

International establishment of doctoral study programme  
in the field of biotechnology and food sciences – MeD-BioFood



**MeD-BioFood**



# BOOK OF ABSTRACTS

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**2<sup>nd</sup> International Workshop for PhD. Students**

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**European Dimension of Internationalization of Doctoral Study  
in Biotechnology and Food Sciences – (EuroDisBioFood)  
2<sup>nd</sup> International online workshop for PhD. students  
6<sup>th</sup> July 2022, Università degli Studi del Molise, Italy**

## **Book of Abstracts**

Institute of Applied Biology  
Faculty of Biotechnology and Food Sciences  
Slovak University of Agriculture in Nitra

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## LIST OF ABSTRACTS

INFLUENCE OF VITAMIN E ON THE MITIGATION OF POLYCYCLIC AROMATIC HYDROCARBONS FORMATION IN CHARCOAL-GRILLED BEEF MEAT .....	6
PROLONGED LEAVENING TIME AS A METHOD TO MITIGATE ACRYLAMIDE IN NEAPOLITAN PIZZA .....	7
EVALUATION OF FUNCTIONAL DIVERZITY IN THE SET OF FAGOPYRUM GENOTYPES USING MOLECULAR MARKERS.....	8
DEVELOPMENT AND OPTIMIZATION OF EDIBLE GEL-BASED FOOD FOR A TARGET GROUP OF CONSUMERS.....	9
EFFECT OF BLUE HONEYSUCKLE ON HUMAN OVARIAN AND CANCER CELLS <i>IN VITRO</i> .....	10
VARIABILITY OF BIOACTIVE SUBSTANCES IN BEAR GARLIC ( <i>ALLIUM URSINUM</i> L.).....	11
VALORIZATION OF PRICKLY PEAR <i>OPUNTIA FICUS-INDICA</i> (L.) MILL: STUDY OF ITS PHYTOCHEMICAL, NUTRACEUTICAL AND FUNCTIONAL PROPERTIES .....	12
MODIFICATION OF CHEMICAL COMPOSITION, ANTIOXIDANT CAPABILITIES AND TECHNO-FUNCTIONAL PROPERTIES OF BREWERS' SPENT GRAIN BY ENZYMATIC TREATMENTS, THERMAL EXPOSURES AND ULTRASONICATION .....	13
GENETIC, PHYSICOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF COMMERCIAL CULTIVARS OF ARTICHOKE FOR ITS PRODUCTIVE AND COMMERCIAL OPTIMIZATION.....	14

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## ABSTRACTS

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## **Influence of Vitamin E on the mitigation of polycyclic aromatic hydrocarbons formation in charcoal-grilled beef meat**

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Coordinator of the PhD Course: Prof. Giuseppe Maiorano*

Polycyclic aromatic hydrocarbons (PAHs), potential carcinogenic agents to humans, are formed during high-temperature cooking methods of meat such as grilling. The European Union has established PAH4 (sum of benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene and benzo[*a*]pyrene) as the most appropriate indicator for the occurrence and carcinogenic potency of PAHs in food. Antioxidants, such as vitamin E due to their mechanisms of action on carcinogenic substances, are of great interest. This research work aimed to evaluate the influence of different levels of addition in ground meat (2, 3, 4.5 and 6 µg/g) of DL- $\alpha$ -tocopheryl acetate on the formation of PAHs in grilled beef hamburgers. Total lipid content and endogenous Vitamin E content of raw meat samples were 1.36 g/100g and 1.32 µg/g, respectively. 14 PAHs were assayed on well-done hamburgers using an acetonitrile based-extraction and high-performance liquid chromatography with fluorescence detection. Data were analysed by one-way ANOVA. No effect ( $P > 0.05$ ) was observed for total PAHs content in samples added with 2 µg/g of vitamin E; while a marked reduction ( $P \leq 0.001$ ) was observed in hamburgers added with 3 and 4.5 µg/g of vitamin E (59 and 46 ng/g respectively) compared to control samples (76 ng/g). Although control samples presented low values of PAH4, far from the maximum established by the European Union (30 ng/g), the addition of vitamin E significantly reduced PAH4 in all experimental groups, especially in those added with 3 and 4.5 µg/g of vitamin E. Although this meat did not generate high PAHs amount, it could be possible to observe that the PAHs formation is greatly affected by vitamin E concentrations added in raw meat samples.

