li while tolerating it at the lowest concentration tested. Alteration of photosynthetic performances and ion content were observed at the highest Li concentration assayed. These preliminary investigations suggest that hemp plants have potential for use in remediation and metal biorecovery strategies in Li-contaminated substrates. However, further trials in real conditions are necessary to confirm these findings.

Keywords: heavy metals, hemp, metal toxicity, photosynthesis, phytoremediation, plant physiology.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Optimization of Sugar Beet Pulp Degradation by Edible Microscopic Fungi

Zydrune Gaizauskaite[1,2], Renata Zvirdauskiene[1], Mantas Svazas[3], Daiva Zadeike[1]

[1]Department of Food Science and Technology, Kaunas University of Technology, Kaunas 50254, Lithuania
[2]Food Institute, Kaunas University of Technology, Kaunas 50254, Lithuania
[3]Department of Applied Economics, Finance and Accounting, Agriculture Academy of Vytautas Magnus University, Kaunas 53361, Lithuania

Corresponding author: daiva.zadeike@ktu.lt

Abstract

The degradation of lignocellulosic biomass is important for its further conversion to value-added bioproducts. Efficient low-cost substrate and fungal strains are the key points addressed for enhanced saccharification and enzyme production at an industrial scale. In this study, ten fungal strains were tested for their cellulose degrading activity and sugar production through hydrolysis of sugar beet pulp (SBP). The production of reducing sugars (RS) was investigated at various solid/water (*s/w*) ratios (1:10–1:20), different incubation temperatures (20–35 ºC), and processing time (60–168 h). The *Aspergillus niger* CCF 3264 and *Penicillium oxalicum* CCF 3438 strains showed the most effective carboxymethyl cellulose (CMC) degrading activity and also sugar recovery (15.9–44.8%) from SBP biomass during the one-factor experiments. The *central composite design (CCD)* and response surface method (RSM) *were used* for the optimization of processing parameters for the cellulase activity and reducing sugar production in the SBP substrate. Mathematical data evaluation indicated that the highest RS concentration (39.15 g/100 g d.w.) and cellulolytic activity (6.67 U/g d.w.) could be achieved by using *A. niger* for the degradation of SBP at 26 ºC temperature with 136 h of processing time and 1:15 *s/w* ratio. This study demonstrates the potential of fungal degradation to be used for the SBP biorefining. The biomass after fungal hydrolysis can be used as a source of carbohydrates for yeast fermentation or microbial cultivation.

Keywords: sugar beet pulp, filamentous fungi, degradation, cellulase activity, process optimisation, biorefinery.

Acknowledgments: The authors would like to acknowledge the Department of Botany of Charles University (Prague, Czech Republic) for the kindly provided fungal strains for the PhD research purposes.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

Enhancing Agricultural Productivity: Nanotechnology's Impact on Cereal Crops and Economic Growth

Yanal Ahmad Alkuddsi[1], Areej Al-Khader[2], Ibrahim Al-Ghoraibi[3], Mohammad Almogdad[4], Saleh Hadi Al-Salem[5], Muhammad Ali Ali[2], Adel Al-Manoufi[2]

[1]Department of Forest Resources, Economics and Policy, Lithuanian Forest Research Institute, Lithuanian Research Centre for Agriculture and Forestry, Lithuania
[2]General Commission for Scientific Agricultural Research, Damascus, Syria
[3]Physics Department, Faculty of Science / University of Damascus, Damascus, Syria
[4]Department of Plant Pathology and Protection, Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Lithuania
[5]Department of Agricultural studies and research in the Iraqi, Ministry of agriculture, Dhi Qar agriculture directorate, Iraq.

Corresponding author: Yanal.alkuddsi@lammc.lt

Abstract

Nanotechnology plays a pivotal role in addressing agricultural challenges and propelling the agricultural sector forward, thereby significantly impacting community welfare and environmental sustainability. This study investigates the efficacy of nanofertilizers in enhancing the productivity of maize (Zea mays L.), a crucial cereal crop in Syria. Specifically, the response of the Ghouta 82 maize variety to chemical and nanofertilizers is examined. Field experiments were conducted at the Qarahta Research Station of the General Commission for Scientific Agricultural Research in Damascus, Syria. The experimental design employed a Randomized Complete Block layout with split plots arrangement, including three replications. Nanofertilizers, namely urea nanoparticles and NPK nanoparticles, were applied foliarly at varying concentrations (30g, 60g, and 90g), alongside traditional chemical fertilizers. Yield-related parameters such as plant height, ear height, grain count per ear row, row count per ear, 100-grain weight, and overall yield were evaluated. Statistical analysis revealed significant enhancements in the 100-grain weight across all fertilizer treatments compared to the control, with urea chemical fertilization registering the highest weight. Notably, nano-urea fertilizers at concentrations of 60g and 90g, along with NPK nanoparticles at 60g and 90g, demonstrated comparable efficacy to chemical fertilizers. Moreover, urea chemical fertilization outperformed other treatments in yield production, followed closely by nano-urea at 90g concentration. The findings suggest the feasibility of replacing chemical fertilizers with specific nanofertilizers to achieve comparable yields, thus advocating for the integration of nanotechnology in agricultural practices.

Keywords: maize, chemical fertilizers, nanofertilizers, Ghouta 82 variety.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

Synthesis and Properties of Thianthrene Derivatives for Oxygen Sensing Applications

Lukas Dvylys, Rasa Keruckienė, Matas Gužauskas, Melika Ghasemi, Juozas Vidas Gražulevičius

Department of Polymer Chemistry and Technology, Kaunas University of Technology, K. Baršausko g. 59, LT-51423, Kaunas, Lithuania

Corresponding author: lukas.dvylys@ktu.edu

Abstract

Room temperature phosphorescence (RTP) is a phenomenon observed in certain materials wherein light emission occurs at ambient temperatures, with a delay following the removal of the excitation source [1]. Unlike fluorescence, which has brief nanosecond lifetimes, RTP involves the transition of excited molecules to long-lasting triplet states, enduring from milliseconds to seconds [2]. RTP displays varying intensity levels between air and oxygen-free environments, indicating the influence of oxygen-sensitive triplets [1]. Organic compounds employed in oxygen sensing play pivotal roles in analytical chemistry and sensor technology, demonstrating distinctive optical or electrochemical attributes that adjust in the presence of oxygen. These compounds are utilized for their sensitivity to fluctuations in oxygen concentration, resulting in changes in emission characteristics [3]. The integration of such advanced organic materials in sensing platforms contributes to advancements in fields such as environmental monitoring, biotechnology, and medical diagnostics, facilitating precise and real-time detection of oxygen levels across various applications [4].

Thianthrene and its derivatives have recently attracted considerable attention due to their promising potential as effective materials in oxygen sensing applications [5]. Thianthrene derivatives feature a conjugated molecular structure that facilitates efficient intersystem crossing, a critical process for phosphorescence. The inclusion of sulphur atoms in the thianthrene molecule enhances spin-orbit coupling, thereby promoting efficient triplet state formation. The structural rigidity and extended conjugation in thianthrene derivatives further stabilize the triplet state, enabling prolonged phosphorescence lifetimes at room temperature [5].

In this work, two thianthrene-based compounds with distinct electron acceptors were synthesized. Their thermal, electrochemical, and photophysical as well as oxygen sensing properties will be discussed.

Keywords: thianthrene, room temperature phosphorescence, oxygen sensing.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Effect of Light Treatment During Pre- and Post-Harvest Storage on Mustard and Kale Microgreens

Ieva Gudžinskaitė, Kristina Laužikė, Giedrė Samuolienė

Lithuanian research centre for agriculture and forestry, Kauno str. 30, Babtai, 54333, Kaunas dist.

Corresponding author: Ieva.gudzinskaite@lammc.lt

Abstract

Microgreens are gaining more and more interest over the past years in the public eye. Its nutritional value and benefits to human health have been one of the topics regarding the advantage of microgreens consumption. Microgreens are early harvested vegetable greens while they are still immature and just developed cotyledonary leaves. These immature leafy greens are considered as a superior replacement for sprouts due to their plentiful nutritional content and distinctive flavor and taste. In this experiment mustard (*Brassica juncea*) microgreens were grown in closed controlled environment chamber with 21/17°C temperature and ~65% relative air humidity. Plants were sowed and grown in a peat substrate for 10 days after germination. Artificial lighting was provided by 4 channel (LED) lighting units, maintaining equal spectral composition consisting of deep red 61 %, blue 20 %, white 15 %, and far red 4 % in all lighting treatments. There were three lighting intensity groups which were set at 150, 200, and 250 µmol/m^2s photosynthetic photon flux density (PPFD). After 10 days from germination plants were harvested and held in the dark/light when +4°C temperature was maintained. Samples were taken on the day of harvest (D0), after one day of postharvest storage (D1), after three (D3) and five (D5) days of postharvest storage. Antioxidant properties of analyzed mustard microgreens evaluated as the FRAP assay, on D1 of postharvest storage, showed higher results when held in the light compared to those held in the dark in all treatments. On the D3 became apparent that significantly the lowest activity was found in mustard grown under 200 µmol/m^2s independently if it was held in the light or dark. Contrary to FRAP assay results, ABTS assay shows that on D1 the highest activity was found to be in microgreens grown under 200 µmol/m^2s and held in the dark. DPPH antioxidant activity on D1 and D3 was determined to be the highest in mustard microgreens grown under 150 µmol/m^2s and held in the light. On D5 the highest value was found to be in microgreens with the most extreme growing conditions -150 and 250 µmol/m^2s, both held in the light. Further analysis of α-tocopherols showed first postharvest storage to exhibit significantly higher contents of α- in plants held in the dark than in the light. Significant differences were determined between the treatments on harvest day, mustard microgreens showed to contain highest amounts of β-carotene and violaxanthin under 200 µmol/m^2s PPFD treatment. In conclusion growing conditions and light intensity may impact microgreens nutritional value and antioxidant activity during growth and during postharvest storage.

Keywords: antioxidants, microgreens, mustard.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

ESBL-Producing *Escherichia coli* in Poultry Farm: Effect of Vaccination on Bacteria Prevalence

Beatrice Kasparaviciene, Jurgita Aksomaitiene, Neringa Kasetiene, Mindaugas Malakauskas, Aleksandr Novoslavskij

Department of Food Safety and Quality, Lithuanian University of Health Sciences, Tilzes g. 18, Kaunas, Lithuania
Corresponding author: beatrice.kasparaviciene@lsmu.lt

Abstract

Escherichia coli, an inhabitant of the avian gastrointestinal microbiota, has emerged as a carrier of extended-spectrum β-lactamase (ESBL) genes, conferring resistance to a wide array of antibiotics. ESBL *E. coli* bacteria pose significant concerns for animal health and the potential transfer of resistant strains to humans via the food chain [1]. Vaccination against *E. coli* has as a promising intervention to reduce the prevalence of antibiotic-resistant strains. Nevertheless, the effectiveness of vaccination within poultry farming contexts and the ESBL *E. coli* prevalence in poultry flocks poses complex questions that this study aims to investigate [2].

In total 460 samples (400 individual cloacal and 60 farm environmental swab samples) were collected from two broiler flocks (vaccinated with the Poulvac® live vaccine and non-vaccinated) in October and November in 2023 at a Lithuanian poultry farm. The presumptive ESBL *E. coli* bacteria were isolated on TBX agar supplemented with 1 mg/L cefotaxime with Petri dish incubated at 37°C for 24 h. All typical *E. coli* isolates were additionally identified with multiplex PCR by the detection of *uspA* and *uidA* genes. Confirmation of ESBL-producing *E. coli* was conducted by a combined antibiotic disk test method according to EUCAST (2017) guidelines.

The findings of the study indicated a significant ($p < 0.05$) differences in prevalence of ESBL *E. coli* between vaccinated and non-vaccinated broiler flocks. The prevalence of ESBL-producing *E. coli* was lower in cloacal swab samples taken from the Poulvac®-immunized flock (29.5%) compared to cloacal swabs collected from the control flock (33.5%). In total 8.33 % of environmental swab samples were positive for ESBL *E. coli* in vaccinated flock in comparison to 13.33 % environmental swab samples from non-vaccinated flock. The peak of ESBL *E. coli* positive cloacal swab samples (65.00 % and 70.00 %, respectively) among vaccinated and non-vaccinated broilers was identified on day 6 and 5 accordingly. Noteworthy, that a significantly ($p < 0.05$) lower prevalence of ESBL *E. coli* in cloacal and environmental swab samples (7.50 % and 0.00 %, respectively) was found in vaccinated flock compared to non-vaccinated birds (20.00 % and 33.33 %, respectively) at the end of broilers breeding cycle. Further research is needed to fully understand the Poulvac® vaccine effect on ESBL *E. coli* prevalence dynamics at farm level.

Keywords: ESBL *E. coli*, vaccination, poultry.

Acknowledgments: This research was funded by RESEARCH COUNSIL OF LITHUANIA, grant number 022-PRO-00037, “Interventions to control the dynamics of antimicrobial resistance from chickens through the environment (ENVIRE)”, JPIAMR-ACTION Joint Transnational Call for Proposals 2021.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Evaluation of Fatty Acids Composition in Roe of Freshwater Fishes

Zhanna Kazangeldina[1], Raushan Uazhanova[1], Gintarė Zaborskienė [2], Artūras Stimbirys[2], Vaida Andrulevičiūtė[2], Ingrida Sinkevičienė[2], Ernestas Mockus[3]

[1]Department of Food Safety and Quality of the Faculty of Food Technology of Almaty technological university, Tole bi st 100, Almaty, 050012, Kazakhstan

[2]Department of Food Safety and Quality of the Veterinary Faculty of Lithuanian University of Health Sciences, Tilžės st. 18, Kaunas, LT-47181, Lithuania

[3]Institute of Animal Rearing Technologies, Lithuanian University of Health Sciences, Tilzes Str. 18, Kaunas, Lithuania

Corresponding author: zhanna.kzb@gmail.com

Abstract

The aim of the research work is to determine the fatty acid composition of freshwater fish roe and to evaluate the amounts of saturated (SFA), monounsaturated (MUFT), polyunsaturated (PUFA), ω-6 and ω-3 fatty acids. Our results present the fatty acids composition of lakes fishes roe from pikeperch - *Sander lucioper*, carp - *Cyprinus carpi*, pike - *Esox lucius*, fathead pike - *Hypophthalmichthys molitrix*, catfish - *Silurus glanis*, perch - *Perca schrenki*. The fatty acids were extracted by n-hexane, methylated by 2 mol/L KOH solution in anhydrous methanol and analysed using GCMS-QP2010 gas chromatograph (Shimadzu, Japan). The following parameters were used: MS ion source temperature: 240°C, MS interface temperature 240°C, helium (carrier gas) flow: 0.90 ml/min, injector: 240°C, oven temperature 50°C (4 min), 10°C/min to 110°C (1 min), 15°C/min to 160°C (2 min), 2.5°C/min to 195°C (1 min), 2°C/min to 230°C (1 min), 2°C/min to 240°C (12 min). The highest amount of saturated fatty acids was in silver carp roe - 45.32±0.11 % of the total fatty acids content (TFAC), the lowest – in pikeperch (11.47±0.31 % of TFAC). The MUFA amount in pikeperch roe was 23.14%, in other fish species varied from 33.0 - 41.21 % of TFAC. PUFA amount varied from 22.06 - 65.39% of TFAC and the highest was in pikeperch roe - 65.39% of TFAC. The ratio of ω-6 to fatty acids ω-3 fatty acids in tested roe ranged from 0.34 to 17.54. Pikeperch roe were characterized as a ω-3 fatty acids source, their levels were 45.45% of TFAC and exceeds levels in carp, pike, catfish and perch roe. Comparative analysis of atherogenicity (AI) and thrombogenicity (TI) indeces of different fish species roe shows the functionality and the lowest indices of pikeperch roe, respectively -0.09 and 0.17, $p<0.05$ in both cases. These results represent the pikeperch roe as a best dietary preventive source for the correction of lipid metabolism in the human body reducing the risk of cardiovascular diseases.

Keywords: freshwater fish roe, fatty acids, atherogenicity, thrombogenicity catfish, carp, silver carp, pikeperch.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

Electrochemotherapy: Combining Bleomycin with Conductive Nanoparticles and Nano/Microsecond Pulse Bursts

Barbora Lekešytė[1], Paulina Malakauskaitė[1], Eglė Mickevičiūtė[1], Eivina Radzevičiūtė-Valčiukė[1,2], Veronika Malyško-Ptašinskė[2], Anna Szewczyka[3], Natalija German[1], Almira Ramanavičienė[1], Julita Kulbacka[1,3], Vitalij Novickij[1,2]

[1]State Research Institute Centre for Innovative Medicine, Department of Immunology and Bioelectrochemistry, Vilnius, Lithuania
[2]Vilnius Gediminas Technical University, Faculty of Electronics, Vilnius, Lithuania
[3]Wroclaw Medical University, Department of Molecular and Cellular Biology, Wroclaw, Poland
Corresponding author: vitalij.novickij@imcentras.lt

Abstract

The use of nanotechnology and nanoparticles for drug and gene delivery has been widely researched in the past decades. Recent findings suggest that conductive nanoparticles can be used for electric field amplification in close proximity to cell membranes [1]. This could improve the current pulsed electric field-mediated drug & gene delivery methods and solve the issues associated with non-uniform electric fields in heterogeneous tissues. However, the effects of conductive nanoparticles have yet to be studied in the context of nanosecond pulse bursts or drug delivery [2]. Therefore, this study aims to explore the potential use of gold nanoparticles (AuNPs: 13 nm) in combination with microsecond: 0.6–1.5 kV/cm × 100 µs × 8 (1 Hz) and nanosecond: 6 kV/cm × 300–700 ns × 100 (1, 10, 100 kHz and 1 MHz) electric field pulses. The results suggest that the synergy between AuNPs and electroporation is significantly affected by the pulse repetition frequency in the nano-pulse range. The study shows that the synergistic effects are profound only with increased burst frequency and are non-existent with low-frequency bursts. AuNPs not only reduce the permeabilization thresholds but also affect pore resealing and the efficacy of electroporation. Finally, the study has tested the most prominent protocols (microsecond and nanosecond) in the context of bleomycin-based electrochemotherapy *in vitro* (4T1 cell line). The study shows that a saturated cytotoxic response with AuNPs can be triggered at significantly lower electric fields, which are considered sub-threshold and/or not applicable for electrochemotherapy otherwise. Furthermore, the nanoparticles themselves are non-toxic for the cells either separately or in combination with bleomycin, indicating the methodology's prospective applicability in the context of electrochemotherapy.

Keywords: nanosecond pulses, nanoparticles, drug delivery, permeabilization, resealing.

Acknowledgments: The research was supported by the Research Council of Lithuania, Grant Nr. S-PD-24-5.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Mitochondrial Depolarization, ATP Depletion and ROS Generation During High Frequency Nanosecond Electroporation

Paulina Malakauskaitė[1], Augustinas Želvys[1], Auksė Zinkevičienė[1], Eglė Mickevičiūtė[1], Eivina Radzevičiūtė-Valčiukė[1,2], Veronika Malyško-Ptašinskė[2], Barbora Lekešytė[1], Jurij Novickij[2], Vytautas Kašėta[3], Vitalij Novickij[1,2]

[1]State Research Institute Centre for Innovative Medicine, Department of Immunology and Bioelectrochemistry, Vilnius, Lithuania
[2]Vilnius Gediminas Technical University, Faculty of Electronics, Vilnius, Lithuania
[3]State Research Institute Centre for Innovative Medicine, Department of Stem Cell Biology, Vilnius, Lithuania
Corresponding author: vitalij.novickij@vilniustech.lt

Abstract

Electroporation can be effectively applied for drug and gene delivery [1], while the efficacy of the procedure is highly dependent on the pulse parameters. Ultra-short electric field pulses can selectively permeabilize intracellular structures, such as mitochondria, without major effects on the outer cell plasma membrane [2]. Nanosecond electroporation would have high applicability in cancer treatment, allowing for the enhanced delivery of chemotherapy drugs and modulating the immunogenic response [3].

Our study aimed to compare the effects of 100 µs x 8 pulses (ESOPE - European Standard Operating Procedures on Electrochemotherapy) and bursts of 100 ns pulses for modulation of the mitochondria membrane potential. We characterized the efficacies of various protocols including cell plasma permeabilization, mitochondrial depolarization, ATP depletion, generation of reactive oxygen species (ROS) and provided the most prominent protocols in the context of Ca^{2+} electrochemotherapy *in vitro*.

We present a proof of concept that 100 ns pulses (7.5–12.5 kV/cm, n = 100–1000, 1 MHz) can be used for permeabilization CHO cell line and modulation of mitochondrial potential. Although both the microsecond and nanosecond pulses depolarize mitochondria, therefore membrane permeabilization is still a pre-requisite for depolarization. In terms of ROS, 100 ns pulses result in lesser ROS generation when compared to ESOPE, even when the energy input is several-fold higher than for the microsecond procedure. ATP depletion is accompanied by mitochondria membrane damage during reversible electroporation, therefore Ca^{2+} electrochemotherapy triggers a more dynamic ATP loss and results in cell death. Finally, our data showed that the efficacy of Ca^{2+} electrochemotherapy can be equally effective between 100 ns and 100 µs pulses, ensuring excellent cytotoxic efficacy.

Keywords: mitochondria, membrane potential, calcium electrochemotherapy, burst compression, high frequency.

Acknowledgments: The research was supported by Research Council of Lithuania, Grant Nr. S-MIP-23-124.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Impact of Bipolar Cancellation Phenomenon on Calcium Nano-Electrochemotherapy for B16 F10 Tumor Treatment

Eglė Mickevičiūtė[1], Eivina Radzevičiūtė-Valčiukė[1,2], Veronika Malyško-Ptašinskė[2], Paulina Malakauskaitė[1], Barbora Lekešytė[1], Nina Rembialkowska[3], Julita Kulbacka[1,3], Vitalij Novickij[1,2]

[1]State Research Institute Centre for Innovative Medicine, Department of Immunology and Bioelectrochemistry, Vilnius, Lithuania
[2]Vilnius Gediminas Technical University, Faculty of Electronics, Vilnius, Lithuania
[3]Wroclaw Medical University, Department of Molecular and Cellular Biology, Wroclaw, Poland
Corresponding author: vitalij.novickij@imcentras.lt

Abstract

Electroporation is a phenomenon which involves the use of electric pulses to create openings in the cell membrane. [1] When bipolar nanosecond electric field pulses are utilised, cancellation effect occurs, resulting in lower electroporation efficiency than unipolar pulses with the same parameters. Essentially, the negative phase of a bipolar pulse reduces the influence of the positive phase.[2] Our study sought to evaluate how bipolar cancellation influences Ca^{2+} electrochemotherapy and cellular response at various electric field intensities and pulse durations (3-7 kV/cm, 100, 300, and 500 ns bipolar 1 MHz repetition frequency pulse bursts, n = 100). For reference, standard microsecond range parametric protocols (100 µs x 8 pulses) were used. We demonstrated that the cancellation effect is exceptionally significant when the pulses are closely spaced (1 MHz frequency), resulting in a lack of cell membrane permeabilization *in vitro*. Finally, we conducted a pilot *in vivo* study to evaluate the efficacy of monophasic (5 kV/cm, 500 ns, n=100) and biphasic (5 kV/cm, 500 ns + 500 ns, n=100) sequences in the setting of Ca^{2+} electrochemotherapy (B16 F10 cell line, C57BL/6 mice, n = 28). The research showed that mice treated with bipolar pulses did not have longer life times than untreated controls (tumor-bearing mice); thus, the bipolar cancellation event occurred *in vivo*, greatly limiting electrochemotherapy. At the same time, monophasic nanosecond pulses were as effective as the ESOPE sequence in reducing tumors and prolonging animal survival after electroporation procedure.

Keywords: bipolar pulse, cancellation effect, high frequency, calcium electrochemotherapy, tumors.

Acknowledgments: The research was supported by Research Council of Lithuania, Grant Nr. S-MIP-23-124.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Evaluating Pyrrolizidine Alkaloids in Latvia's Predominant Weeds and their Transfer to Medicinal Plants

Emīls Francis, Arta Kronberga, Ieva Mežaka, Gundars Skudriņš, Ilva Nakurte

Institute for Environmental Solutions, "Lidlauks", Priekuļu parish, Cēsis county, Latvia.
Corresponding author: emils.francis.lv@gmail.com

Abstract

Pyrrolizidine alkaloids (PAs) are a diverse group of naturally occurring, toxic secondary plant metabolites synthesized primarily as a defence mechanism against herbivores and insects. Recently, there has been a significant rise in the number of food alerts reported on the Rapid Alert System for Food and Feed (RASFF) concerning these compounds, emphasizing their significance as a food safety issue. PAs are an increasingly concerning issue given the overall decrease in herbicide usage in Europe [1], the growing market of organic agriculture, and the regulations implemented by the European Commission concerning the permissible content of PAs. PAs have been scarcely researched in the Baltic region, and their content in weeds has been noted to differ across geographic locations [2]. The aim of this study was to examine the pyrrolizidine alkaloid content of several common weeds, including *Myosotis arvensis, Anchusa arvensis, Anchusa officinalis, Senecio vulgaris*, and *Jacobaea vulgaris*, in the territory of Latvia. Additionally, the study sought to assess the concept of "horizontal natural product transfer" in the context of PAs from the most widely distributed weeds and their residues to the soil and to medicinal and aromatic plants: *Matricaria recutita L.*, *Mentha x piperita L.*, and *Petroselinum crispum L.* Experimental plants were grown in pots, with three replications. The tested treatments involved the co-cultivation of acceptor plants with PA-containing weeds, the cultivation of acceptor plants with soil containing PA weed residues, and controls where donor and acceptor plants were cultivated separately. Donor and acceptor plant material, as well as soil material, were collected for PA analysis at the acceptor plant harvesting stage. Samples for PA analyses were prepared using ultrasonification and a modified QuEChERs method. Analysis was conducted using an Agilent 1260 Infinity HPLC (high performance liquid chromatography) system equipped with an Agilent 6530 Q-TOF (quadrupole time of flight) mass spectrometer. The method has been thoroughly validated according to European Pharmacopoeia 10.6. As a result, only a few parsley samples exhibited a PA concentration over 1000 µg/kg, indicating that this might be a factor in PA transfer. Conversely, all other samples either fell below the level of detection or contained low levels of PAs. Soil analysis assessed minor transfer during autumn harvesting time and indicated decomposition over a six-month period. This observation occurred despite optimal conditions conducive to the phenomenon of "horizontal natural product transfer". Additionally, our results reveal a significant disparity in the PA content of weeds collected in the territory of Latvia compared to those from elsewhere [3].

Keywords: pyrrolizidine alkaloids, weeds, medicinal plants, liquid chromatography, mass spectrometry.

Acknowledgments: The authors gratefully acknowledge the Latvian Council of Science for their financial support in the lzp-2022/1-0543 project.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Multifunctional Nutraceutical Combinations Based on Bioconverted Milk Permeate, Apple Juice, Bovine Colostrum, Grapefruit Essential Oil and Berry By-Products

Emilis Radvila[1], Vita Lele[1,2], Domantas Medonas[1], Deimante Ivanauskaite[1], Edikas Vysniauskas[1], Paulina Sakaite[1], Greta Kotinskaite[1], Laurynas Marciulionis[1], Viktorija Klein[1], Ieva Tatarunaite[1], Paulina Gliaubiciute[1], Monika Zimkaite[1], Evaldas Kentra[1], Egle Zokaityte[2], Vytaute Starkute[1,2], Paulina Zavistanaviciute[1,2], Dovile Klupsaite[2], Ernestas Mockus[2], Ernesta Tolpeznikaite[2], Vadims Bartkevics[3], Modestas Ruzauskas[4,5], Pranas Viskelis[6], Elena Bartkiene[1,2]

[1]Department of Food Safety and Quality, Faculty of Veterinary, Lithuanian University of Health Sciences, Mickeviciaus str. 9, LT-44307 Kaunas, Lithuania

[2]Institute of Animal Rearing Technologies, Faculty of Animal Sciences, Lithuanian University of Health Sciences, Mickeviciaus str. 9, LT-44307 Kaunas, Lithuania

[3] Institute of Food Safety, Animal Health and Environment "BIOR," Riga, Latvia

[4]Department of Anatomy and Physiology, Faculty of Veterinary, Lithuanian University of Health Sciences, Mickeviciaus str. 9, LT-44307 Kaunas, Lithuania

[5]Institute of Microbiology and Virology, Faculty of Veterinary, Lithuanian University of Health Sciences, Mickeviciaus str. 9, LT-44307 Kaunas, Lithuania

[6]Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Kauno str. 30, LT-54333 Babtai, Lithuania

Corresponding author: emilis.radvila@stud.lsmu.lt

Abstract

The aim of this study was to develop multifunctional nutraceutical combinations (MNC) based on *Citrus paradisi* essential oil (CO), bioconverted (B) milk permeate (BMP), apple juice (BAJ), and bovine colostrum (BBC), as well as berry by-products (chokeberries (By-CH), blackcurrants (By-Bl), elderberries (BY-Eld), and raspberries (By-Ras)). Gelatine was used as a texture-forming ingredient and two sweetening agents were tested (saccharose (Sach) and xylitol (Xy)) for MNC preparation. In total thirteen MNC were prepared (BMP+CO+Sach or Xy (I and II, respectively), By-Bl+BBC+Sach or Xy (III and IV, respectively), BAJ+Sach or Xy (V and VI, respectively), By-Eld+BBC+Sach or Xy (VII and VIII, respectively), By-Ras+BBC+Sach or Xy (IX and X, respectively), By-CH+BBC+Sach or Xy (XI and XII, respectively), By-Eld+By-Ras+BBC+Xy (XIII)). Developed MNC were evaluated on lactic acid bacteria (LAB) count after 0, 14, and 30 days of storage. Also, antimicrobial properties against pathogenic and opportunistic strains (*Klebsiella pneumoniae*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Acinetobacter baumani*, *Proteus mirabilis*, methicillin-resistant *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Bacillus cereus*, *Streptococcus mutans*, *Enterobacter cloacae*, *Citrobacter freundii*, *Staphylococcus epidermis*, *Staphylococcus haemolyticus*, *Pasteurella multocida*) and overall acceptability (OA) of MNC were tested. The highest number of tested pathogens (13 out of 15 tested) was inhibited by BAJ and BMP. BBC inhibited 3 out of 15 pathogens tested, while By-CH, By-Bl, BY-Eld, and By-Ras inhibited 3, 9, 2, and 3, respectively, out of 15 pathogens tested. The layers of MNC with the highest OA were combined into a single formulation: II (BMP+CO+Xy) + IV (By-BC+BBC+Xy) + VI (ACV+Xy) + XIII (BBC+By-Eld+By-Ras+Xy). Finally, MNC OA was, on average, 8.14 scores, however, despite, that the viable LAB count in freshly prepared MNC was, on average, 5.74 $\log_{10}$ CFU/g, after 30 days of storage, viable LAB were not detected. It can be concluded that the developed MNC are very promising as an antimicrobial product prepared in a sustainable manner.

Keywords: nutraceuticals, bioconversion, bovine colostrum, milk permeate, apple cider vinegar, berry by-products, antimicrobial properties.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

Assessment of Environmental Genotoxicity in the Šešupė River Using Mussels (*Unio tumidus*) in 2023

Miglė Ragelytė, Grita Skujienė

Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania

Corresponding author: migle.ragelyte@gmc.stud.vu.lt

Abstract

Anthropogenic pollution can disturb freshwater ecosystem function. Changes in the integrity of genetic material in sentinel organisms can serve as an early indicator of potentially drastic shifts in ecosystems. *Unio tumidus* Retzius, 1788 are particularly suitable for measuring local chemical stressors, as they interact with pollutants found in both sediments and the water column [1].

Šešupė is one of the rivers with a uniquely good mollusc condition at its source in Poland [3], while situation in Lithuania is satisfactory [2], perhaps because of the influence of anthropogenic pollution. One of the largest known cases of pollution happened near Antanavas, when unknown pollutants foamed and killed thousands of fish in 5 km stretch in 2015. This ecological disaster and other cases could have genotoxic affect. Mussels were collected from two sites – before Antanavas (near Sidory, Poland and near Kalvarija, Lithuania) and after (near Šapalai and Bosai, Lithuania) to determine environmental genotoxic impact upon *U. tumidus* mussels.

The level of DNA damage was determined in the epithelial gill cells of the freshwater bivalve *U. tumidus.* Water and sediment parameters, such as phthalates, phosphates, nitrates, ammonium, water hardness, ph, salinity, concentration of organic carbon and heavy metals (Cu, Zn, Ni, Pb, Cr, Cd, and Hg) were determined. Statistical analysis was performed using PAST, one-way ANOVA and Kruskal–Wallis tests.

It was found that U. tumidus biomarkers of genotoxicity did not show a consistent trend, but the cumulative criterion of damages revealed the highest impact of pollution near Kalvarija and Šapalai and the lowest in Bosai. Compared cumulative damages do differ in investigated sites ($p>0.05$) but are statistically insignificant. The highest pollution with phthalates (DIBP = 0.33 µg-1 and DEHP = 0.23 µg/L) was measured near Sidory. Heavy metals (Cd, Cr, Ni, Pb, Zn, Hg) in Šešupė water did not exceed permissible levels. The highest physico-chemical water quality parameters including water temperature, salinity, dissolved oxygen (DO), pH, total dissolved solids (TDS), conductivity values were measured in Bosai.

To conclude, there are no traces of genotoxic effect in molluscs because of the disaster near Antanavas. However, to enhance confidence in the data it is necessary to perform more tests to state what environmental factors lead to adverse outcomes for the condition of molluscs in the Šešupė river.

Keywords: water pollution, mussels, genotoxicity, biomarkers, phthalates, heavy metals.

Acknowledgments: We would like to thank Jonas Aistis Skuja for help in field and laboratory work. This project has received funding from the Research Council of Lithuania (LMTLT), agreement No. P-SV-23-424.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Anti-inflammatory Activity and Amino Acids Profile of Bee Pollen After Fermentation and Enzymatic Hydrolysis

Vaida Damulienė[1], Donata Drapanauskaitė[2], Karolina Barčauskaitė[2], Vilma Kaškonienė[1], Paulius Kaškonas[3], Audrius Maruška[1]

[1]Instrumental Analysis Open Access Centre, Vytautas Magnus University, LT-44404 Kaunas, Lithuania
[2]Lithuanian Research Centre for Agriculture and Forestry, LT-53362 Akademija, Kaunas District, Lithuania
[3]Institute of Metrology, Kaunas University of Technology, LT-51368 Kaunas, Lithuania

Corresponding author: vaida.damuliene@vdu.lt

Abstract

Contemporary health-conscious consumers prefer value-added products. These products allow them to replace traditional food ingredients with high-nutrient alternatives, thereby enhancing the nutritional quality of processed foods. A balanced human diet should provide adequate energy and essential nutrients to support physical, mental health, and development [1]. Bee pollen presents a valuable supplement to such a diet, offering a rich nutrient profile that can contribute significantly to daily nutritional intake. Pollen is recognized for its health and immunity-boosting advantages, characterized by its anti-inflammatory, anticarcinogenic, antioxidant, antimicrobial, antiallergenic, antiulcer, hepatoprotective, and chemo-preventive properties [2, 3]. The aim of this research was to determine anti-inflammatory activity and amino acid profile of bee pollen after fermentation and enzymatic hydrolysis. Ten bee pollen samples from different counties of Lithuania were enzymatically hydrolyzed using *Viscozyme L, Clara-diastase* and cellulase. Additionally, fermentation was conducted spontaneously and bacterially using *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, and *Bifidobacterium animalis*. Anti-inflammatory activity was measured using spectrophotometric method, while UHPLC was employed for amino acids profile determination [4, 5]. A positive effect of fermentation and enzymatic hydrolysis on the anti-inflammatory activity of bee pollen was observed. Bacterial fermentation increased anti-inflammatory activity by 1.1-1.4 times, spontaneous fermentation – 1.1-1.3 times and enzymatic hydrolysis – 1.2-1.6 times. Furthermore, analyzed bee pollen samples contained varying quantities and types of free amino acids, with proline, glycine, alanine, isoleucine, leucine, and phenylalanine being the most predominant in all samples. Moreover, an analysis of the amino acid profile revealed significant increases in all essential amino acids after fermentation (1.2-1.5 times) or enzymatic hydrolysis (1.3-2.0 times) of the pollen.

Keywords: bee pollen, enzymatic hydrolysis, fermentation, amino acids, anti-inflammatory.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

FLASH PRESENTATIONS

ISSN:2335-8653
ONLINE ISSN: 2335-8718

Multi-cropping Influence on Biomass Productivity and Energy-Environmental Indicates

Jovita Balandaitė, Kęstutis Romaneckas, Rasa Kimbirauskienė, Aušra Sinkevičienė, Austėja Švereikaitė, Ugnius Ginelevičius

Vytautas Magnus University, Agriculture Academy, Studentu str. 11, 53361, Akademija, Kaunas reg., Lithuania

Corresponding author: jovita.balandaite@vdu.lt

Abstract

Multi-crops produce better results than single-crops [1]. Growing multi-crops on the same field at the same time can lead to more sustainable agricultural systems, where it is possible to increase biodiversity, reduce damage from diseases, pests and weeds, reduce the need for mineral fertilisers, and maintain the fertility and quality of the soil [2]. Multi-crops enable farmers to obtain more yields throughout the cropping season, which generates higher incomes for the marketing of produce and the provision of food for humanity [3]. Recently, faba bean (*Vicia faba* L.), hemp (*Cannabis sativa* L.) and maize (*Zea mays* L.) have the highest biomass productivity, energy and nutritional potential. Little research has been carried out in Lithuania and abroad on technologies for the combined cultivation of these crops under short growing conditions. The aim of this study was to assess the biomass productivity of single and multi-crops and their impact on energy and environmental performance.

A stationary field experiment was conducted in 2020–2022 at Vytautas Magnus University, Agriculture Academy, Research Station (54º52′ N, 23º49′ E). The soil of the experimental field is a deeper gleyic saturated loam (*Endohypogleyic-Eutric Planosol-Ple-gln-w*) [4]. The water regime is regulated by closed drainage, the micro-relief is levelled. The crops grown in the experiment were: maize (*Zea mays* L.), hemp (*Cannabis sativa* L.) and faba bean (*Vicia faba* L.), which were sown as single, binary and ternary crops. Totally 7 treatments were tested. The dried biomass of the ternary crop was significantly the highest (1868.0 g/m^2), followed by the dried biomass of the maize single–crop (239.9 g/m^2), which was 7.8 times lower than it. The green biomass of the ternary crop was 1.7 times lower than the green biomass of the maize single–crop. As a result, energy consumption was highest in the ternary crop or almost twice as high as in the single–crop of maize, single–crop of hemp, binary maize–hemp crop. The highest total GHG emissions were produced by the binary crop of maize and hemp and binary crop of maize and faba bean (1729.84 and 2067.33 CO_2eq/ha). Ternary crop provided the highest energy return and only initiated an average GHG emission (1541.90 kg/ha CO_2eq).