*Keywords:* grilled meat, PAHs, vitamin E

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## **Prolonged leavening time as a method to mitigate acrylamide in Neapolitan pizza**

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Acrylamide is a chemical compound, first discovered in 2002 and classified as a potential carcinogenic for humans (International Agency for Research on Cancer, group 2A) and as genotoxic and neurotoxic for animals. In 2015, the European Food Safety Authority has defined the presence of acrylamide in food a public health concern, recommending the need to identify measures to reduce consumer exposure to this substance. The Commission Regulation (EU) 2017/2158 established mitigation measures and reference levels to mitigate the presence of acrylamide in foods. Food categories most subjected to acrylamide formation (in the range 40 – 4000 µg/kg) are potato-based products, bakery products and coffee, due to their composition and the high temperatures reached during processing. In cereal-based products, acrylamide occurs mainly through Maillard reaction, starting from asparagine and reducing sugars during the cooking process at temperatures above 120 °C and low moisture. In this context, this study focused on the investigation of acrylamide content in Neapolitan pizza, an icon of the Italian gastronomic and cultural panorama, where it can be developed for the natural composition of the ingredients and the high temperatures (430 - 485 °C) reached in the ovens. Acrylamide has been evaluated in different sections of Neapolitan pizza (external part, internal part, slice) with and without the presence of topping, and in pizza samples obtained with leavened doughs for different times, to evaluate fermentation with microbial starters as a possible mitigation strategy for the formation of acrylamide. Particularly, after the extraction and purification phase, freeze-dried samples were injected in an HPLC-UV instrumentation for acrylamide determination. The research highlighted a different distribution of acrylamide both in the different sections of pizza and in the pizza with and without topping, suggesting a likely influence of topping on acrylamide formation as a result of the effect on factors such as thickness, pH and moisture, that affect the development and course of the Maillard reaction. A decreasing value of acrylamide has been observed for the samples of Neapolitan pizza subjected to various leavening time, indicating the prolonged fermentation as a possible mitigation strategy.

*Keywords:* acrylamide mitigation, prolonged leavening time, Neapolitan pizza

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## Evaluation of functional diversity in the set of *Fagopyrum* genotypes using molecular markers

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The genus *Fagopyrum* includes two cultivated species known as common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum* Gaertn.). These cultivated species are intended for cultivation and human consumption. Buckwheat is grown as a traditional crop in Asia, Central Europe and Eastern Europe. Buckwheat is a pseudo-cereal and has potential as a functional food material. In certain areas of Asia, it is already used as a functional crop. *Fagopyrum* could replace potatoes or rice in the diet with the respect to chemical composition of buckwheat. It contains high-quality gluten-free proteins also high level of phenolic compounds and antioxidants, especially rutin. *Fagopyrum* is in general characterized by a high level of B vitamins. Molecular markers are appropriate tools for the analysis of genetic diversity mainly PCR-based markers. SCoT markers are designed based on the short conserved region flanking the ATG start codon in plant gene. For the analysis of 5 genotypes of common buckwheat and 5 genotypes of tartary buckwheat coming from different countries 5 SCoT markers were used. A total of 81 amplified fragments were detected, of which 97.5 % (79 fragments) were polymorphic. The number of polymorphic fragments ranged from 7 (SCoT 8) to 21 (SCoT 30) with an average of 15.8 per primer. The average percentage of polymorphism was 98.91 %. Polymorphism information content values ranged from 0.819 (SCoT 8) to 0.979 (SCoT 29) with an average of 0.918. Using hierarchical cluster analysis using the UPGMA algorithm, a dendrogram was created. Two main clusters were created in the dendrogram, one containing the genotypes of common buckwheat and the other tartary buckwheat genotypes. Genetically the closest were genotypes of common buckwheat, AIVA and BALLADA, originating from Lithuania and Russia, respectively. Based on the results, we can conclude that the SCoT technique is suitable for the genetic diversity study of buckwheat. Its advantage is that the markers are obtained from the functional part of the genome, so we can use the SCoT technique directly to monitor economically important traits in the research as well as in the breeding process.

**Keywords:** common buckwheat, tartary buckwheat, SCoT markers, polymorphism, dendrogram

**Acknowledgement:** Work was supported by the Operational Program Integrated Infrastructure within the project: Demand-driven Research for the Sustainable and Innovative Food, Drive4SIFood 313011V336, cofinanced by the European Regional Development Fund (50 %) and KEGA project No. 027SPU-4/2021 (50 %).



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## Development and optimization of edible gel-based food for a target group of consumers

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As the evidence suggests, our population is aging rapidly. For the first time in history, the number of people aged 65 and over is exceeding the number of children under the age of 5. By 2050, one in six people in the world will be over 65, as the number of people in this age category is projected to double. It is essential to ensure that people live to old age in good health so that they can lead full and meaningful lives. As nutrition is a very important component that affects our health, it is essential to ensure safe and sufficient food consumption for the elderly. In older age, age-related diseases (including inflammatory, cognitive, gastrointestinal, cardiovascular, cancer, and diabetes, among others) affect food intake. Age-related changes or diseases such as presbyphagia, xerostomia, tooth loss, and sarcopenia also affect the swallowing and chewing mechanism. Medication use, which is usually higher at older ages, also leads to hyposalivation, and thus to problems with food consumption. Understanding the changes and diseases that are typical of older age is essential for the development of new foods for older people that do not expose them to the risks of aspiration, choking, and even death (suffocation). As edible gels have a soft and stable structure, they represent a very promising form of food to ensure adequate nutrient and energy intake for the elderly. Due to its technological properties, the gel is an ideal matrix for the incorporation of bioactive substances. The main objective of this thesis is to develop an edible gel containing bioactive compounds that will have a protective effect in aging as well as in the prevention and treatment of age-related diseases and will be safe to swallow. In order to achieve the main objective, we have set sub-objectives: a.) to propose the composition and technological process for the production of edible gel and analyze the chemical, physical, organoleptic, and hygienic/health parameters. b.) To implement geroprotective compounds in the gel matrix and optimize the prototype. c.) To design and conduct consumer tests for general acceptance of the product. d.) To finalize the product.

*Keywords:* aging population, age-related diseases, edible gel, easy to swallow, food for seniors

**Acknowledgment:** This work was supported by the Slovak Research and Development Agency under Grant No. APVV-20-0078. “Development of edible gel-based foods and applications in a target segment of the aging population.”

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**Effect of blue honeysuckle on human ovarian and cancer cells *in vitro***  
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*Lonicera caerulea* (blue honeysuckle) has gained popularity in recent years due to its beneficial effects on health. Its fruit is known for antioxidant, anti-inflammatory, anti-cancer activities, etc. The aim of this study was to determine the effect of *L. caerulea* var. *kamtschatica* ethanol extract (concentrations 5 - 100 µg/mL for 24 hours) on human ovarian carcinoma cell line (OVCAR-3) and human ovarian granulosa cell line (HGL5). Cell viability was evaluated by AlamarBlue™ cell viability assay. The secretion of steroid hormones (17β-estradiol and progesterone) by HGL5, the amount of apoptosis-inducing factor (AIF), and the production of transforming growth factor β2 (TGF-β2) and its receptor (TGFBR2) from HGL5 and OVCAR-3 cell lysates were evaluated by ELISA. In our research, blue honeysuckle extract did not observe any changes in viability of ovarian cells. It appears the blue honeysuckle extract did not affect on the amount of AIF not even the production of TGF-β2 and its receptor TGFBR2 in cell lysates. Similarly, the extract did not have a significant impact on the secretion of progesterone and 17β-estradiol by HGL5 cells. According to our research, blue honeysuckle in our study did not make any significant changes in HGL5 and OVCAR-3 cell lines.