Keywords: maize, hemp, faba bean, multicropping, biomass, energy, GHG emissions.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

New Bipolar Derivatives with Diphenylsulfone or Dibenzophenone as TADF Based Emitters for OLEDs

Gintare Krucaite[1], Raminta Beresneviciute[1], Daiva Tavgeniene[1], Saulius Grigalevicius[1], Yi-Ting Chen[2], Yu-Hsuan Chen[2], Chih-Hao Chang[2]

[1]Department of Polymer Chemistry and Technology, Kaunas University of Technology, Radvilenu plentas 19, Kaunas, LT50254, Lithuania

[2]Department of Electrical Engineering, Yuan Ze University, Chungli, Taoyuan, 32003, Taiwan

Corresponding author: saulius.grigalevicius@ktu.lt

Abstract

There has been huge interest in developing energy-efficient, cleaner and more affordable lighting technologies to resolve the energy crisis. Organic light emitting diodes (OLEDs), mainly formed of thin films of organic molecules, have been regarded as a sustainable and desirable strategy to achieve the goal [1–3].

New thermally activated delayed fluorescence (TADF) properties having derivatives are prepared and characterized as TADF emitters of OLEDs. The bipolar compounds have 3-(N,N-diphenylamino)carbazol-9-yl and carbazol-9-yl fragments as donor moieties and diphenylsulfone or benzophenone as acceptors. The fully amorphous materials have high thermal stability with temperatures of 5% weight loss in the range 381–408 ˚C as well as very high glass transition temperatures of 140 ˚C and 143 ºC.

Third generation OLEDs were formed by using the new emitters. The blue emitting device using diphenylsulfone based emitter showed turn-on voltage of 3.6 V, maximum luminance of about 11400 cd/m^2 and peak efficiency values of 5.1% (10.5 cd/A and 9.6 lm/W). The green emitting device using benzophenone-based emitter demonstrated superior performance with low turn-on voltage of 2.2 V, maximum luminance of 60155 cd/m^2 with high peak efficiency values of 12.1% (35.4 cd/A and 46.3 lm/W). At practical luminance levels recorded at 100 cd/m^2 (1000 cd/m^2), the EQE values of the green device dropped by 0% (8.3%) from the respective peak value. The results confirm that some of the new emitters are very promising material for preparation of highly efficient devices.

Keywords: bipolar derivative, glass transition temperature, amorphous material, TADF, OLED, efficiency.

Acknowledgments: This research was conducted in the frame of the project with support from the Research Council of Lithuania (Grant No. S-LLT-19-2).

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

New Triphenylethylene Fragments Containing Compounds for the Realization of Blue and White Aggregation-Induced Emission OLEDs with High Luminance

Gintare Krucaite[1], Cheng-Yung Ho[2,3], Raminta Beresneviciute[1], Dovydas Blazevicius[1], Wei-Han Lin[2], Jhao-Cheng Lu[2], Chang-Yu Lin[3], Saulius Grigalevicius[1], Chih-Hao Chang[2]

[1]Department of Polymer Chemistry and Technology, Kaunas University of Technology, Radvilenu plentas 19, LT50254, Kaunas, Lithuania

[2]Department of Electrical Engineering, Yuan Ze University, Chung-Li, 32003 Taiwan

[3]Department of Mechanical Engineering, Chung Yuan Christian University, Chung-Li, 32023 Taiwan

Corresponding author: gintare.krucaite@ktu.lt

Abstract

Organic light-emitting diodes (OLEDs) are becoming increasingly popular for use in display or phototherapy lighting applications due to their various advantages such as high-quality color, low energy cost, light weight, and flexibility. Among the potential molecular structures in blue-emitting aggregation-induced emission (AIE) compounds, tetraphenylethene has been shown to be an effective core molecule for generating excellent AIE [1-3].

Molecules **1**-**3** (1,1,2-triphenyl-2-(9-[4-(phenylsulfonyl)phenyl)*]*-carbazol-2-yl)ethylene(**1**), 1,1,2-triphenyl-2-(9-(4-*benzonitril)*-carbazol-2-yl)ethylene(**2**), 1,1,2-triphenyl-2-(9-(*4-benzophenon*)-carbazol-2-yl)ethylene (**3**)) with different substitutions were designed and synthesized. The photophysical, thermal, and electroluminescence characteristics of the designed emitters were investigated to clarify the molecular structure-property-performance relationship.

The doped blue-emitting OLED with compound **2** exhibited satisfactory peak efficiency of 8.7 cd/A and high maximum luminance of 18474 cd/m^2. The fabricated white-emitting OLEDs with **2** achieved a maximum efficiency of 5.6% (8.0 cd/A and 7.5 lm/W). These results indicate that the triphenylethene-carbazole structure designs for AIE molecules could simultaneously harvest wide energy bandgap and high luminance, demonstrating their high potential for EL applications.

Keywords: triphenylethene, carbazole, aggregation-induced emission (AIE), organic light-emitting diodes (OLEDs), non-doped.

Acknowledgments: We gratefully acknowledge the funding support from the Ministry of Science and Technology of Taiwan, under the grant number (MOST 110-2221-E-155-036- and MOST 110-2221-E-155-033-MY2) and support from the Research Council of Lithuania (grant No. S-MIP-22-84).

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Total Phenolic Content and Antioxidant Activity of Invasive *Amelanchier* × *Spicata* (Lam.) Koch Leaves: Variation from Different Habitats Lithuanian

Sandra Saunoriūtė[1,2], Lina Raudonė[1,3]

[1]*Department of Pharmacognosy, Lithuanian University of Health Sciences, Sukileliu Av. 13, 50162 Kaunas, Lithuania*
[2]*Research Institute of Natural and Technological Sciences, Vytautas Magnus University, Universiteto Str. 10, 53361 Akademija, Lithuania*
[3]*Laboratory of Biopharmaceutical Research, Institute of Pharmaceutical Technologies, Lithuanian University of Health Sciences, Sukileliu Av. 13, 50162 Kaunas, Lithuania*
Corresponding author: sandra.saunoriute@lsmu.lt

Abstract

The *Amelanchier* (Medik.) genus plants accumulate numerous health-beneficial biologically active compounds such as phenolics, anthocyanins, triterpenes, minerals, and vitamins [1-2]. Most *Amelanchier* species and cultivars are also distinct by strong antioxidant properties [3]. They have a high potential for use in the food, pharmaceutical and cosmetics industries [4].

The aim of the study was to determine the amount of total phenolic compounds in leaf extracts of invasive *Amelanchier* × *spicata* (Lam.) K. Koch) and to assess their antioxidant activity.

Leaves of *A. spicata* were collected in July from urbanized areas and natural and semi-natural Lithuanian habitats. The total phenolic content was determined according to Folin–Ciocalteu assay [5], while antioxidant activity was determined using ABTS [6], FRAP [7], and CUPRAC [8] assays.

In our study, the total amount of phenolic compounds varies significantly from 66.02 to 117.48 mg GAE/g DW. The greatest amount of total phenolic compounds (117.48±1.95 mg GAE/g DW) and the strongest reducing activity by FRAP (4192.63±60.78 µmol TE/g DW) was determined in leaf extracts collected in Marcinkonys (natural habitats in South part of Lithuania). The strongest reducing activity by CUPRAC (1349.68±23.05 µmol TE/g DW) and antiradical activity by ABTS (841.17±85.45 µmol TE/g DW) was determined in leaf extracts collected in Bartlaukis and Nevardėnai (both semi-natural habitats in West part of Lithuania). The lowest content of phenolic compounds (66.02±5.47 mg GAE/g DW) and the weakest reducing activity by CUPRAC (177.98±12.13 µmol TE/g DW) was found in leaf extracts collected in Kęsai (semi-natural habitats in West part of Lithuania). The weakest reducing activity by FRAP (3697.93±172.67 µmol TE/g DW) and antiradical activity by ABTS (147.41±54.40 µmol TE/g DW) was found in leaf extracts collected in Marcinkonys (natural habitats in South part of Lithuania). In conclusion, leaves of *A. spicata* are sources of phenolic compounds and natural antioxidants. Our data indicated that different habitats have a significant impact on the variation of total phenolic content and antioxidant activity in leaf extracts.

Keywords: *Amelanchier* × *spicata*, invasive plant, phenolic compounds, antioxidant activity.

Acknowledgments: This project was supported from the Research Council of Lithuania, agreement No. P-PD-23-050.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Antiplatelet Properties of Fractions Isolated from Various Organs of Sea Buckthorn (*Hippophae rhamnoides*) Measured by Flow Cytometry

Bartosz Skalski[1,4], Joanna Rywaniak[2], Jerzy Żuchowski[3], Anna Stochmal[3], Beata Olas[4]

[1]Department od Plant Physiology and Biochemistry, Institute of Experimental Biology, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland
[2]Department of Immunology and Infectious Biology, Institute of Microbiology, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland
[3]Department of Biochemistry, Institute of Soil Science & Plant Cultivation, Puławy, Poland
[4]Department of General Biochemistry, Institute of Biochemistry, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland

Corresponding author: bartosz.skalski@biol.uni.lodz.pl

Abstract

Sea buckthorn (*Hippophae rhamnoides*) is a shrub whose natural habitat is coastal areas. It naturally occurs in Siberia, the Caucasus, Russia and the coast of the Baltic Sea. In Poland, small cultivated areas of this plant were established in the Suwałki region. Sea buckthorn belongs to the olive family (*Elaegnaceae*). Its characteristic feature is the presence of thorns. It has lanceolate leaves and intensely orange fruit-berries. Sea buckthorn is a dioecious plant [1].

Numerous studies on the chemical composition of various organs of sea buckthorn show that this plant is a rich source of vitamins, phenolic compounds, tocopherols, carotenoids, amino acids, fatty acids, micro- and macroelements. A wide range of experiments conducted on preparations from various organs of this plant have proven their anticancer, antiulcer and hepatoprotective effects. Moreover, sea buckthorn has antibacterial and antiviral properties [2].

The primary goal of this research task was to assess the impact of fractions from sea buckthorn fruits, leaves and twigs on hemostasis in the *in vitro* model (fraction A (polyphenols rich fraction from fruits), fraction B (triterpenic acids rich fraction from fruits), fraction C (polyphenols rich fraction from leaves), fraction D (triterpenic acids rich fraction from leaves), fraction E (polyphenols rich fraction from twigs), and fraction F (triterpenic acids rich fraction from twigs)). The biological material was whole human blood collected from healthy volunteers.

In this task, platelet activation and their reactivity were measured in the presence of two physiological platelet agonists (10 and 20 μM adenosine diphosphate (ADP) and 10 μg/ml collagen) in whole blood incubated with six different sea buckthorn fractions with using three-color flow cytometry [3].

The experiment suggests that the inhibition of platelet aggregation is confirmed in our previous works it is correlated with low activity of the GPIIb/IIIa receptor. Moreover, it was determined that the antiplatelet potential of the tested fractions was not dependent on the P_2Y_{12} receptor [3].

Keywords: sea buckthorn, blood platelets, flow cytometry.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

New Bipolar Derivatives Using as Potential TADF Emitters for OLEDs

Daiva Tavgenienė[1], Raminta Beresnevičiūtė[1], Gintare Kručaitė[1], Sujith Sudheendran Swayamprabha[2], Jwo-Huei Jou[2], Saulius Grigalevičius[1]

[1]Department of Polymer Chemistry and Technology, Kaunas University of Technology, Radvilenu plentas 19, LT 50254, Kaunas, Lithuania

[2]Department of Materials Science and Engineering, National Tsing-Hua University, No.101, Kaungfu Rd. Hsin-Chu 30013 Taiwan, ROC

Corresponding author: daiva.tavgeniene@ktu.lt

Abstract

Previously widely investigated phosphorescence (Ph) metallic complexes and Ph OLEDs contain noble metals, were quite expensive and environment-hazardous [1]. The mentioned derivatives especially endure from stability of the OLED devices and from unsatisfied light colours [2]. Achieving of a suitable right colour and suitable morphological stability in clean organic emitters is a very important investigations field in last some years. TADF (thermally activated delayed fluorescence) emitters, which are currently very actively researched, have also solved the drawback that traditional OLEDs only use single excitons [3].

Here I present well defined new electroactive bipolar compounds containing 3-(N,N-diphenylamino)-9H-carbazole as donor. Meanwhile, (bis)phenylsulfone or benzophenone were using as acceptor fragments. The obtained results showed that new electroactive bipolar compounds can form homogeneous solid amorphous with a glass transition temperature in the range of 111–173 ºC. These new materials, which were soluble in common organic solvents like chloroform, acetone, ethylacetate and methanol, were tested as emitting derivatives dispersed in 4,4′-bis(N-carbazolyl)-1,1′-biphenyl host. The newly developed OLEDs with the emitter bis[4-{3-(N,N-diphenylamino)carbazol-9-yl}phenyl] sulfone exhibited the best overall performance. The OLEDs using bis[4-{3-(N,N-diphenylamino)carbazol-9-yl}phenyl] sulfone as emitter demonstrated quite low turn-on voltage of about 3.0 V. Meanwhile, these new OLED devices demonstrate the maximum brightness of more than 2600 cd/m^2, the power efficiency of 2.2 lm/W, the current efficiency of 3.2 cd/A and EQE of about 1.7 % at 100 cd/m^2. For the technically important brightness of 10000 cd/m^2 efficiencies above 2.4 cd/A were obtained. The obtained results demonstrate that a few of the compounds could be further investigated as potential TADF emitters.

Keywords: OLED, carbazole, TADF, emitter.

Acknowledgments: This research was conducted in the frame of the project with support from the Research Council of Lithuania (grant No. S-LLT-19-2).

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Research of Preparation Techniques for Medical Plant Raw Materials to Optimize Extraction of Biologically Active Compounds

Domantas Armonavičius, Audrius Maruška

Instrumental Analysis Open Access Centre, Institute of Research of Natural and Technological Sciences, Vytautas Magnus University, Kaunas, Lithuania
Corresponding author: domantas.armonavicius@vdu.lt

Abstract

The research of medical plants as a reservoir of therapeutic compounds has been an indispensable part of both traditional and contemporary medicinal practices across diverse cultures. The unique chemical composition of these plants often harbours biologically active compounds with potential health benefits. In recent years, there has been a growing interest in unlocking the full potential of medical plants through the optimization of preparation methods in raw materials [**1,2**]. For numerous years, scientists at Vytautas Magnus University have been engaged in research focused on isolating phyto-compounds from herbal medicinal raw materials and studying their antioxidant properties [**3,4**].

This study aims to explore and assess various preparation methodologies employed for raw medical plant materials, including techniques such as freezing at -18°C, -80°C and -196°C, drying at room temperature, 35°C and 60°C, direct extraction using 0%, 25%, 50% and 75% MeOH solution and aerobic and anaerobic fermentation. The primary objective is to elucidate the influence of these techniques on the extraction efficiency of bioactive compounds, with the goal of maximizing the potency of medical plant-derived products. As an objective, Fireweed (*Chamerion angustifolium)* was selected. Raw material of plant was separated intro three main parts: leaves, blossoms and stems. All samples were divided in 10 groups; in total there were 240 extracts prepared.

Total phenolic and flavonoid contents and free radical-scavenging assays were determined using a UV-VIS spectrometer. All measurements were compared to a standard curve prepared using rutin solution and expressed as rutin equivalents (means) in mg of rutin per 1g of dry raw material: RE mg/g. 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 [**5**].

Results of the study shows that most effect for extraction of biological compounds were observed using fresh sample aerobic fermentation following 35°C drying process technique. Freezing different plant parts in liquid nitrogen for 3 and 6 months had the lowest impact on total content of phenolic compounds and flavonoids of *Chamerion angustifolium*.

Keywords: medical plants, preparation methodologies, phyto-compounds, antioxidant properties, extraction efficiency.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Investigation of the Antimicrobial Activity of Fresh and Dried Extracts of Rosehip (*Rosae pseudo-fructus*) and Guelder Rose (*Viburni fructus*) and Rosehip Seed Oil (*Rosae semen oleum*)

Paula Batutytė, Rūta Mickienė, Audrius Sigitas Maruška

Instrumental Analysis Open Access Centre, Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania
Corresponding author: paula.batutyte@stud.vdu.lt

Abstract

The ever-increasing spread of infectious diseases has a significant and negative impact on global and human health, and the alarming and increasing rate of antibiotic resistance is prompting the pharmaceutical industry to research new drugs to address this critical situation. In this context, plant phenolic compounds are an interesting source of new phytocompounds with a broad spectrum of activity, especially against gram-negative bacteria. In terms of their mechanism of action, each compound interacts with a different cell component due to the multiple structures they possess. Plants rich in phenolic compounds have antibacterial effects, these compounds cause an increase in membrane permeability, nutrient deficiency, and inhibition of extracellular enzymes. Studies have shown that many species of rosehip have antibacterial effects against both gram-positive and gram-negative bacteria. Methanolic extracts 70% made from fresh rosehip fruit (*Rosae pseudo-fructus*) and guelder rose fruit (*Viburni fructus*) have a inhibitory effect against *Lactiplantibacillus plantarum* and *Azotobacter vinelandii* bacteria. 70% methanol extracts made from dried rosehip fruits (*Rosae pseudo-fructus*) have an inhibitory effect only against *Azotobacter vinelandii* bacteria. Rosehip seed oil (*Rosae semen oleum*) showed an inhibitory effect against *Lactiplantibacillus plantarum* and *Azotobacter vinelandii* bacteria.

Keywords: rosehip fruit (*Rosae pseudo-fructus*), guelder rose fruit (*Viburni fructus*), rosehip seed oil (*Rosae semen oleum*), *Lactiplantibacillus plantarum*, *Azotobacter vinelandii*.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

OLEDs Achieving High Efficiency Through Naphtalimide-Based Bipolar Derivatives

Raminta Beresneviciute[1], Prakalp Gautam[2], Mangey Ram Nagar[2], Gintare Krucaite[1], Daiva Tavgeniene[1], Jwo-Huei Jou[2], Saulius Grigalevicius[1]

[1]Department of Polymer Chemistry and Technology, Kaunas University of Technology, Radvilenu pl. 19, Kaunas, Lithuania

[2]Department of Materials Science and Engineering, National Tsing Hua University, No. 101, Section 2, Guangfu Rd., East District, Hsinchu, Taiwan

Corresponding author: ramintaberesneviciute@ktu.lt

Abstract

Organic light-emitting diodes (OLEDs) surpass the abilities of conventional diodes, demonstrating superior performance, longevity, and manufacturing techniques. [1]. OLEDs stand out for their self-illumination, expansive viewing angles, swift response times, vivid color contrast, low operating temperatures, remarkable color rendering index (CRI), soft and diffuse emission, comprehensive color reproduction, flat design, tailored spectra, robust construction, lightweight and slim profile, flexibility, transparency, straightforward molecular design, utilization of sustainable materials, energy efficiency, user and environmentally friendly features, and low driving voltages [2-3].

Organic electroactive materials undergo thorough synthesis and examination for their potential application in the mentioned devices. Bipolar organic derivatives, in particular, find utility as the emitting layer materials in OLEDs. In our investigation, we introduce new potential emitters incorporating a naphtalimide core as an electron acceptor and carbazole or arylcarbazole fragments serving as electron donors. Several of these newly developed materials exhibit promising electroluminescent characteristics when employed as emitters in OLED devices. The experimental results showed that one incorporating 5 wt% RB-11 emitter demonstrated superior performance, achieving maximum power efficacy of 7.7 lm/W, maximum current efficacy of 7.9 cd/A and maximum external quantum efficiency of 3.3%.

Finally, the synthesized naphtalimide-based compounds exhibit significant promise as efficient green emitters intended for OLED applications. These economically viable materials boast appropriate photophysical, electrochemical, and thermal properties, rendering them well-suited for various display and solid-state lighting uses.

Keywords: organic light-emitting diode, electroactive compound, emitter, naphtalimide-based material.

Acknowledgments: This work was supported by the project funded by the Research Council of Lithuania (Grant No. S-MIP-22-84) and by the Ministry of Science and Technology (MOST), Taiwan (Grant No. 109-2923-M-007-003-MY3).

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

PFOA *vs* PFOS: the Different Responses of Selected Plants to Perfluoroalkyl Compounds (PFAS)

Lidia Błażałek[1*], Angelika Łacwik[1*], Monika Olczyk[1*], Massimo Zacchini[2], Fabrizio Pietrini[2], Anna Wyrwicka-Drewniak[1⁂]

[1]University of Lodz, Faculty of Biology and Environmental Protection, Department of Plant Physiology and Biochemistry, Banacha 12/16, 90-237 Lodz, Poland
[2]Research Institute on Terrestrial Ecosystems (IRET), National Research Council of Italy (CNR), Via Salaria km 29.300, Monterotondo Scalo, 00015 Roma, Italy
[*]Section of Plant Physiology and Ecobiotechnology of the Student Scientific Society of Biologists of the University of Lodz, operating in the Department of Plant Physiology and Biochemistry
[⁂]Scientific supervisor of the Section of Plant Physiology and Ecobiotechnology of the Student Scientific Society of Biologists of the University of Lodz
Corresponding author: lidia.blazalek@edu.uni.lodz.pl

Abstract

Among the "forever chemicals" present in the environment, some of the most widespread are the perfluoroalkyl compounds (PFAS), two of which are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). These compounds can attribute their long half lives to *inter alia* their high carbon-fluorine binding energy. Due to the simultaneous hydrophilic and hydrophobic nature, making them ideal for high-performance surfactants, PFAS are widespread in industry and everyday products; however, due to their toxicity, mobility and bioaccumulation potential, this is a matter of great concern. Furthermore, while PFAS are known to be harmful to human and animal health, their effects on plant growth, development, physiology and biochemistry remain unclear. Hence, the present study examines the effects of PFOA and PFOS on the development of selected monocotyledonous and dicotyledonous seedlings (*Sorghum saccharatum, Lepidium sativum* and *Sinapis alba*) in hydroponic cultivation. The seedlings were germinated and then transferred to liquid medium, without (control) or with the addition of PFOA or PFOS at concentrations ranging from 10 mg/L to 100 mg/L. After seven days of cultivation, they were harvested. The roots and green parts were subjected to morphological examination, and their fresh weight and morphometric parameters (such as root and hypocotyl length) were recorded. In addition, the levels of the assimilatory pigments, e.g. chlorophyll a and b, carotenoids and total phenolic compounds were measured. It was found that PFOS had a stronger negative effect than PFOA. *Sinapis alba* was the most sensitive to PFAS in the medium compared to controls, and *Lepidium sativum* the least. The research was carried out as part of the project titled "Assessment of the capacity of uptake and accumulation of toxic perfluoroalkyl.

Keywords: PFAS, perfluoroalkyl compounds, PFOA, perfluorooctanoic acid, PFOS, perfluorooctane sulfonic acid, phytotoxicity, hydroponic cultivation.

Acknowledgments: The research work was carried out as part of the project titled "Assessment of the capacity of uptake and accumulation of toxic perfluoroalkyl compounds (PFAS) by plants being a component of human diet. Identification of plants' potential for remediation of PFAS contaminated matrices" financed by the University of Lodz, Initiative of Excellence – Research University (IDUB) on the basis of the decision number 64/2021. The exchange of the researchers during the work was granted by the project: "Perfluoroalkyl substances (PFAS) pollution and plants: accumulation and toxic effects, possible transfer into human diet and potential for bioremediation of PFAS-contaminated substrates" co-financed by the Italian Ministry of Foreign affairs and International Cooperation (MAECI) and Polish National Agency for Academic Exchange (NAWA), decision number PPN/BIT/2021/1/00100/U/00001.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

Bicarbazole-Substituted Benzophenone Derivatives as Blue-Emitting Materials for Organic Light-Emitting Diodes

Dovydas Blazevicius[1], Saulius Grigalevicius[1], Daiva Tavgeniene[1], Iram Siddiqui[2], Prakalp Gautam[2], Jayakumar Jayachandran[2], Sushanta Lenka[2], Jwo-Huei Jou[2]

[1]Department of Polymer Chemistry and Technology, Kaunas University of Technology, Radvilenu plentas 19, LT-50254 Kaunas, Lithuania

[2]Department of Materials Science and Engineering, National Tsing Hua University, Hsinchu 30044, Taiwan

Corresponding author: dovydas.blazevicius@ktu.lt

Abstract

The benefits offered by organic light emitting diode (OLED) technologies, such as brightness, viewing angle, contrast ratio, and production cost, are unparalleled by liquid crystal-based displays [1]. Thermally activated delayed fluorescence (TADF) has emerged as a well-established emission mechanism, theoretically capable of achieving 100% internal quantum efficiency [2]. TADF materials are particularly appealing due to their capacity to emit light without relying on noble metal atoms in molecular design [3]. Here, we present novel derivatives based on bicarbazole with a benzophenone fragment. The synthesis was conducted through a three-step synthetic route, by firstly oxidising carbazole with iron (III) chloride to obtain 3,3'-bicarbazole, then substituting one of the amino groups with alkyl chain and another one with benzophenone.

We presented a series of twisted donor-acceptor (D-A) carbazole-benzophenone-based ambipolar compounds as potential TADF emitters for OLEDs. These compounds were synthesized via nucleophilic substitution reactions of 4-fluorobenzophenone with mono-alkylated 3,3'-bicarbazoles. Differential scanning calorimetry (DSC) measurements indicate that the novel materials are amorphous, with glass transition temperatures ranging from 57 °C for the octyl-substituted derivative to 102 °C for the ethyl-substituted compound. Thermogravimetric analysis (TGA) experiments revealed high thermal stability of the benzophenone derivatives, with destruction temperatures exceeding 370°C for all compounds. Photoluminescence quantum yield (PLQY) measurements showed that the materials can achieve elevated PLQY levels ranging from 45.3% to 75.5%. Moreover, the energy levels of HOMO, LUMO, singlet, and triplet states are all suitable for the application of these novel materials as blue-emitting compounds in OLEDs. These properties, combined with good film-forming properties and excellent solubility, resulted in efficient OLED devices with emissive layers containing the synthesized compounds. The most promising performance is observed in OLEDs incorporating 15 wt% of the emitter 4-(9'-{2-ethylhexyl}-[3,3']-bicarbazol-9-yl)benzophenone in the emissive layer. External quantum efficiency reached 2.7%, while power and current efficiencies were, respectively, 4.1 lm/W and 5.7 cd/A. Furthermore, the OLED demonstrate a high maximum luminance exceeding 3500 cd/m². These improved characteristics can be explained by enhanced solubility and film-forming properties for wet-processed OLEDs, facilitated by the introduction of long and branched aliphatic chains in the molecular structure.

Keywords: OLED, organic semiconductor, blue emitter, benzophenone, carbazole.

Acknowledgments: This work was funded by the Research Council of Lithuania (Grant No. S-LLT-19-2).

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Evaluation of Total Phenolic Content, Total Flavonoid Content and Radical Scavenging Activity in Fractions of *Epilobium angustifolium* L. Extracts and their *in Vitro* Anticancer Activity

Jūratė Bliumaitė, Audrius Sigitas Maruška, Domantas Armonavičius, Mantas Stankevičius, Arvydas Kanopka, Ona Ragažinskienė

[1]*Instrumental Analysis Open Access Centre, Faculty of Natural Sciences, Vytautas Magnus University, Vileikos g. 8, LT-44404 Kaunas, Lithuania*

Corresponding author: jurate.bliumaite@vdu.lt

Abstract

Epilobium angustifolium L. is one of the 200 species of medical plant belonging to the Onagraceae family that is populated all over the world. Extracts of *Epilobium* species are studied to determine not only bioactive compounds composition but also its antitumor properties, wound healing properties, and beneficial effect for many other health disorders like gastrointestinal inflammation, benign prostate hyperplasia and others [1]. The aim of this research is to evaluate *Epilobium angustifolium* L. plant extracts fractions bioactive compounds – total phenolic compounds (TPC), total flavonoid content (TFC), and radical scavenging activity (RSA). Extracts were prepared from dried material, from separated parts – blossoms, stems, and leaves. Extraction was done using maceration method with 15 (15/85) and 60 (60/40) methanol and bidistilled water. Four fractions – 0%, 30%, 60% and 100% methanol (v/v) were collected using solid phase C18 cartridge from every extract. TPC, TFC and RSA were determined using spectrophotometric analysis methods. DPPH (2,2 – diphenyl – 1 picrylhydrazyl) radical was used for bioactive compounds with antioxidant properties determination. [2]. Results of analysis were presented using rutin equivalents (mg RE/g) and suggested largest number of phenolic compounds in 30% methanol (v/v) fraction of both extract concentrations (15% and 60% methanol (v/v)) of all *Epilobium angustifolium* L. plant parts, with values from 28.8 mg RE/g to 337.6 mg RE/g. Largest amount of total flavonoids was calculated from 60% methanol (v/v) fraction of all extracts and varied from 1.64 mg RE/g to 22.1 mg RE/g. Quantity of bioactive compounds that has antiradical properties had correlation with phenolic compound amount and the largest amount of antioxidants were determined in 30% methanol (v/v) fraction with values from 58.8 mg RE/g to 181.2 mg RE/g. Anticancer properties of *Epilobium angustifolium* L. extract fractions were estimated by *in vitro* analysis applying MTS assay for HTC116 and Caki-1 cell lines. MTS assay was carried out after 24, 48 and 72 hours with different bioactive compound concentrations. Results suggest that extract concentration and time duration have impact on cancerous cells growth reduction.

Keywords: bioactive compounds, DPPH, antioxidants.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Honey - a Natural Medicine; Its Antimicrobial Activity

Dominika Błońska[1], Bogusław Buszewski[2]

[1]Nicolaus Copernicus University in Toruń, Faculty of Chemistry, Chair of Environmental Chemistry and Bioanalytics, Gagarina 7, 87-100 Toruń, Poland
[2]Prof. Jan Czochralski Kuyavian-Pomeranian Research & Development Centre, Krasińskiego 4, Toruń, Poland
Corresponding author: d.blonska@doktorant.umk.pl

Abstract

Bacterial infections and the development of antibiotic resistance among microorganisms around the world, resulting in a limited selection of drugs for therapy, pose a huge threat to human health and life. In the case of multidrug-resistant pathogens, patient treatment becomes more problematic. The health properties of honey as a natural medicine have been talked about for years, and its antibacterial properties are of particular importance [1]. The composition and diversity of substances and microbiome vary depending on the type of honey and the region it comes from. An important aspect influencing the health properties of honey is also the microbiome, dominated by spore-forming bacteria and yeasts [2]. Research indicates that microorganisms present in honey samples can produce specific metabolites responsible for antimicrobial activity. Identification of honey's microbiome contributes to a better understanding of its microbial landscape and health-beneficial properties, and it is also important from the point of view of environmental protection [3,4]. The aim of the work is to develop conditions for isolation (e.g. capillary electrophoresis) and cultivation of various microorganisms from honey samples from various geographical and botanical regions. The key step is the identification of microorganisms using the MALDI-TOF/MS method and the initial determination of the antibacterial properties of microorganisms contained in honey. This may constitute a contribution to the development of new drugs as potential therapeutic and cosmetic preparations.

Keywords: honey microbiome, pathogens, MALDI-TOF/MS, capillary electrophoresis, bacteria identification.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Investigation of the Electroporation-Induced Bystander Effect using Nanosecond and Microsecond Pulses

Ugnė Borinskytė, Neringa Barauskaitė Šarkinienė, Paulius Ruzgys, Saulius Šatkauskas

[1]Biophysical Research Group, Faculty of Natural Sciences, Vytautas Magnus University, Lithuania
Corresponding author: ugne.borinskyte@stud.vdu.lt

Abstract

In 2020 there were 4 million new cases of cancer diagnoses and almost 1,9 million cancer-related deaths in the European Union [1]. At the same time, 19,35% of all the deaths in Lithuania that were reported were due to cancer [2]. Therefore, new cancer treatment techniques are highly desired. Electrochemotherapy is a relatively recent treatment modality that enhances the uptake of anticancer drugs by the cells when combining electric fields with chemotherapy. During treatment, the anticancer drug bleomycin and the calcium used in calcium electroporation can be added into the cell's cytoplasm [3].

Cell electroporation uses electrical pulses with the duration between microseconds and nanoseconds. Electroporation usually is occurring when applying electrical pulses of around 100 µs (intensity 1-1,6 kV/cm) or 100-300 ns (10-19 kV/cm). It is thought that classical electroporation and nanosecond electroporation can have rather different impacts on the cell [3].

Ruzgys et al. [4] used microsecond electric fields to apply electrochemotherapy. Additionally, they were able to demonstrate the use of a bystander effect where neighbouring cells are indirectly impacted when chemotherapy drugs are electroporated into them. Two publications were found suggesting that the bystander effect is possible after electrochemotherapy or electroablation [4]. In any case, the impact of nanosecond electrical fields on this impact is still unexplored.

The investigation is focused on the bystander effect following *in vitro* calcium electroporation and bleomycin electrotransfer. For such experiments, three cell lines had been used: mouse mammary cancer cells (4T1), human adenocarcinoma alveolar basal epithelial cells (A-549), and Chinese hamster ovary cells (CHO-K1). The bystander effect occurs using an identical methodology following both electroporation treatments ($CaCl_2$ (1 mM) and BLM (20 nM). One electric pulse with an amplitude of 1400 V/cm and a length of 100 µs was applied for electroporation using microsecond electric fields. Also, the cells were exposed to 10 200 ns duration nanosecond pulses with electric fields of 13 kV/cm. Using a flow cytometer, we were able to see the diffusion of propidium iodide into the cells, which worked as a fluorescent agent to differentiate between living and dead cells. Cell viability was evaluated as well using a colony assay using crystal violet dye.

With nanosecond electroporation, there was little to no decrease in cell viability observed with either calcium electroporation or bleomycin electrotransfer. An exception was found in A-549 cells, where bleomycin electrotransfer resulted in zero cell viability (using the clonogenic assay). Following both treatments with calcium and bleomycin, there is a notable drop in cell viability as compared to the control following microsecond electroporation. After microsecond electroporation, a bystander effect (lower cell viability) was observed between CHO-K1 and 4T1 cell cultures; however, after nanosecond electroporation using the clonogenic assay this impact was not observed.

Keywords: electroporation, bystander effect, bleomycin electrotransfer, calcium electroporation**.**

Acknowledgments: Funded by project No. S-MIP-23-124.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Total Phenolic Content and Antioxidant Activity in Buckwheat Honey from Lithuania

Ernesta Zaksaitė[1], Mindaugas Liaudanskas[1,2], Sonata Trumbeckaitė[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Lithuanian University of Health Science, Sukilėlių av. 13, LT- 50162 Kaunas, Lithuania
[2]Institute of Pharmacuetical Technologies of the Faculty of Pharmacy of Lithuanian University of Health Science, Sukilėlių av. 13, LT-50162 Kaunas, Lithuania

Corresponding author ernesta.zaksaite@stud.lsmu.lt

Abstract

Background: Honey is one of the most valued natural products. Honey is used not only as a nutritional product, but also as an alternative remedy for improvement of health. Honey has antioxidant properties due to its chemical composition, mainly phenolic compounds. The chemical composition and biological properties of honey highly dependent on the source of the floral origin and on the qualitative and quantitative composition of compounds. Therefore, it is important to investigate the phytochemical composition and biological properties of honey samples obtained from different regions of Lithuania.
Aim: to determine the total phenolic content (TPC) and the antioxidant activity *in vitro* of monofloral buckwheat honey samples, collected in different regions of Lithuania.
Methods: buckwheat honey samples were colected in 2023 from six different apiaries. They were located in Trakai, Alytus, Panevėžys, Druskininkai and Varėna districts. 1 g honey was dissolved in 10 ml 70 % ethanol. TPC was investigated by Folin-Ciocâlteu assay, antioxidant activity *in vitro* by ABTS and CUPRAC assays [1]. Statistical analysis was performed using Microsoft Office Excel 2023 (Microsoft, Redmond, Washington, USA) and SPSS 29.0.2 (Chicago, Illinois, USA).
Results: TPC determined in the buckwheat honey samples ranged between 0.46±0.01 mg GAE/g and 1.94±0.03 mg GAE/g. The highest TPC was evaluated in a sample, that was collected in north-eastern part of Varėna district and in Trakai district (1.94±0.03 and 1.89±0.04 mg GAE/g, respectively). The lowest TPC was found in a sample collected from Panevėžys district (0.46±0.01 mg GAE/g, $p<0.05$). The reducing activity of buckwheat honey varied between 1.22±0.02 µmol TE/g and 3.61±0.24 µmol TE/g The highest reducing activity *in vitro* using CUPRAC assay was determined in a sample from north-eastern part of Varėna (3.61±0.241 µmol TE/g, $p<0.05$). The lowest reducing activity was detected in a sample from Druskininkai district (1.22±0.02 µmol TE/g, $p<0.05$). The *in vitro* ABTS test indicated that the sample from the Trakai region had also the highest antiradical activity (75.45±3.52 %, $p<0.05$) comparing to other samples.
Conclusion: the results of our research have shown that all investigated samples of buckwheat honey contain polyphenols and possess antioxidant (both, antiradical and reducing) activities. The total phenolic content in the buckwheat samples depends on the floral source and geographical origin of honey. The highest reducing and antiradical activity *in vitro* and the highest total phenolic content contained buckwheat honey collected from southern (Varėna district) and southeastern (Trakai district) part of Lithuania.

Keywords: honey, total phenolic content, antioxidant activity, ABTS, CUPRAC, buckwheat honey.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Enhancing Oral Chemotherapy: Synthesis and Analysis of Lactoferrin-Cisplatin Complexes

Paweł Fijałkowski[1], Oleksandra Pryshchepa[2], Paweł Pomastowski[2], Katarzyna Rafińska[1]

[1]Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University in Torun, J. Gagarina 7, 87-100 Toruń, Poland
[2]Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University in Torun, Wileńska 4, 87-100 Toruń, Poland

Corresponding author: katraf@umk.pl

Abstract

Platinum-based drugs are highly effective in treating a broad spectrum of cancers; however, they are currently limited to being administered via intravenous injections [1]. This method of delivery comes with several drawbacks, including a rapid spike in drug levels in the bloodstream, exceeding the maximum safe concentration, followed by a swift decline below the effective therapeutic threshold. The challenge lies in developing an approach that enables the effective absorption of these drugs in the gastrointestinal tract when taken orally. The method should aim to accurately target the drugs to the intestinal epithelial cells while minimizing side effects, such as the chemical reactivity of platinum ions and their compounds, as well as gastrointestinal issues. In our study we have developed solution that involves using a carrier that can both be recognized by intestinal epithelial cells and potentially protect the digestive system from harmful effects. Lactoferrin (LTF) was used as the carrier for platinum compounds.

The aim of the study was to develop a method for synthesizing the lactoferrin-cisplatin complex and then to conduct its physicochemical characterization. The complexes were synthesized in two different buffers. Various analytical techniques, such as FTIR, Raman, and UV-Vis spectroscopy, were used to characterize the resulting compounds. Inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis was performed to determine the amount of bound platinum. Additionally, MALDI-TOF MS and SDS-PAGE studies were conducted, along with dynamic light scattering (DLS) particle size measurements. The cytotoxicity of the obtained complexes was also evaluated using the MTT assay on two cell lines, Caco-2 and L929.Full abstract (one page) should fit in the same page.

Keywords: bovine lactoferrin, cisplatin, chemotherapy, instrumental methods, cytotoxicity.

Acknowledgments: K.R., P.P. and O.P. are members of Toruń Center of Excellence “Towards Personalized Medicine” operating under Excellence Initiative-Research University.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Concentration and Diversity of Micromycete Species in Raw Material of *Aegopodium podagraria* L.