*Keywords:* blue honeysuckle, ovarian steroidogenesis, viability, TGF-β2, AIF

**Acknowledgments:** This work was supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects APVV-18-0312, DS-FR-19-0049, VEGA 1/0266/20, KEGA 033SPU-4/2021 and the Operational Program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund.

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## Variability of bioactive substances in bear garlic (*Allium ursinum* L.)

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Bear garlic (*Allium ursinum* L.) is a bulbous perennial flowering plant. It grows in humid forests of Europe and Asia. Although the most popular and used part of bear garlic is its leaves, all parts of the plant, including the flowers, are edible and have culinary uses. Bear garlic is considered rich source of secondary metabolites, including polyphenols, flavonoids, and other substances with significant antioxidant activity. It is used as a spice and a traditional folk medicine, and a number of *in vitro* and *in vivo* experiments have shown that it has a high potential for the prevention and treatment of diseases of the cardiovascular system. Samples of plant material were obtained in the spring of 2021 from three different locations in Slovakia (Šoporňa, Rumanová, Hlohovec). The wild garlic samples were divided into three parts, bulb, leaf and stem, which were analyzed separately. The total polyphenol content (TPC) was determined by spectrophotometric method using the Folin-Ciocalteu reagent, the total flavonoid content (TFC) was determined by spectrophotometric method using aluminum chloride, and the antioxidant activity (AA) was determined by FRAP spectrophotometric method. Average TPC in bulbs was 167.67 mg GAE.kg<sup>-1</sup> FW, in stems 151.78 mg GAE.kg<sup>-1</sup> FW and in leaves 776.33 mg GAE.kg<sup>-1</sup> FW. Average TFC in bulbs was 16.1 mg CE.kg<sup>-1</sup> FW, in stems 11.3 mg CE.kg<sup>-1</sup> FW, and in leaves 70.1 mg CE.kg<sup>-1</sup> FW. Average AA in bulbs was 0.87 mmol TE.kg<sup>-1</sup> FW, in stems 0.95 mmol TE.kg<sup>-1</sup> FW and in leaves 2.18 mmol TE.kg<sup>-1</sup> FW. The total content of polyphenols, flavonoids and antioxidant activity differed depending on the morphological part of the plant and the location. Highest values of TPC, TFC and AA were determined in the leaves. Based on the results, we confirmed a strong positive relationship between the content of total polyphenols and the content of total flavonoids, between the content of total polyphenols and antioxidant activity, and between the content of total flavonoids and antioxidant activity.

**Keywords:** bear garlic, *Allium ursinum*, antioxidant activity, total polyphenol content, total flavonoid content

**Acknowledgments:** This work was supported by the Operational Program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund.

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## Valorization of prickly pear *Opuntia ficus-indica* (L.) Mill: Study of its phytochemical, nutraceutical and functional properties

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*Opuntia ficus-indica* (L.) Mill., commonly known as prickly pear, cactus pear, or nopal cactus, is a tropical or subtropical plant which can grow in arid and semi-arid climates. Prickly pear is native to Mexico, the country with the largest area under cultivation, but at present is naturalized in all continents. This plant is mainly known by their fruits, popularly named “figs” or “tunas”, but their cladodes are also consumed. There is ample evidence of the health benefits of prickly pear: it is a source of nutrients and vitamins, it shows antioxidant properties due to its phenolic content and antioxidant activity and presents medicinal use: anticancer effect, treatment of hyperglycemia, and treatment of high levels of cholesterol among others. In addition to their use for human consumption, the prickly pear botanical parts (fruits, cladodes, and seeds) can be used for other purposes, such as in the pharmaceutical and cosmetic industries, animal feeding, CO<sub>2</sub> uptake, biofuel production and phytoremediation of soils, among other uses. The objective of this Doctoral Thesis was to determine the phytochemical, nutraceutical, and functional qualities of the different parts of *Opuntia ficus-indica* (L.) Mill. (fruits, cladodes and seeds) in order to evaluate their use for human diet, animal feeding or industrial use. To reach the main purpose, four specific objectives were determined. Objective 1: Phytochemical, nutraceutical, and functional characterization of the fruit, cladodes, and seeds. About this objective, antioxidant activity, bioactive compounds, (poly)phenolic profile, fatty acids and nutritional composition of prickly pear were analyzed and four papers were published. Objective 2: Sensory analysis. Regarding this objective, volatile compounds were analyzed in prickly pear fruit pulp and results were published. Objective 3: Evaluation of the quality parameters of prickly pear fruits during their conservation under different conditions. Concerning this objective, the results on changes in fruit quality, antioxidant activity, bioactive compounds and ethylene and respiration rate under cold and shelf-life storage were published. Objective 4: Economic estimation of prickly pear production and its feasibility in Spain. About this objective, the results on cost production structure and economic evaluation were published