Ugnė Gabrytė, Rūta Mickienė, Audrius Sigitas Maruška

Instrumental Analysis Open Access Centre, Vytautas Magnus University, Kaunas, Lithuania
Corresponding author: ugne.gabryte@stud.vdu.lt

Abstract

World Health Organization states that approximately 80% of the global population relies on various medicinal plants and spices that are collected in forests or other areas, for treatment of their health issues. However, several environmental factors, such as suitable temperature, rainfall and relative humidity in the collection area, can induce the proliferation of various microscopic fungi [1]. Although some of them form symbiotic relationships with plants or their roots, they are considered a microbial hazard because they synthesize secondary metabolites and mycotoxins that could cause problems both economically and for human or animal health [2]. Therefore, it is necessary to examine collected herbs, in order to ensure their quality.

The aim of this research was to determine micromycete species, associated with different parts of *Aegopodium podagraria* L. and their concentrations. In this experiment different parts of plant (leaves, stems and roots) were cut into 1-2 cm pieces and placed symmetrically onto a MAE medium in a Petri dish. The plates with plant parts were incubated at a thermostat at 25 °C for 5–7 days. After a week, the growth of molds and yeasts was analysed and micromycetes were identified morphologically. Nine species of micromycetes were determined in leaves, stems and roots of *A. podagraria*: *Trichoderma vidire*, *Alternaria alternata*, *Penicillium olsonii*, *Phytophthora nicotianae*, *Aspergillus glaucus*, *Aspergillus terreus*, *Aspergillus niger*, *Candida krusei*, *Cryptococcus neoformans.* To determine the quantity of colony forming units, dilution method was used, and the concentration of micromycetes was calculated based on the dilution factor. Micromycetes with the highest concentrations in leaves and roots were *Trichoderma viride* (9125 cfu/g and 850 cfu/g respectively), in stems - *Candida krusei* (950 cfu/g). This research helped to determine the distribution of micromycetes in *Aegopodium podagraria* L. raw material and estimate potential risks, associated with it.

Keywords: *Aegopodium podagraria*, raw material, fungi, yeasts, micromycetes.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Determination of Volatile Organic Compounds in Refill Fluids of Electronic Cigarettes by Headspace Solid-Phase Micro Extraction and Gas Chromatography–Mass Spectrometry

Radvilė Gaučaitė, Kristina Bimbiraitė-Survilienė, Mantas Stankevičius

Instrumental Analysis Open Access Centre, Vytautas Magnus University, Vileikos 8, Kaunas LT-44404, Lithuania
Corresponding author: radvile.gaucaite@stud.vdu.lt

Abstract

An optimized analytical method was employed to detect volatile organic compounds (VOCs) in refill fluids of electronic cigarettes (EC) using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS). The GC-MS conditions, including injector, oven, interface, and ion source temperatures, were optimized for the analysis of volatile compounds in EC refill fluids. The experimental parameters for GC-MS were as follows: the injector temperature was set to 280 °C, the oven temperature program initiated at 40 °C for 2 minutes, then ramped at 8 °C/min up to 80 °C, followed by a rapid ramp at 60 °C/min up to 250 °C. The interface and ion source temperatures were maintained at 260 °C and 270 °C, respectively. Samples underwent incubation at 50 °C for 5 minutes and were then extracted for 20 minutes using a SPME fiber composed of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), which proved to be the most effective for analyte extraction [1,2].

The optimized method was applied to study the volatile profile of 19 EC refill fluid samples with different aromas. In total, 68 compounds were identified. The dominant compounds were esters (benzyl acetate, linalyl acetate), aldehydes and ketones (menthol, vanillin), terpenes and terpenoids (limonene, camphor, linalool) and carboxylic acids (acrylic acid, valeric acid). The results substantiated that the optimized HS-SPME GC-MS method could provide an efficient and convenient approach to study the flavor characteristics of EC refill fluid.

Keywords: solid phase microextraction, electronic cigarettes, refill fluid, flavor, HS-SPME, GC-MS.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Unravelling the Potential of Phenothiazine and Tetraphenylethylene as Emitters and HTMs for Efficient Doping Free Fluorescent and Multiple-Resonance TADF OLEDs

Melika Ghasemi[1], Ramakant Gavale[2], Faizal Khan[2], Dmytro Volyniuk[1], Juozas Vidas Grazulevicius[1], Rajneesh Misra[2]

[1]Department of Polymer Chemistry and Technology, Kaunas University of Technology, K. Baršausko g. 59, 51423 Kaunas, Lithuania

[2]Department of Chemistry, Indian Institute of Technology Indore, Khandwa Rd, Simrol, Madhya Pradesh 453552, India

Corresponding author: melika.ghasemi@ktu.edu

Abstract

Since the invention of organic light-emitting diodes (OLEDs) in 1987 by C. W. Tang and S. A. Vanslyke, considerable advances have been made towards achieving high efficiency in solid-state lighting and display technologies through intensive research [1]. According to spin statistics, by electronic excitation singlet and triplet excitons are theoretically generated in a 1:3 ratio, where triplet excitons are lost as heat or through other non-radiative relaxations, and only singlet excitons are converted into photons, utilizing 25% of the exciton generation factor, leading to an external quantum efficiency (EQE) of about 5%-7.5% in devices [2–4]. The efficiency and stability of OLEDs depends on factors such as photoluminescence quantum yield (PLQY), internal quantum efficiency (IQE), electron-hole transportation and recombination, energy levels of each implemented layers in the device [5]. Twisted organic compounds play a significant role in the development of solid-state fluorescent materials and have gained substantial attention owing to their potential applications in optoelectronic devices [6]. A series of novel metal-free twisted organic compounds integrating phenothiazine and tetraphenylethylene moieties were designed, synthesized, and characterized to ascertain their viability as blue/green emitters as well as hole transporting layers (HTLs) within OLEDs. The study delved into discerning the impact on the efficiencies of the devices based on the presence and absence of donor moiety. The photophysical studies unveiled that all the compounds exhibit fluorescence, photoluminescence quantum yields of up to 39.64%, commendable thermal stability and proficient hole transporting capabilities (1.56×10^{-4} cm^2/Vs and 1.27×10^{-4} cm^2/Vs at 5×10^5 V/cm electric field), thus rendering them suitable for OLED applications. The compounds were evaluated as emissive layers within doping-free fluorescent devices, achieving an EQE of 4.33% with a turn on voltage of 3.60V with near zero efficiency roll-offs at 1000 cd/m^2. Furthermore, the compounds were also used as HTLs in multiple-resonance thermally activated OLEDs, yielding EQEs of up to 7.45% and exhibiting a low turn-on voltage of 4.60V.

Keywords: Organic light-emitting diodes, fluorescence, hole transporting, phenothiazine, tetraphenylethylene.

Acknowledgments: This project has received funding from the Research Council of Lithuania (Project "ELOS" No S-MIP-21-7).

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Nutritional Composition of *Tenebrio molitor* Larvae Reared on Substrates Derived from By-Products

Agnė Jankauskienė, Aistė Kabašinskienė

Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Tilzes St. 18, Kaunas LT-47181, Lithuania

Corresponding author: agne.jankauskiene@lsmu.lt

Abstract

The larvae of *Tenebio molitor* are increasingly recognized as a sustainable protein source, offering a potential solution to the challenges of food waste, essential amino acids, omega acids, and unsaturated fatty acids [1]. In pursuit of economic, safe, and sustainable practices, there is a growing interest in rearing mealworms using by-products from production and agriculture. The impact of these by-products on mealworms varies based on the chosen raw materials and their proportions [3]. Our study aims to identify local by-products or food residues suitable for mass larval production, specifically focusing on sprouted and green potatoes, wheat bran, and by-products from brewers' production as a comprehensive substrate for rearing mealworms. Conducted at the "DIVAKS" company in Lithuania, the larvae were grown, lyophilized, and subjected to tests in an accredited laboratory. The results showed that the highest protein content (59.18 ± 0.07 %) was observed in samples grown on brewers' spent grain, while wheat bran yielded the highest fat content (34.22 ± 0.491 %). The amount of FAs in the larvae was influenced by the substrate used, with variations in monounsaturated, omega-3, and oleic acids. Notably, wheat bran showed the highest content of total polyunsaturated FAs (36.23 %). In the analysis of 16 distinct amino acids, a sample with brewers' spent grain, consistently demonstrated the highest content in 11 instances. This adaptation renders the larvae suitable for diverse purposes, including animal or human nutrition and health enhancement. In conclusion, our study underscores the significance of substrate optimization in harnessing the full potential of *Tenebio molitor* larvae for targeted applications.

Keywords: mealworms, fatty acid, amino acid, sustainability.

Acknowledgments: We would like to thank the "Divaks" company for providing mealworm larvae for research.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Antioxidant Effect of Silver Nanoparticles on Mouse Hepatoma *MH-22A* Cells and Their Electroporation

Laura Kairytė, Raminta Rodaitė

Department of Biology, Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania
Corresponding author: laura.kairyte@stud.vdu.lt

Abstract

Electroporation is a process in which short electrical pulses create pores in the plasma membrane through which various molecules and ions can enter cells [1]. The electrical impulses cause stress to the cells, resulting in the production of reactive oxygen species [2]. Usage of chemotherapy drugs during cancer treatment can also cause oxidative stress because mechanisms of action of many of these drugs promote the production of reactive oxygen species and other free radicals [3]. Reactive oxygen species can break important organic molecules [4]. Free radicals are neutralized by antioxidants [5]. Silver nanoparticles also have antioxidant properties [6]. It is important to understand the cellular effects of silver nanoparticles in the presence of cellular oxidative stress, as oxidative stress complicates cancer treatment [3]. In this work the antioxidant effect of silver nanoparticles on the viability and electroporation of mouse hepatoma *MH-22A* cells was investigated. It was established that reactive oxygen species are formed during electroporation of mouse hepatoma *MH-22A* cells. By increasing the intensity of the electric field, cell viability decreases due to the formation of reactive oxygen species. The damage caused by active forms of oxygen is reduced by antioxidant silver nanoparticles. Silver nanoparticles, acting as free radical scavengers, increase cell viability both after hydrogen peroxide-simulated oxidative stress and after electroporation.

Keywords: electroporation, silver nanoparticles, amplex red, mouse hepatoma cells.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

New Carbazole-Substituted Diphenyl Sulfones as Host Materials for Solution Processed TADF OLEDs

Mantas Kirstukas[1], Sujith Sudheendran Swyamprabha[2], Kiran Kishore Kesavan[2], Iram Siddiqui[2], Dovydas Blazevicius[1], Jayakumar Jayachandran[3], Marius Eidimtas[1], Sandhya Rani Nayak[4], Mangey Ram Nagar[2], Rohit Ashok Kumar Yadav[2], Gintare Krucaite[1], Sivakumar Vaidyanathan[4], Saulius Grigalevicius[1], Jwo-Huei Jou[2]

[1]Department of Polymer Chemistry and Technology, Kaunas University of Technology, Radvilenu Plentas 19, LT50254, Kaunas, Lithuania
[2]Department of Materials Science and Engineering, National Tsing Hua University, Hsinchu, 30013, Taiwan
[3]Department of Chemistry, National Tsing Hua University, Hsinchu, 30013, Taiwan
[4]Department of Chemistry, National Institute of Technology Rourkela, India

Corresponding author: mantas.kirstukas@ktu.lt

Abstract

The advantages that organic light emitting diode (OLED) based technologies offer in terms of brightness, viewing angle, contrast ratio, production cost, etc. are not rivalled by liquid crystal-based displays [1]. Thermally activated delayed fluorescence (TADF) is a now well-known emission mechanism which make it theoretically possible to achieve 100% internal quantum efficiency [2]. While TADF emitters have been extensively reported, the design and development of potential host materials are overlooked. In this work, a series of diphenyl sulfone-based solution-processable host materials were utilized to fabricate green TADF OLEDs. Diphenyl sulfone-based objective materials are: bis(4-(3-phenylcarbazol-9-yl)phenyl)sulphone (ME4), bis(4-(3-naphthylcarbazol-9-yl)phenyl)sulphone (ME5); bis{4-[3-(4-carbazol-9-ylphenyl)carbazol-9-yl]phenyl} sulphone (ME9), bis(4-[3-(4-trifluoromethylphenyl)carbazol-9-yl]phenyl)sulphone (ME10) and 4,4'-bis[3-(1-naphtyl)carbazol-9-yl] benzophenone (ME13).

Among the five materials, ME9 showed best performance. The resultant device exhibited a maximum power efficacy of 40.0 lm/W, current efficacy of 44.7 cd/A, external quantum efficiency of 14.1%, and maximum luminance of 16,220 cd/m^2. The better performance of ME9 may be attributed to the low ΔE_{ST} and the unique porous morphology of the host material, which may improve the carrier mobility and facilitate efficient electron injection into the emission layer. In addition, the higher PLQY (88.56 ± 2%) value of ME9 doped with 4CzIPN emitter also played a crucial role in enhancing the performance of OLED.

Keywords: TADF, OLED, organic synthesis, host material, carbazole.

Acknowledgments: This work was funded by the Research Council of Lithuania (Grant No. S-LLT-19-2).

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Influence of Lighting Conditions and Soil Supplementation with Trichoderma Fungi and Polymers Used in the Food Industry on the Growth of Herbs and the Profile of Biologically Active Compounds in *Thymus vulgaris* and *Thymus serpyllum*

Liliana Kozlowska[1], Kamila Kulbat-Warycha[2], Justyna Nawrocka[1], Dorota Żyżelewicz[2]

[1]Katedra Fizjologii i Biochemii Roślin, Wydział Biologii i Ochrony Środowiska, Uniwersytet Łódzki, ul. Banacha 12/16, 90-237 Łódź

[2]Instytut Technologii i Analizy Żywności, Wydział Biotechnologii i Nauk o Żywności, Politechnika Łódzka, ul. Stefanowskiego 4/10, 90-924 Łódź

lilianakozlowska@gmail.com

Abstract

The research concerned the impact of various lighting conditions and soil supplementation with polymers used in the food industry and polymers in combination with *Trichoderma* fungal spores on the growth and development of herbs - *Thymus vulgaris* and *Thymus serpyllum*. The metabolic analysis focused on detecting changes in the content of plant pigments, such as chlorophyll a and b, anthocyanins and carotenoids, and the content of biologically active compounds, including phenolic compounds (including flavonoids), terpenoids and volatile organic compounds with health-promoting properties. The research aimed to assess the impact of environmental conditions on the growth and chemical composition of selected plants and to consider ways to optimize the cultivation of these herbs in order to improve the quality and efficiency of the production of bioactive ingredients.

Under the influence of additional lighting (174 and 600 µmol/m^{2}s), the growth of *T. vulgaris* and *T. serpyllum* was significantly accelerated. Herbs belonging to both varieties showed greater biomass and shoot length, and in the case of *T. serpyllum*, an increase in the content of carotenoids and anthocyanins was observed. With regard to secondary metabolites, the most pronounced changes were observed in the flavonoid content and total antioxidant capacity, which increased significantly under the influence of additional lighting. Simultaneous or separate application of *Trichoderma* spores and polymers resulted in an increase in the content of flavonoids in the leaves of both thyme species. The increase in terpenoid content was related to the effect of additional lighting, *Trichoderma* spores and polymers. The nature of these changes depended on the thyme species. Using an electronic nose method, eight volatile compounds produced by *T. vulgaris* and *T. serpyllum* were tentatively identified: α-pinene, myrcene, α-terpinene, γ-terpinene, 1,8-cineole (eucalyptol), thymol, carvacrol and eugenol. Additional lighting resulted in an increase in the percentage of thymol and γ-terpinene in the total pool of volatile compounds. The results also showed a positive effect of polymers and, to a lesser extent, *Trichoderma* fungi on the increase in the content of volatile compounds with health-promoting properties in vapors, especially thymol and γ-terpinene.

Keywords: *Thymus vulgaris*; *Thymus serpyllum*; *Trichoderma*; food polymers.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

An Evaluation of Food Waste Treated with Physical and Biotechnological Methods

Ieva Krivaitė, Vilma Kaškonienė, Audrius Maruška

Instrumental Analysis Open Access Center, Faculty of Natural Sciences, Vytautas Magnus University, Vileikos g. 8, LT-44404 Kaunas

Corresponding author: ieva.krivaite@stud.vdu.lt

Abstract

During food processing, a large amount of food waste is generated. Some of it can be used as fertilizers, integrated into animal feed, or composted. However, most of these wastes are usually discarded, which not only endangers the environment, but also results in the loss of valuable compounds. Food waste, such as peels, shells, husks, skins, pomace, stems, etc., typically contains a higher concentration of biologically active compounds compared to edible parts [1]. These wastes are also rich in carbohydrates, such as sucrose, starch, lignocellulose. Additionally, food waste is inexpensive and produced in large quantities, making it an excellent biomass alternative for producing valuable products. Biologically active extracts derived from food waste can be used as food additives or applied in pharmaceutical industry. Furthermore, bioethanol can be obtained through the fermentation of this biomass.

Due to the highly variable composition of food waste as a biomass, no single method can be universally applied. To obtain value-added compounds and maximize bioethanol yield, pretreatment of food waste is necessary. Preferably, environmentally friendly methods, such as enzyme hydrolysis or ultrasound should be utilized for pretreatment.

Four different food wastes were selected for this study: pomelo (Citrus grandis) peel (flavedo, albedo), peanut (Arachis hypogaea) shells, and onion (Allium cepa L.) shells. The aim was to evaluate the potential of these food wastes by subjecting them with three different pretreatment methods (ultrasound, enzyme hydrolysis, and combined treatment), all using water as a solvent. After extraction, the remaining sediment was treated with 75% methanol. The total content of phenolic compounds (TPC), flavonoids (TFC), and antiradical activity (DPPH) were determined for both water-based and methanolic extracts.

To evaluate bioethanol potential of food waste, pretreated food wastes were fermented by Saccharomyces cerevisiae, and the resulting bioethanol content was evaluated using gas chromatography with flame ionization detector.

The results indicated that onion shells had the highest TPC and TFC (103.44 ± 5.06 and 16.37 ± 0.81 mg/g, respectively), whereas peanut shells – the lowest (4.18 ± 0,20 and 0.54 ± 0.01 mg/g, respectively). Pomelo albedo and flavedo peel parts displayed comparable levels of these compounds. Optimal extraction of TPC and TFC occurred when ultrasound and enzyme hydrolysis were used combined. Onion shells also had the highest antiradical (DPPH) activity (55.15 ± 1.13 mg/g), while peanut shells, pomelo albedo and flavedo parts had much lower values (1.43-2.64 mg/g). The highest bioethanol yield (0.4%) was obtained from pomelo albedo peel part when treated with combination of ultrasound and enzymes. The lowest bioethanol yield (0.05%) was from onion shells treated solely with enzymes.

Keywords: food waste, bioactive compounds, bioethanol.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Exploring the Physicochemical and Sensory Profiles of Hybrid Spreadable Cheese Analogues Incorporating White Kidney Beans and Cow's Milk

Ibaratkan Kurbanova[1], Lina Lauciene[2], Loreta Serniene[2], Mukarama Musulmanova[1]

[1]Kyrgyz State Technical University, 66, Chyngyz Aitmatov ave, 720044 Bishkek, Kyrgyz Republic
[2] Lithuanian University of Health Sciences, Veterinary Academy, Department of Food Safety and Quality, Tilžės str. 18, LT-47181 Kaunas, Lithuania

Corresponding author: ibaratk@gmail.com

Abstract

As consumer interest grows in plant-based alternatives to traditional dairy products, incorporating legumes like white kidney beans presents a promising avenue for developing nutritious and flavourful cheese substitutes. The aim of our study was to explore the physicochemical and sensory attributes of hybrid spreadable cheese analogues formulated by blending white kidney beans with cow's milk at different ratios.

Dry matured white kidney beans (*Phaseolus vulgaris*) were sourced from a local market in Talas, Kyrgyzstan with a protein content of approximately 18%. The beans were soaked overnight in tap water at room temperature, drained, rinsed, and ground with water. The resulting bean slurry was filtered to obtain white kidney bean milk, which was then stored at 4°C. To prepare fresh spreadable cheese, cow's milk and white kidney bean milk were mixed in various proportions (0%:100%, 30%:70%, 50%:50%, 70%:30%, 100%:0%) and pasteurized at 95°C for 15 minutes. Curd was obtained by adding $CaCl_2$ 40% solution to the hot milk blend, followed by draining the whey and distributing the curd into portions. The portions were sealed and stored at 4°C.

The production of suitable spreadable cheese was achieved only when using blends containing 50% or less bean milk. Experimental cheeses incorporating bean milk exhibited significantly higher spreadability compared to control cheese made solely from milk. Moreover, among the cheeses with bean milk added, we observed that spreadability increased with higher amounts of bean milk in the mixture, attributed to the higher moisture content in such cheeses. Additionally, cheese samples containing bean milk showed higher acidity levels compared to the control sample made with cow's milk. While no notable variations were observed in the L* coordinate (brightness), the cheese samples with bean milk displayed a more pronounced reddish hue in the a* coordinate, contrasting with the control sample, which exhibited more bluish and yellowish tones in the b* coordinate. Flavor differences were noted by panelists between the experimental cheese samples and the control sample; however, overall acceptability did not significantly differ among the samples.

These findings suggest that cheeses made from blended milk compositions can offer unique characteristics and may appeal to consumers who appreciate diverse flavor profiles and textures in cheese products. The acceptability of these cheeses demonstrates their potential for commercial production and consumption.

Keywords: white kidney bean, cow milk, blends, spreadable cheese analogues.

Acknowledgments: This work was supported by funding of GIZ project “Vocational training for economic growth sectors in Central Asia”, No. 2023-PRO-000999; No. 81295537)

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Comparison of Antioxidant Activity in Flowers, Leaves and Stems of *Lythrum salicaria* L. by ABTS and FRAP Assays

Goda Lukoševičiūtė, Audronis Lukošius

[1]*Department of Pharmacognosy at the Faculty of Pharmacy, Lithuanian University of Health Sciences, Sukilelu av. 13, Kaunas LT-50161, Lithuania*

Corresponding author: goda.lukoseviciute1@stud.lsmu.lt

Abstract

Lythrum salicaria L. – is a herbaceous perennial plant of the *Lythraceae* family, native to Europe, Asia and Northern Africa [1, 2]. The plant is widely used for the treatment of inflammatory diseases such as dysentery, chronic intestinal catarrh and eczema. *In vitro* and *in vivo* studies have shown that extracts of the aerial part of *Lythrum salicaria*, obtained using various solvents, have a wide range of therapeutic effects, such as anti-inflammatory, hypoglycaemic, antimicrobial, antioxidant and anticancer [3-5]. The aim of present study is to evaluate the antioxidant activity by comparing the response of two assays using different parts of the aerial part of *L. salicaria*. The two spectrophotometric assays selected were free radical scavenging activity assay (ABTS) and Ferric ion Reducing Antioxidant Power assay (FRAP). It was found that the FRAP assay produces higher results of antioxidant activity in all extracts compared to the ABTS. Leaf extracts shown the highest values ($p<0.05$) of antioxidant activity in both used assays (respectively 11.20±1.73 µmol TE/g and 5.80±1.38 µmol TE/g). Flower extracts antioxidant activity varied from 10.86±0.57 µmol TE/g to 7.45±0.28 µmol TE/g by the FRAP assay and from 6.01±0.18 µmol TE/g to 3.32±0.76 µmol TE/g by ABTS. The weakest antioxidant activity was obtained in stem extracts, ABTS – 3.73±0.45 µmol TE/g and FRAP – 7.16±1.08 µmol TE/g.

Keywords: *Lythrum salicaria* L., flowers, leaves, stems, antioxidant activity, ABTS, FRAP.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Comparative Analysis of Physicochemical and Sensory Characteristics of Kurut Drink Based on Different Types of Dairy Whey

Askar M. Mametjanov[1,3], Loreta Serniene[2], Lina Lauciene[2], Agne Vasiliauskaite[2], Kristina Kondrotiene[2], Mukarama Musulmanova[3]

[1]Alaiku Organics Company, Osh, Kyrgyzstan
[2]Lithuanian University of Health Sciences, Department of Food Safety and Quality, Kaunas, Lithuania
[3]Kyrgyz State Technical University, Bishkek, Kyrgyzstan

Corresponding author: mametask@gmail.com

Abstract

The study aimed to explore the possibility of replacing the primary production ingredient of the Kurut beverage, drinking water, with dairy whey, which in Kyrgyzstan is an unprocessed waste product of dairy manufacturing. This approach considered the potential for: a) conserving drinking water as a valuable resource in Central Asia, b) valorizing dairy whey and its permeate, c) enhancing the nutritional properties of the final product.

Kurut, as the main ingredient of the beverage, was selected from a random batch at a manufacturing plant in Kyrgyzstan. Ground Kurut was mixed in a 1:10 ratio in various liquid mediums: distilled water, acid whey, acid whey permeate, sweet whey, and sweet whey permeate. To closely replicate the production process at the manufacturing plant in Kyrgyzstan, all samples underwent homogenization, pasteurization, and cooling. The selected samples were stored at a temperature of 5 degrees Celsius in tightly sealed glass containers. Physicochemical and sensory parameters of the original water-based Kurut drink were measured against samples made with acid and sweet wheys and their permeates.

During the study, it was found that within the CIELAB color system, all samples did not show significant differences in the L*, a*, and h* coordinates. However, notable differences were observed in the b* and c* coordinates, especially in the sample based on sweet whey permeate, which is attributed to the yellow color of the permeate. This observation was also confirmed by panelists during sensory analysis.

The pH and acidity levels varied among all samples. Nonetheless, panelists did not notice a significant difference in terms of acidic smell or taste between the water-based samples, acidic whey, and acidic whey permeate. Only in the sample based on sweet whey permeate did panelists note an excessively sweet taste. In terms of overall acceptability, the sample based on sweet whey was much closer to the original water-based sample than the sweet permeate was.

Nutritional analysis revealed a significant difference between the original water-based sample and all other samples ($p<0.05$) in terms of an increase in protein, fat, and carbohydrate content.

Based on the study's results, it can be concluded that replacing water with acid whey and its permeate supports the hypothesis of whey valorization and the enhancement of the final product's nutritional properties. While the hypothesis of using sweet whey permeate was rejected based on the study results, the use of sweet whey remains viable.

Keywords: kyrgyz kurut, kurut drink, kurut beverage, milk whey, circular economy, zero waste, valorization, Kyrgyzstan.

Acknowledgments: This work was supported by funding of GIZ project "Vocational training for economic growth sectors in Central Asia", No. 2023-PRO-000999; No. 81295537)

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Effect of Newly Synthesized Compounds on Gene Expression in Cancer Cells

Aidan Marmion[1], Inga Pečiulienė[2]*

[1]Department of Immunology, Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio Ave. 7, Vilnius LT-10257, Lithuania
[2]Department of Biological DNA modification, Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio Ave. 7, Vilnius LT-10257, Lithuania
Corresponding author: inga.peciuliene@bti.vu.lt

Abstract

Oncological diseases remain one of the most urgent public health problems. Despite great efforts, the number of patients suffering from oncological diseases is very high [1]. The latest technologies provide all the conditions to synthesize chemicals quickly and in large quantities, only about 5% of newly synthesized molecules for chemotherapy are confirmed as clinically appropriate [2]. For these reasons, there is a great need for new medicinal compound, as the synthesis of safe, effective and suitable chemical compounds is a major challenge.

Cancer can be described as a disease of altered gene expression. Cancer genes are involved in the dysregulation of a broad array of normal cellular functions including cell proliferation, cell–cell communication, angiogenesis, DNA repair, chromosome stability, tumor invasion, metastasis, and apoptosis [3].

A current goal in molecular medicine is the development new compounds to interfere with gene expression in living cells in the hope that novel therapies for human disease will result from these efforts. To achieve the same goals, our study investigates the effect of a group of newly synthesized chemical compounds on the gene expression of cancer cells.

Keywords: synthesized compounds, gene expression, cancer cells.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Auxin Effect on *in Vitro* Growth of Larch Endophytic Fungus

Yana Martynova, Jonas Žiauka

Vytautas Magnus University, Faculty of Natural Sciences, Universiteto g. 10, Akademija, Kaunas District, Lithuania
Corresponding author: yana.martynova@vdu.lt

Abstract

Plant hormones are chemical messengers that, acting in micromolar or even smaller concentrations, control plant growth and development. They include auxins, cytokinins, gibberellins, abscisic acid, ethylene, and a few other chemical compounds. Plant hormones are produced not only by plants but also by various microorganisms and fungi [1]; however, the effect of plant hormones on the growth and development of fungi is relatively poorly researched, with data coming only from several fungal species [2-3].

In this study, the effect of auxins indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) was investigated on an endophytic fungus, isolated from surface-disinfected European larch (*Larix decidua*) shoot buds. The fungus, after being isolated from larch explants, was grown *in vitro* on an artificial nutrient medium in Petri plates. For experiments, the fungus was planted either on the medium containing auxins (1 µmol/L and 3 µmol/L of IAA or IBA), or on the hormone-free medium shared by apical or lateral larch buds in the same Petri plate. Hormone-free medium without larch explants was used as control in these experiments.

The results showed that the growth of fungus was not significantly affected by larch explants planted in the same Petri plate; meanwhile, both auxins had negative effect on fungal growth. However, the effect of IAA was significant only at concentration of 3 µmol L^{-1}; in contrast, both concentrations of IBA significantly diminished the average size of fungal colony on the nutrient medium, with 3 µmol/L having no advantage over 1 µmol/L. The results of this study indicate that auxins can play a role in preventing the outgrowth of plant-colonizing fungi.

Keywords: auxins, endophytic fungus, fungal colony, *in vitro*, larch.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Sensitivity of *Fusarium* spp. Isolated from Weeds and Crops to Metconazole, Prothioconazole and Tebuconazole

Neringa Matelionienė[1], Skaidrė Supronienė[1], Renata Žvirdauskienė[1], Gražina Kadžienė[2], Evelina Zavtrikovienė[1]

[1]Microbiology Laboratory, Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto al. 1, Akademija, LT-58344 Kedainiai, Lithuania

[2]Department of Soil and Crop Management, Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto al. 1, Akademija, LT-58344 Kedainiai, Lithuania

Corresponding author: neringa.matelionienė@lammc.lt

Abstract

Fusarium spp. are widespread plant pathogens that cause significant losses in agriculture. *Fusarium* spp. colonizes not only the main plants – crops, but also alternative plants – weeds [1]. Fungicides of the triazole group: metconazole, prothioconazole and tebuconazole are often applied for the protection of cash crops against fungal diseases [2]. This study compares the sensitivity of *F. graminearum*, *F. culmorum* and *F. sporotrichioides* species isolated from crops (oilseed rape, field pea, sugar beet, spring wheat) and weeds (scentless false mayweed, field pansy, shepherd's purse, wild buckwheat) to different concentrations of metconazole, prothioconazole and tebuconazole. The effect of fungicides was determined by the percentage inhibition of *Fusarium* fungal mycelial growth *in vitro*. The 50% effective concentration (EC50) values of all isolates on metconazole were lower than prothioconazole and tebuconazole. It was observed that *Fusarium* isolates from weeds were more sensitive to low concentrations of fungicide than isolates from crop plants. These findings may show that *Fusarium* strains in crop plants have developed some resistance to triazole fungicides, as opposed to those in weeds.

Keywords: *Fusarium*, disease control, triazoles, fungicide, alternative plants.

Acknowledgments: This study was supported by the long-term research program 'Harmful Organisms in Agro and Forest Ecosystems' implemented by the Lithuanian Research Centre for Agriculture and Forestry.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Sucrose Effect on Shoot and Root Development in the *in Vitro* Culture of Black Pine (*Pinus nigra*) Embryos

Jokūbas Nagulevičius, **Jonas Žiauka**

Vytautas Magnus University, Faculty of Natural Sciences, Universiteto g. 10, Akademija, Kaunas District, Lithuania
Corresponding author: jokubas.nagulevicius@vdu.lt

Abstract

European black pine (*Pinus nigra*) is an ecologically and economically important conifer species which is used in various *in vitro* studies, including somatic embryogenesis [1] and cryopreservation [2]. One of the potential nutrient medium components for plant *in vitro* cultures is sugar, usually sucrose. It can be either present in or absent from the medium, depending on explant type and the aim of *in vitro* culturing. It is important to test experimentally for each *in vitro* propagated plant species, which morphological features are induced by different sucrose concentrations in the early stages of *in vitro* development.

In this study, *Pinus nigra* embryos were excised from seeds and planted on the nutrient medium *in vitro*. The three experimental variants included sucrose-free nutrient medium and two sucrose-supplemented media, containing either 5 g/L or 20 g/L sucrose. Such characteristics of developing *P. nigra* embryos, as root, shoot, and needle length, were evaluated after 20 and 30 days after planting *in vitro*.

It was shown that different concentrations of sucrose, and even its absence from the medium, did not affect *P. nigra* shoot development; the average length of shoots and needles did not differ significantly among the three medium variants. In contrast, root length analysis proved that higher concentration of sucrose, 20 g/L, was required for the increase and maintenance of *P. nigra* root growth. After 20 days, the average root length on the medium with 20 g/L sucrose was three times higher than either on sucrose-free or 5 g/L sucrose medium. After 30 days, this difference only increased, as *P. nigra* roots continued to grow on 20 g/L sucrose medium and their average length here became approximately 4.5-times higher than on the other two medium variants. Thus, exogenous sucrose was shown as a factor necessary for root, but not shoot, growth in the *in vitro* culture of excised *P. nigra* embryos.

Keywords: embryo, *in vitro*, *Pinus nigra*, root growth, sucrose.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Toxic Effect Of Di(2-Ethylhexyl)Phthalate and Dibutylphthalate on Hippocampus Neuron Density And Region Thickness

Laurynas Orla[1], Edita Paulikaitė[1], Rasa Aukštikalnienė[1], Urtė Neniškytė[1], Violeta Žalgevičienė[2], Grita Skujienė[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
[2]Department of Anatomy, Histology and Anthropology, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Lithuania

Corresponding author: laurynas.orla@gmc.stud.vu.lt

Abstract

Phthalates are a group of man-made chemical compounds that have mutagenic and endocrine disrupting properties and tend to accumulate in the environment [1]. In Lithuania, the discharge of phthalates into wastewater is not fully controlled. Although only microgram quantities are discharged, their accumulation in water and soil can have serious environmental and human health consequences. The aim of this project was to evaluate the effects of phthalate (di(2-ethylhexyl)phthalate – DEHP and dibutylphthalate – DBP) exposure on the density of hippocampal neurons and the thickness of CA1 and CA3 regions in male offspring. The material comes from the study carried out on 2022-08/09, which was reviewed and approved by the Ethics Committee for Animal Experiments of the State Food and Veterinary Service (No. G2-221).

Female rats of the Wistar strain, 5-8 weeks old, were divided into control and 5 experimental groups, which received standard food and additional piece of an ecological biscuit infused with different doses of phthalates dissolved in olive oil: 1) DEHP 200 μg/kg; 2) DEHP 1000 μg/kg; 3) DBP 100 μg/kg; 4) DBP 500 μg/kg; 5) mixture of phthalates (DEHP 200 μg/kg, DBP 100 μg/kg). Control animals received only a piece of the same biscuit with oil and without phthalates. After 2 months, females were mated with unaffected males. At the postnatal day 21, male offspring were selected and euthanised in a CO_2 chamber. The brain was collected and placed in formaldehyde. The brains were cut in half, one half was embedded in paraffin, cut into 5 μm-thick sections, and stained with Nissl dye. Changes in neuronal density in the CA1 and CA3 regions and changes in the thickness of the regions were calculated to determine the effects of phthalates [2,3]. Data were analysed using one-way ANOVA. Statistical analysis was performed using GraphPad Prism 9.0.0.

The smaller dose of DBP found in Lithuanian wastewater significantly reduced neuronal density and CA1 region and CA3 region thickness in the rats. Neural density and thickness of both regions were also significantly reduced in rats receiving higher doses of DEHP and DBP phthalates and in rats receiving a mixture of phthalates.

Keywords: rat, pups, hippocampus, neuron, density, thickness, CA1 region, CA3 region.

Acknowledgments: We would like to thank dr. Virginija Bukelskienė for help in organizing the research, medical students Justina Alčauskaitė and Evita Sėrikovaitė for assisting in the experiment. The project was financed by the Science Promotion Fund for Scientific projects of Vilnius University, agreement No MSF-JM-18/2022.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Evaluation of Irreversible Electroporation in Suspension and Monolayer Cell Cultures

Aras Rafanavičius[1], Ingrida Šatkauskienė[2], Saulius Šatkauskas[1,2]

[1]Department of Biochemistry, Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania
[2]Cell and Tissue Biotechnology Research Group, Institute of Research of Natural and Technological Sciences, Vytautas Magnus University, Akademija, Lithuania

Corresponding author: aras.rafanavicius@vdu.lt

Abstract

Pulsed field ablation (PFA) is a method used to treat malignant tissues by subjecting them to strong electrical pulses. In brief, these electrical pulses create transient pores in the cell plasma membrane, and due to membrane damage and loss of homeostasis, the treated cells eventually die. This process is called irreversible electroporation and has been utilised for tissue ablation since 2005 [1]. Recently, this ablation modality has been researched for treatment of atrial fibrillation – a type of arrhythmia diagnosed in at least 60 million patients worldwide [2] – particularly due to its purportedly non-thermal effect and tissue specificity, compared to conventional thermal ablation methods [3]. While studies in this field are abundant, the optimum parameters for safe, non-thermal ablation have not been reported, and the pulse parameters used in clinical trials are currently undisclosed. Therefore, it is important to determine the conditions that would result in the highest cell mortality and, preferably, low energy cost.

In this study, Chinese hamster ovary cells were utilised. The pulses were administered using stainless steel electrodes. Viability of suspended cells was determined via clonogenic assay and membrane integrity assay using a flow cytometer. Reversible and irreversible electroporation zones in cell monolayers were identified via fluorescence microscopy. For imaging, the cells were stained using propidium iodide either before electroporation or 30 min after, respectively. Then, a composite image was analysed, and total affected areas were evaluated.

A variety of pulse parameters were analysed: number of pulses, pulse duration, amplitude and frequency, while maintaining the same single pulse energy. For cell suspensions, it was determined that applied pulse amplitude is the main factor decreasing cell viability, and the number of pulses increase mortality rates until a plateau is reached for a high number of pulses (10-50). Conversely, in monolayer cultures, both irreversibly and reversibly electroporated area continually increases with increasing pulse number. The ratio of irreversible and reversible electroporation zone areas remains mostly stable across a wide number of pulses (1-50). These results were contrasted with experiments that were conducted under high-frequency electroporation conditions.

Keywords: pulsed field ablation, electroporation, irreversible electroporation.

Acknowledgments: This study was supported by the grant no. S-ST-23-214 by the Research Council of Lithuania.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Chitosan as a Seed Treatment: Investigating its Antifungal Properties

Aurelija Ramanauskaitė[1,2], Povilas Mulerčikas[2], Sonata Kazlauskaitė[2], Vykintas Baublys[1]

[1]Department of Biology, Faculty of Natural Science, Vytautas Magnus University, Universitetas street. 10-314, Akademija, Kaunas district, LT-53361 Lithuania
[2]Department of Agroecosystems and Soil Sciences, Faculty of Agronomy, Vytautas Magnus University, Studentų street 11, Akademija, Kaunas district, LT-53361 Lithuania

Corresponding author: aurelija.ramanauskaite@vdu.lt

Abstract

Chitosan is soluble in weak acids, it can form gels and films. [1]. Its antifungal activity in agriculture is influenced by various factors including molecular activity, type of micromycetes, and environmental interactions [2]. The study aims to investigate the potential of chitosan, with different molecular weights and concentrations, to inhibit the growth of micromycetes on natural, non-sterilized seeds of spring wheat and spring barley.

The experiment was designed by different concentrations (0.5 g/l, 1 g/l, and 2 g/l) of chitosan with varying molecular weights (high, medium, and low) were tested. Chitosan gels were prepared by dissolving chitosan powder in weak acetic acid (1%), and seeds were coated with these gels by dipping. Coated seeds were then sown on potato agar in Petri dishes, and micromycete colonies were identified and counted after five days.

Fungicidal activity generally increases with higher concentrations of chitosan. However, there are cases where excessive concentrations may suppress the growth of micromycetes or even stimulate it, possibly due to the enzymatic breakdown of chitosan into glucosamine units [3]. Different concentrations and molecular weights of chitosan exhibited varying degrees of effectiveness against different micromycete genera. For example, low molecular weight chitosan was effective against *Bipolaris* spp., *Alternaria* spp., and *Saccharomyces* spp., while high molecular weight chitosan was effective against *Bipolaris* spp., *Penicillium* spp., and *Mycelia sterilia*. There's a correlation between concentration and fungicidal efficacy, with higher concentrations showing a greater negative effect on the amount of all identified micromycete genera.

Conclusion and Recommendations: The study shows the potential of using chitosan in pre-sowing seed treatment to reduce farmers' losses caused by micromycetes. However, further research is necessary to determine the optimal concentration of the most effective chitosan preparations.

Keywords: chitosan, seed treatment, micromycetes, fungicidal activity.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Investigating the Effect of Pulse Number on Cell Survival and the Efficiency of Concurrent *vs* Separate Electroporation of Proteins and Small Molecules *in Vitro*

Evaldas Ramanciuškas, Justinas Venckus, Saulius Šatkauskas

Department of Biology, Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania
Corresponding author: evaldas.ramanciuskas@vdu.lt

Abstract

Exogenous proteins can be introduced into cells using electroporation, which is useful for processes like CRISPR-Cas9 gene editing and detecting intracellular signalling. However, because of their varied sizes, complex structures, and wide range of biological functions, proteins are difficult to transport. Moreover, no well-established theory exists currently to explain how proteins enter cells through electrotransfer [1,2]. Investigating how this technique interacts with other migrating molecules, like propidium iodide (PI), during electroporation could offer necessary insights how effective this approach is as this could highlight interferences or synergies between proteins and small molecules. It's also important to investigate how this method affects cell survival because it may increase endocytosis and cause more harm to the cell membrane when proteins and PI pass through it simultaneously. The aim of this study was to investigate the influence of the number of high voltage (HV) electrical pulses on cell viability and the efficiency of simultaneous and separate electroporation of proteins (bovine serum albumin, BSA) and PI into Chinese hamster ovary (CHO) cells *in vitro*. CHO cells were electroporated using a BTX BT820 Electro Square Porator with 1, 9 or 17 HV pulses (60 μs, 1000 V/cm, 1 Hz) in the presence of Alexa Fluor 488-conjugated BSA (final concentration ~10 μg/ml) and/or PI (final concentration ~4 μM). Cells were resuspended in low-conductivity electroporation medium (10 mM phosphate, 250 mM sucrose, 1 mM $MgCl_2$, pH 7.4) prior to electroporation [3]. Cell viability, BSA and PI uptake efficiency and fluorescence intensity were assessed by flow cytometry using a BD Accuri C6 flow cytometer 30 minutes after electroporation. For each sample, 10000 events were collected using a 22 μm core size and a flow rate of 66 μl/min. BSA and PI fluorescence were excited with a 488 nm laser and detected with 530/30 (FL1) and 585/40 (FL2) bandpass filters, respectively. Proper fluorescence compensation was performed using single stained controls to accurately distinguish BSA^+ and PI^+ cells. Cells with PI fluorescence, higher than 1000000 a.u., were considered dead. Statistical analysis was performed using descriptive statistics and unpaired Student's t-tests with a significance level of $p \leq 0.05$. The control was treated with BSA, albeit it was not subjected to the electric pulses. The experiments were repeated three times for each condition (n=3). Results are expressed as mean ± standard error of the mean (SEM). The method's effectiveness was confirmed by the preliminary results, which showed that co-electroporation of BSA and PI decreased PI uptake from 96.12% to 93.4% ($p<0.05$), with BSA-Alexa_Fluor 488 increasing to 12.92% at 17 HV pulses. These results highlight the intricate interactions between PI and BSA in CHO cells.

Keywords: electroporation, intracellular delivery, protein delivery, small molecule delivery.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Optimizing Intracellular Delivery of Proteins *in Vitro* Through Electroporation

Aditya Rout, Justinas Venckus, Saulius Šatkauskas

Department of Biology, Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania
Corresponding author: aditya.rout@vdu.lt

Abstract

Intracellular delivery of exogenous proteins is advantageous over DNA/RNA approaches because it avoids transcription and translation and offers direct effects and controlled dosing within cells. Thus, it is particularly beneficial for CRISPR-Cas9 gene editing and examining subcellular processes. However, because of their size and charge, proteins, like nucleic acids (NA), have difficulty passing through plasmalemma naturally. One approach to mitigate this is through electroporation which creates transient pores in the membrane by utilizing pulsed electric fields inducing the transmembrane potential across the membrane [1]. However, the effectiveness of this technique for larger proteins is called into question due to their lower surface charge than on NA resulting in diffusion-dominant migration as opposed to the electrophoretic-driven movement of NA [2,3]. While concerns about cargo retention and cell viability persist over time, varying the number of electric pulses could improve delivery by giving proteins more time to enter cells. Despite the challenges, optimizing parameters responsible for electroporation can help deliver the proteins successfully.

The aim of this study is to determine the dependence of protein electroporation *in vitro* efficiency and post-procedure cell viability on the number of high-voltage (HV) electric pulses. In our experiment, we subjected CHO cells with BSA conjugated protein with Alexa Fluor 488 and exposed them varying numbers (1,5,9,13, and 17) HV pulses (60 µs, 1000 V/cm, 1 Hz). The comparative analysis was carried out at three distinct post-electroporation time points: 30 minutes, 6 hours, and 24 hours using the BD AccuriTM C6 cytometer to estimate fluorescent viable cell percentage at the specified time intervals. In addition to evaluating the transfection efficiency, our study also investigated the impact of electroporation on cell viability. Cell viability was quantified using MTS/PMS assay 24 hours after the electroporation, with results expressed as a percentage of the viability of the control group that received BSA protein cargo but was not subjected to the electric pulses.

The results suggested a dose-dependent relationship between the number of pulses and the efficiency of cargo uptake. The data indicated that while lower numbers of pulses (1 HV, 5 HV) resulted in a slight decrease or minimal increase (12.26%, 2.30%) in fluorescence after 30 min., higher numbers of pulses (13 HV, 17 HV) improved the delivery efficiency by 17.80% and 11.81% ($p \leq 0.05$). The findings indicated a propensity for electro transfer efficiency to improve with time, with the strongest impacts being shown 24 hours after electroporation using 17 HV. While the control group exhibited 99.00% viability which serves as the reference point, the lower number of pulses (1 HV, 5 HV) maintained high viability with 98.10% and 99.08%. However, we observed a reduction in cell viability with increased number of pulses (9 HV,13 HV, 17 HV) which resulted in a viability of 68.28%, 58.11% and 49.67% ($p \leq 0.05$). The results demonstrated a dose-dependent decrease in cell viability, indicating that higher HV pulse numbers may compromise cell viability. Therefore, the findings of this research demonstrate that while optimizing electroporation parameters, the trade-off between achieving high protein electroporation rates and maintaining cell integrity must be carefully considered.

Keywords: protein electroporation, cell viability, electroporation efficiency.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Calcium Delivery to Breast Cancer Cells via Sonoporation

Reda Rulinskaitė[1], Saulius Šatkauskas[1], Renaldas Raišutis[2], Martynas Maciulevičius[1,2]

[1]Biophysical Research Group, Vytautas Magnus University, Lithuania
[2]Ultrasound Research Institute, Kaunas University of Technology, Lithuania
Corresponding author: reda.rulinskaite@vdu.lt

Abstract

Chemotherapy affects cancerous cells more efficiently than normal cells due to their rapid growth, proliferation and metastasis. After chemotherapy treatment, adverse short-term and long-term side effects may occur, including infertility, alopecia, and cardiotoxicity [1].

Calcium ions (Ca^{2+}) play a crucial role in signal transduction pathways and are the most common intracellular signal transmitters within the cell [2]. Moreover, calcium ions are vital for intracellular Ca^{2+} homeostasis in a cell; therefore, their disruption could play a fundamental role in the destruction of cancerous cells. Ca^{2+} has been shown to be efficient in cancer treatment in the field of electroporation [3].

Sonoporation (SP) involves non-invasive application of ultrasound (US) in combination with microbubbles (MBs) and can be utilised for the treatment of deep-seated pernicious tumors. Therefore, efficient treatment of cancer with Ca^{2+} SP could substantially improve conventional chemotherapy.

Our study indicates that the mortality of mouse breast cancer (4T1) cells increases from 5mM Ca^{2+} concentration, as indicated by metabolic activity loss and reduced clonogenicity. Our initial findings suggest that the Ca^{2+} transport induced by MB cavitation in 4T1 cells is not fatal in the presence of 200 kPa US, as evidenced by the clonogenic assay showing cell viability recovery within 6 days.

The performed ANOVA test revealed a trend indicating that increasing Ca^{2+} concentrations at 200 kPa US tend to elevate cell mortality. Furthermore, the differences between the control (0 mM Ca^{2+}) and the therapeutic (Ca^{2+}+SP) group are up to 30% (according to PI and MTT tests), which indicates the permeabilization of the cell plasma membrane and the disturbance of metabolic activity.

Keywords: sonoporation, ultrasound, cancer, microbubbles.

Acknowledgments: this work was supported by the grant: "Krūties vėžio gydymas taikant Ca^{2+} pernašą ultragarsu (S-ST-23-211)" from the Research Council of Lithuania.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Determination of Phenolic Compounds Composition of Water Extracts of *Equisetum arvense* (L.)

Agnė Siaurusevičiūtė[1], Kristina Zymonė[1,2], Agnė Mazurkevičiūtė[1]

[1]Institute of Pharmaceutical Technologies of the Faculty of Pharmacy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Department of Analytical and Toxicological Chemistry, Lithuanian University of Health Sciences, Kaunas, Lithuania
Corresponding author: agne.siauruseviciute@stud.lsmu.lt

Abstract

Equisetum arvense (L.), commonly known as horsetail, is generally used in traditional medicine as a therapeutic and pain reliever in wound, skin diseases, hair loss. Wide range of skin and hair cosmetics contains *E. arvense* extract. In skin cosmetics it acts as anti-aging, moisturizer, antiwrinkle, anti-acne, antiperspirant, conditioner. It prevents grey hair, strengthen the hairs and maintain hair tone [1,2]. *E. arvense* contains many phenolic components.

The aim of this study is to determine the phenolic compounds composition of water extracts of *E. arvense* stem by high performance liquid chromatography (HPLC).

Water extracts samples of horsetail stem were extracted by comparing raw materials and water with different extractants in a ratio of 1:100. Horsetail water extracts were prepared with PEG 400, polyethylene glycol, β-cyclodextrin, $NaHCO_3$ and extraction utilizing a magnetic stirrer or in an ultrasonic bath for 30 minutes.

Studies have shown that there are differences between the content of the released bioactive substances in water extracts of *E. arvense* and selected different parameters of extraction.

In our results the highest contents of detected phenolic compounds of *E. arvense* stem were determined in water extracts with polyethylene glycol and using an ultrasonic bath. The components of horsetail stem water extract with polyethylene glycol were determined as hydroxycinnamic acids and flavonoids. *E. arvense* water extract with polyethylene glycol gave the following result: total content of detected flavonoids (25.85±0.352 µg/ml); total content of detected hydroxycinnamic acids (21.36±0.291 µg/ml). In total, about 47.21±0.642 µg/ml different phenolic compounds were detected by HPLC in water extract with polyethylene glycol. In conclusion, these results revealed that polyethylene glycol could be used in *Equisetum arvense* water extracts to improve release of biologically active substances.

Keywords: phenolic compounds, *Equisetum arvense* L., water extract.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Quantitative Estimation and Comparison of Vitamin C in *Actinidia* (*Actinidia Arguta* (Siebold & Zucc.) Planch. ex Miq. and *Actinidia Kolomikta* (Rupr. & Maxim.) Maxim.) Berries by HPLC-DAD-MS Method

Livija Skėrytė[1], Kristina Zymonė[1,2], Mindaugas Marksa[1], Laima Česonienė[3,4]

[1]*Department of Analytical and Toxicological Chemistry, Lithuanian University of Health Sciences, Sukilelu av. 13, Kaunas LT-50161, Lithuania*
[2]*Institute of Pharmaceutical Technologies, Lithuanian University of Health Sciences, Sukileliu av. 13, Kaunas LT-50161, Lithuania*
[3]*Research Institute of Natural and Technological Sciences, Vytautas Magnus University, LT-40444 Kaunas, Lithuania*
[4]*Botanical Garden, Vytautas Magnus University, LT-46324 Kaunas, Lithuania*

Corresponding author: livija.skeryte@stud.lsmu.lt

Abstract

Actinidia arguta (Siebold & Zucc.) Planch. ex Miq. and *Actinidia kolomikta* (Rupr. & Maxim.) Maxim. – are a perennial, climbing plants of Actinidiaceae Engl. & Gilg. family [1, 2]. Accumulation of biologically active compounds in *Actinidia* berries have been studied both by foreign and Lithuanian scientists. Antilipidemic, antiproliferative, antimutagenic, anticholinergic and antioxidant effects have been reported [3-5]. Vitamin C, ascorbic acid ($C_6H_8O_6$) – water soluble vitamin, not synthesised in the human body. It is known that *Actinidia* berries are a great source of vitamin C. The present research examined variability of the quantitative content of vitamin C in different *A. arguta* and *A. kolomikta* cultivars/clones berries by high performance liquid chromatography – diode array detector – mass spectrometry (HPLC-DAD-MS) method. It was found that the accumulation of vitamin C in *Actinidia* berries depended on *Actinidia* cultivars/clones. Higher ($p < 0.05$) amounts of ascorbic acid were found in *A. kolomikta* berries than in *A. arguta* berries. The determined amount of vitamin C in *A. arguta* berries varied from 1.16 ± 0.04 mg/g to 3.5 ± 0.11 mg/g, in *A. kolomikta* berries – from 1.17 ± 0.04 mg/g to 23.88 ± 0.74 mg/g. The highest amount of ascorbic acid was found in berries of *A. kolomikta* F_{ELE} clone (23.88 ± 0.74 mg/g).

Keywords: *Actinidia arguta* (Siebold & Zucc.) Planch. ex Miq., *Actinidia kolomikta* (Rupr. & Maxim.) Maxim., berry, ascorbic acid, HPLC-DAD-MS.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

In Vitro Studies on Plasmid DNA Transfer into Cells

Gerda Skindaraitė[1], Ernestas Urbanskas[2], Saulius Šatkauskas[2]

[1]Department of Biology, Faculty of Natural Sciences, Vytautas Magnus University, Vileikos str. 8, LT-44404, Kaunas, Lithuania
[2]Department of Biology, Research Institute of Natural Sciences and Technology, Vytautas Magnus University, Vileikos str. 8, LT-44404, Kaunas, Lithuania
Corresponding author: gerda.skindaraite@vdu.lt

Abstract

Electroporation is a physical method in which short and intense electrical pulses are applied that cause temporary permeability of the plasma membrane of a cell to molecules that do not pass through it naturally, such as DNA. [1] Properly selected electrical impulses (their duration, strength) create pores in the cell membrane, through which the plasmid DNA is transported to the cytoplasm of the cell, and then to the nuclusei, where the transmitted gene is expressed [2]. The experiments conducted over the past three decades are aimed at optimizing the protocol of electrical impulses and other parameters, but the results of studies assessing the optimal parameters of the electrotransfer are very different among different authors and do not provide an unambiguous, properly selected protocol for effective electrotransfection [3].

The study aim was to determine dependence of plasmid DNA electrotransfer into Chinese hamster ovary cells (CHO-K1) on plasmid DNA size. Two different plasmid DNA were transfected into CHO cells at the same time. Flow cytometry was used to quantify the number of viable cells, also the percentage of transfected cells and gene expression.

The results showed that gene electro transfer is influenced by the size of the plasmid DNA, since the electro transfer of smaller plasmid DNA was better than that of large plasmid DNA. The results show that larger plasmid DNA is more difficult to transfer and has a greater cytotoxic effect. Also, lower plasmid affected cell viability to lower extent. The efficiency of pEGFP plasmid DNA transport is significantly reduced by simultaneous transfer of mCardinal plasmid DNA. Also, gene expression was found to be higher of smaller plasmid DNA in contrast with bigger plasmid DNA gene expression, which suggests that smaller plasmid DNA is more suitable for gene transfection to CHO cells. Overall GET of smaller plasmid DNA was found higher after transfection of two plasmid DNA's.

Keywords: electroporation, plasmid DNA, transfection, stainless-steel.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Tonic Compositions Based on Mineral-Rich Waters from Ciechocinek

M. Sugajski[1,2], M. Buszewska-Forajta[3], B. Buszewski[2]

[1]Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University in Torun, Gagarina 7, 87-100 Torun, Poland
[2]Prof. Jan Czochralski Kuyavian-Pomeranian Research and Development Center, Krasińnskiego str. 4, 87-100 Toruń, Poland
[3]Institute of Veterinary Medicine, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń, 1 Lwowska St., 87-100 Toruń, Poland

Corresponding author: mateusz.sugajski@o2.pl

Abstract

With the development of civilization and technological progress, the demand for high-quality drinking water increases. Surface and deep water are the main source of consumption resources. Currently, in accordance with WHO recommendations, we distinguish several types of water: medicinal, mineral, spring and tap.

Medicinal and mineral water contain specific minerals or other ingredients, such as calcium or magnesium, which contribute to their healing effects and beneficial effects on health. Spring water, obtained from natural sources, is unpolluted and meets quality standards. Tap water supplied from water networks is the most common type of water used for drinking and must meet sanitary drinking water quality standards.

Polish health resorts, famous for their wealth of natural resources and experienced medical staff, are widely recognized as competitive health tourism destinations. This study focuses on Ciechocinek, the "pearl" of Polish health resorts. This health resort is famous for its mineral-rich healing waters, known for their effectiveness in treating a wide range of diseases.

The primary goal is to conduct a comprehensive physicochemical and microbiological analysis of water collected from four intakes in Ciechocinek. The above-mentioned comprehensive analyzes were carried out to assess the suitability of these waters for food purposes, as bottled mineral drinks and for therapeutic purposes (balleontology). The aim of the analysis was to highlight the potential of Kujawy's water resources and their health-promoting properties. In addition, comparative studies were carried out on bottled medicinal water "Krystynka" with other commonly available bottled waters distributed in popular retail chains.

The research used classic instrumental and microbiological analytical methods as well as chemometric analysis. Through this analysis, protocols were developed for classifying water and its derivatives as consumer products. The research culminated in the formulation of a model enabling a simple and accessible assessment of the usefulness of bottled water from the consumer's point of view.

Keywords: water analysis, health promotion, isotachophoresis, isotonics.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

Development of an Open-Source Ozonometer for Citizen Scientists

Vladas Šatas, Benas Gabrielis Urbonavičius

[1]*Department of Physics, Kaunas University of Technology, Studentu str. 50, LT-51368 Kaunas, Lithuania*
Corresponding author: vladas.satas@ktu.lt

Abstract

This paper introduces the development of an open-source filter ozonometer, uniquely designed to boost public engagement and awareness in environmental monitoring. The instrument, practical in its approach and ideal for citizen scientists, operates using specialized filters that isolate wavelengths of 300, 330, and 360 nm. This functionality enables sequential measurements of intensity with a UV sensor for estimating the atmospheric ozone layer thickness. The Huggins System [1], selected for its simplicity and appropriateness for non-expert users, facilitates the practical approximation of ozone column density.

The design process, rooted in open-source principles, is central to the project's mission of democratizing scientific research and promoting community-level involvement in environmental science. This ozonometer is not just a functional scientific tool but also a medium to enhance public understanding and encourage active participation in environmental observation. Its potential impact in educational settings and to engage the public in environmental science is significant. By lowering the barriers to atmospheric monitoring, the instrument is poised to foster a more informed and engaged society.

Keywords: open-source filter ozonometer, environmental monitoring, citizen scientist, UV sensor, ozone column density.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Determination of Volatile Compounds in Food Supplements by HS-SPME Coupled with GC-MS

Laura Šimaitytė, Kristina Bimbiraitė-Survilienė, Juozas Šiurkus, Audrius Maruška

Vytautas Magnus university, Instrumental Analysis Open Access Center

Corresponding author: kristina.bimbiraitė-surviliene@vdu.lt

Abstract

Maughan *et al*. 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 [1]. Bioactive compounds and volatile compounds [2] are abundant in natural dietary supplements, including herbal extracts, herbal products, and superfoods. These compounds affect the gut microbiota and can help maintain endocrine balance by enhancing different metabolic indicators [3].

The aim of this work was to use gas chromatography - mass spectrometry headspace solid phase microextraction technique to analyze volatile compounds in food supplement. Task was to identify volatile compounds in food supplement samples using the gas chromatography-mass spectrometry method.

A total of 30 compounds were identified with HS-SPME gas chromatography – mass spectrometry analysis. Main compounds were detected in all food supplement samples: benzaldehyde accounted for up to 9%, linalool 8.7%, benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- 5.7%.

Keywords: food supplements, GC–MS, volatile compounds.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Effect of DNMT1 Inhibitors on Cell Viability

Arnas Treimakas, Eglė Jakubauskienė

Department of Biological DNA modification, Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio Ave. 7, Vilnius LT-10257, Lithuania
Corresponding author: egle.jakubauskiene@bti.vu.lt

DNA methylation is a key mechanism of epigenetic regulation carried out by enzymes known as methyltransferases (DNMTs) [1]. DNMT1 is required to maintain DNA methylation in mammals, and its alterations trigger different cellular mechanisms involved in various types of cancer [2]. As such, there is high interest in the therapeutic applications of DNMT1 inhibitors. However, due to the variability of methylation between different cell types, inhibition of DNMT1 is likely to affect the vitality of specific cells or tissues to a varying degree. In this study, we investigated the effect of two DNA methyltransferase 1 inhibitors – GSK1 and GSK2 – on the vitality of two different mouse cell lines (MEF and LLC1). The effect of inhibitors on cell viability was analyzed using MTT assay, based on the reduction of tetrazolium salt to formazan crystals by metabolically active cells.

Keywords: DNMT1 inhibitors, cell viability, MTT assay.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Modeling Oil-in-Water Dispersed System Utilizing *Sorbus aucuparia L.* Extract: A Study on Physical Properties Assessment

Augustė Morta Vaitkutė[1], Agnė Mazurkevičiūtė[1], Kristina Zymonė[1,2]

[1]Institute of Pharmaceutical Technologies of the Faculty of Pharmacy, Lithuanian University of Health Sciences, Sukilėlių av. 13, LT-50162 Kaunas, Lithuania
[2]Department of Analytical and Toxicological Chemistry, Lithuanian University of Health Sciences, Sukilėlių av. 13, LT-50162 Kaunas, Lithuania

Corresponding author: auguste.morta.vaitkute@stud.lsmu.lt

Abstract

Sorbus aucuparia L. contains carotenoid compounds, that enhance skin's innate resistance against UVB-induced erythema, act as an antioxidant and prevent aging-related collagen I degradation in the dermis [1]. In order to formulate an oil-in-water (O/W) type dispersion system, gelling agents are employed to provide the necessary stability and viscosity. Carbomer polymers, derived from acrylic acid, are hydrophilic, white powders, providing acidic pH to the formulations, thus their pH value is adjusted to skin using a 1% NaOH solution [2].

The aim of this study was to analyze the physical properties of the (O/W) type dispersion system using Carbomer 980 as a gelling agent. Rheological properties (rheometer MCR102, temperature controlled via Peltier system) and particle size (Mastersizer 3000) were assessed on diverse emulgel formulations: containing almond oil and containing *S. aucuparia* extracts. The concentrations of *S. aucuparia* and almond oil in the emulgels varied as follows: 10%, 20%, 30%, and 40%, with an additional 5% consisting of the surfactant Tween 80, and the rest being Carbomer 980.

Particle size measurements, indicated by percentiles (Dx10, Dx50, and Dx90), showed consistent particle sizes across different concentrations of almond oil in the studied emulgels. Moreover, the freeze–thaw test was completed, which consisted of 72 hr freezing (−20 ± 1 °C) followed by 24 hr thawing (20 ± 1 °C). Although emulgels subjected to freezing remained visually stable, with no apparent phase separation, particle size measurements revealed that after the freezing process, the increase of Dx50 is found to be negligible, but Dx90 showed significant increase (43,70 (8,98) μm) compared to pre-freezing particle size (3,30 (0,16) μm). Post-freezing emulgels with a 10% oil concentration exhibited the largest particle sizes. The incorporation of rowanberry extract into the O/W dispersed system resulted in particle sizes (Dx90) remaining relatively consistent (3,23 (0,20) μm), compared to emulgel particles composed solely of oil and not subjected to freezing. A temperature-based test was employed to determine how the consistency of formulations fluctuates with temperature changes. Temperature tests were conducted on formulations containing *S. aucuparia* extract and those with only almond oil. Results indicated changes in emulgel structure as storage modulus varied. The obtained data showed that over the temperature range (0-40°C) emulgels maintained their semi-solid consistency, regardless of the oil content, indicating stability. Similar research results are evident in emulgels containing both rowanberries extract and almond oil.

Keywords: carbomer 980, *Sorbus aucuparia L.*, emulgels, physical properties.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Evaluation of Antioxidant Activity and Antifungal Potential of Bee Product Extracts

Aušrinė Venckaitytė, Vilma Kaškonienė, Rūta Mickienė, Audrius Maruška

Instrumental Analysis Open Access Centre, Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania
Corresponding author: ausrine.venckaityte@stud.vdu.lt

Abstract

In pursuit of enhanced quality of life, contemporary consumers increasingly opt for natural functional foods, known for their bioactive compounds of natural origin, which are considered safer compared to synthetic drugs. Scientific research has revealed a broad spectrum of health benefits associated with bee products, including antioxidant, antibacterial, anti-inflammatory, antitumor, and antiviral properties [1,2]. Of particular significance is their potent antioxidant capacity, which aids in warding off certain illnesses by shielding cells from oxidative damage caused by free radicals. Bee products boast exceptionally high biological and therapeutic properties, owing to their rich array of bioactive compounds such as phenolic compounds, flavonoids, and terpenoids. According to literature findings, propolis emerges as the most potent antioxidant among bee products, containing the highest levels of phenols and flavonoids, followed by pollen, bee bread, and royal jelly [3]. Methanolic and ethanolic compounds made from propolis have shown an inhibitory effect against *Cryptococcus laurentii*, *Aspergilus niger, Aspergilus fumigatus* and *Memnoniella echinata* fungi. Methanolic and ethanolic compounds made from bee pollen have shown an inhibitory effect against *Aspergilus niger* and *Aspergilus fumigatus.*

Keywords: bee products, bee pollen, bee bread, propolis, antifungal, antioxidant activity.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

The Microsatellite Analysis of the Genome of *Tripolium pannonicum* (Jacq.) Dobrocz.

Miłosz Wasicki[1], Agnieszka Ludwiczak[1,2], Małgorzata Szultka-Młyńska[1,3]

[1]Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University in Torun, 87-100 Torun, Poland
[2]Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, 87-100 Torun, Poland
[3]Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University in Torun, 87-100 Torun, Poland

Corresponding author: milos13w@outlook.com

Abstract

Microsatellites (MSs) are known for their high polymorphism and co-dominant inheritance patterns, which are crucial for molecular genetic studies. Therefore, we used microsatellite sequencing and identification to utilize MS as genetic markers for their potential in studying genetic diversity, linkage mapping, and gene identification, especially in plant genomes. The isolated *Tripolium pannonicum* genome was sequenced using a next-generation sequencing (NGS) platform (Illumina) known for its high-throughput capabilities. The 128845 microsatellite sequences were identified utilizing advanced bioinformatic tools such as MaSuRCA version 3.3.3 for sequence assembly and QDD version 3.1.2 for primer design targeting specific microsatellite regions. At the same time, tri-nucleotide sequences such as AAT/ATC/AAG/AAC/ACC were also noted, accounting for nearly 13% of all motifs. The tri-, tetra-, penta-, and hexanucleotide sequences were less expected. The sixteen MS were selected based on their diverse lengths and nucleotide compositions for further bioinformatic analysis (BLASTN, Plants Esembl, and Uniprot). The chosen sequences primarily appeared in exons and UTR regions of homologous sequences. Comparative genomic analysis showed a distinctive distribution of microsatellites within the genome of *T. pannonicum*, with 66.7% in exons, 27.8% in 3'UTR, and 5.6% in introns, challenging previous expectations about microsatellite locations in other species. This comprehensive analysis highlighted the efficacy of Illumina sequencing in microsatellite identification, underscoring its potential to enhance the understanding of genetic structures and functions in halophytes, which is pivotal for ecological and evolutionary studies.

Keywords: microsatellite, halophyte, sequencing, genetic variability, DNA.

ISSN:2335-8653
ONLINE ISSN: 2335-8718

Effect of Phthalates on Physical Activity in Rat Model

Rokas Zalūba[1], Edita Paulikaitė[1], Justina Alčauskaitė[2], Violeta Žalgevičienė[2], Rokas Buišas[1], Grita Skujienė[1]

[1]Institute of Biosciences, Life Sciences Centre, Vilnius University, Vilnius, Lithuania
[2]Department of Anatomy, Histology and Anthropology, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania

Corresponding author: rokas.zaluba@gmc.stud.vu.lt

Abstract

Phthalates are ubiquitous environmental contaminants produced in large quantities and widely utilised as plasticizers, which tend to build up in both the environment and human body. Exposure to phthalates has neurotoxic effect, for example, Di-2-Ethylhexyl Phthalate (DEHP) can increase internalising behaviours and decrease child mental and motor development [1]. Despite being present in small amounts (micrograms) in environment, the effects of high doses are mostly studied. The aim of this project was to determine whether phthalates found in the Lithuanian environment could influence human behaviour. Therefore, as usual, model organisms – 36 outbred *Wistar* female rats, aged 1 month, – were used. All experimental procedures were reviewed and approved by the Ethical Committee of State Food and Veterinary Service of the Republic of Lithuania (2022-08/09, No G2-221) and were conducted following the European Communities Council Directive (2010/63/EU) and the local Animal Welfare Act. To evaluate rats' physical activity, a voluntary running test and its data analysis were carried out. Standard laboratory food and tap water were provided *ad libitum* throughout the experimental period. Rats were divided into control and 5 experimental groups that received a piece of ecological biscuit with different doses of phthalate (DEHP or Di-Butyl Phthalate (DBP)) dissolved in olive oil: 1) DEHP 200 µg/kg; 2) DEHP 1000 µg/kg; 3) DBP 100 µg/kg; 4) DBP 500 µg/kg; 5) DEHP 200 µg/kg and DBP 100 µg/kg. In the control group, animals were given an identical biscuit to the experimental groups, except it only contained oil and no phthalates.

Voluntary wheel running test was performed – after one week and two months of phthalate consumption. Physical activity was monitored using specialised activity recording system and analysed with LabChart software (ver. 7-8, ADInstruments Ltd, UK). Motor activity was measured by counting the number of revolutions of the wheel during a 24-hour period: 12-hour day cycle and 12-hour night cycle (no lighting). Statistical analysis was performed using RStudio 4.3.1, one-way ANOVA and Kruskal–Wallis tests were used.

It was found that daily, uninterrupted consumption of small doses of phthalates over the long-term (2 month) has no significant influence on physical activity of rats ($p < 0.05$), although a decrease in activity is seen in all phthalate-treated groups. However, it has been observed that daily, uninterrupted short-term (1 week) consumption of phthalate mixture (DEHP 200 µg/kg and DBP 100 µg/kg) showed a reliable decrease in number of wheel revolutions comparing to 500 µg/kg DBP group.

We can conclude that phthalates have tendency to influence rats' behaviour by reducing their locomotor activity. It is plausible that phthalates may demonstrate synergistic interactions. Yet, to enhance confidence in the data it is necessary to perform more tests to state that phthalates lead to adverse outcomes.

Keywords: behaviour, rat, physical activity, phthalates, ecotoxicity.

Acknowledgments: Financial support by the Vilnius University Science Promotion Fund for Scientific Projects, No MSF-JM-18/2022.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Evaluation of Biodegradation of Plastics by White Rot Fungi

Cemre Sahin, Nicola Tiso

Instrumental Analysis Open Access Centre, Faculty of Natural Sciences, Vytautas Magnus University

Abstract

Improper disposal of plastics has led to a harmful buildup of plastic waste in our environment, posing a significant global threat [1]. The biodegradation process is the most efficient and desirable method for degrading plastics among forms of biodegradation because it is environmentally friendly, economical, and has a non-polluting mechanism. Fungi can grow on plastic surfaces, and they produce several enzymes including cutinase, lipase and lignocellulolytic enzymes that can break down the plastic [2]. Laccases are ligninolytic enzymes and they degrade lignin and other aromatic compounds. Laccases are used for wastewater treatment and bioremediation due to their ability to act on both phenolic and non-phenolic lignin related compounds and environmental pollutants [3]. The ability of fungi to enzymatically degrade high-density polyethylene (HDPE) and low-density polyethylene (LDPE) has been observed, but it was thought to be limited to only a few fungal species. Nevertheless, some studies emphasized the potential of diverse fungal strains in the degradation of plastics. This paper aims to evaluate the biodegradation of polystyrene (PS) and polyethylene terephthalate (PET) by white rot fungi *P. ostreatus* and *B. adusta.* Fungal strains were grown in solid and liquid media at 28°C supplemented with pieces of dry plastics. Two different concentrations of malt extract were used for solid medium: 3% and 0.5%. Dry weight loss of plastic samples is evaluated periodically, starting from 30 days after incubation. So far, *P. ostreatus* degraded 0.5% of PET after 48 days in liquid medium. More time is needed to obtain a higher percentage of biodegradation. Furthermore, the biodegradation by enzyme crude extract at various temperature will be evaluated.

Keywords: plastic biodegradation, *P. ostreatus, B. adusta,* polyethylene terephthalate, polystyrene, polyethylene.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Influence of Room Temperature on the Volatile Compounds of Tea Tree Oil (*Melaleuca alternifolia* Cheel) Studied by Gas Chromatography

Miglė Kazlauskaitė, Kristina Bimbiraitė-Survilienė, Audrius Maruška

Instrumental Analysis Open Access Centre, Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania

Corresponding author: migle.kazlauskaite2@stud.vdu.lt

Abstract

Terpenoids present in essential oils are sensitive to changes during storage, which can deteriorate the quality of essential oils. Light, temperature, and oxygen availability are key external factors influencing the changes in essential oils. The stability of essential oils depends on several chemical and environmental factors, and oxidation of essential oils can impair their quality [1]. Tea tree essential oil is extracted from the Melaleuca alternifolia plant. According to international standards, tea tree oil contains 15 main components, including terpinen-4-ol, γ-terpinene, α-terpinene, 1,8-cineole, p-cymene, terpinolene, α-terpineol, α-pinene, sabinene, aromadendrene, ledene, δ-cadinene, limonene, globulol, and viridiflorol [2].

The aim of this study was to identify and quantify the volatile compounds in different tea tree (*Melaleuca alternifolia* Cheel) essential oils and evaluate the changes in their composition during 15 days of storage at room temperature, excluding direct UV radiation exposure, and at room temperature in the dark using gas chromatography analysis. Components present in the essential oils were determined using a gas chromatography with mass spectrometry analysis.

The results showed that 16 compounds were identified under unaffected storage conditions: α-pinene, camphene, β-pinene, α-phellandrene, 2-carene, p-cymene, limonene, eucalyptol, γ-terpinene, α-terpinolene, terpineol, cyclohexanol, terpinen-4-ol, α-terpineol and caryophyllene. After 15 days of storage at room temperature without exposure to direct UV radiation, 16 compounds were identified: α-pinene, camphene, β-pinene, α-phellandrene, 2-carene, p-cymene, limonene, eucalyptol, γ-terpinene, α-terpinolene, terpineol, cyclohexanol, terpinen-4-ol, α-terpineol and caryophyllene. Comparing the sample that was not exposed to storage conditions with the sample that was exposed to room temperature without direct UV radiation, it was observed that: α-pinene, camphene, β-pinene, α-phellandrene, 2-carene, p-cymene, limonene, eucalyptol, cyclohexanol and terpinen-4-ol increased from 5.10% to 28.81%, but α-Terpinolene, Terpineol, α-terpineol and Caryophyllene decreased from 8.14% to 11.35%. After 15 days of storage at room temperature in the dark, 16 compounds were identified: α-pinene, camphene, β-pinene, α-phellandrene, 2-carene, p-cymene, limonene, eucalyptol, γ-terpinene, α-terpinolene, terpineol, cyclohexanol, terpinen-4-ol, α-terpineol and caryophyllene. However, comparing the sample that was not exposed to the storage conditions with the sample that was stored at room temperature in a dark environment, the detected peaks increased from 13.19 to 27.28%. Unfortunately, after 15 days, the majority of identified compounds increased in both samples, indicating that these storage conditions may affect the chemical stability of the essential oil.

Keywords: essential oil, tea tree essential oil, storage conditions.

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ISSN:2335-8653
ONLINE ISSN: 2335-8718

Micromycete Contamination Analysis of Peanut (*Juglans regia*), Hazelnut (*Corylus avellana*) and Walnut (*Arachis hypogaea*)

Greta Tverskytė, Rūta Mickienė, Audrius Maruška

Instrumental Analysis Open Access Centre, Faculty of Natural Sciences, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania

Corresponding author: tverskyt.greta@gmail.com

Abstract

Fungi are one of the most common contaminant groups in nuts and are considered normal plant inhabitants that can easily survive periods of drying and storage. Nuts are susceptible to pathogenic fungi. *Aspergillus, Fusarium, Penicillium* and *Alternaria* are the most common pathogenic fungi that produce harmful compounds - mycotoxins. Currently, about 400 mycotoxins have been discovered, but the most widely known mycotoxin is aflatoxin, which is produced by *Aspergillus flavus, Aspergillus parasiticus* and other types of fungi under favorable conditions [1]. Direct nut contamination was detected in nutshell and in shelled and unshelled nut kernels. Using sterile tweezers, nut parts were placed 1-2 cm apart on Petri dishes with medium. 10 units of material were placed in one Petri dish and incubated for 7-10 days at a temperature of 25 ± 2°C. During the assessment of nut contamination, 10 g of nuts were added to a flask of 90 ml of sterile distilled water and shaken in a shaker for 20 minutes. The resulting suspension was diluted to a concentration of 10^{-3}(0.001%). 1 ml of the obtained suspension was poured into Petri dishes with medium and incubated at a temperature of 25 ± 2°C for 5-7 days. Nut moisture was measured using an Adam MPB 53 analyzer. During the investigation of direct micromycete contamination, 6 species of fungi were identified on peanut (*Juglans regia*), hazelnut (*Corylus avellana*) and walnut (*Arachis hypogaea*) parts. After the assessment of nut contamination, it was found that walnuts were contaminated the most: the total average number of colonies was 2111 CFU/g in walnuts, 2000 CFU/g in hazelnuts and 1333 CFU/g in peanuts.

Keywords: moisture content, mycological pollution, nuts.

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