*Keywords:* prickly pear, antioxidant activity, nutritional composition, bioactive compounds, cost production

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## **Modification of chemical composition, antioxidant capabilities and techno-functional properties of brewers' spent grain by enzymatic treatments, thermal exposures and ultrasonication**

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Brewers' spent grain (BSG) is the main byproduct of the brewery industry which has been well known for its nutritional value and its potential as a functional food ingredient. The study aimed to evaluate the modification of chemical composition, techno-functional properties and biological activity of BSG as an impact of enzymatic treatment (protamex and flavourzyme), thermal treatment (water-bath and autoclave heating) and ultrasound treatment (bath- and probe-type). The enzymatic treatments demonstrated an enhancement of antioxidant activities (FRAP, ORAC, and ABTS) of BSG protein (BSGPs). After separation, BSGPs had higher antioxidant capacities than those in sediments. The study demonstrated that the amount of polyphenolic content is responsible for FRAP value while BSGPs is responsible for ORAC and ABTS capabilities. Protamex and flavourzyme increased the antioxidant capabilities of BSGPs and phenolic content of the sediments; enhanced higher oil-holding capacity, foaming properties and lower emulsion capability. Thermal treatments (autoclave and water-bath heating) and bath-ultrasonication converted saturated fatty acids into polyunsaturated fatty acids, increased the amount of polyphenolic compounds, improved antioxidant activities and modified the volatile compounds of BSG. Furthermore, an improvement in certain techno-functional properties was also identified. In conclusion, studied treatments including enzymatic treatment, thermal exposure and ultrasound treatment on BSG performed promising benefits for improving the quality of BSG as a functional food and nutraceutical ingredient.

*Keywords:* antioxidant, ultrasonication, grain, brewers

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## **Genetic, physicochemical and functional characterization of commercial cultivars of artichoke for its productive and commercial optimization**

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Nowadays there is a growing interest in the consumption of foods with functional properties and among them globe artichokes (*Cynara cardunculus L. var. scolymus (L.) Fiori*) stands out for its high content of bioactive substances and phenolic compounds. Artichoke is one of the vegetables with higher content in phenolic compounds, which are responsible for their taste, flavor and health beneficial effects. Nevertheless, this high content of polyphenols makes artichoke a vegetable very susceptible to browning, which may difficult the use during processing and in ready-to-eat products. Besides, phenolic profile and concentration depends on many factors, such as genotype, harvest date, and environmental and agronomical conditions. For that reason, a greater knowledge in this field will allow the selection of artichoke cultivars based on their phenolic profile and know the adaptability of each one for: fresh consumption or industrial processing. The main objective of this research is to improve the competitiveness of the artichoke in all phases of the production chain, from seeds or plant material commercialization, passing through agricultural production and industry, to commercialization stage. To achieve that, three different articles have been published at the moment analysing different globe artichoke cultivars obtained by different propagation methods. Main results report that artichoke cultivars are highly influenced by three factors: the flower head order (main, secondary and tertiary head), gibberellic acid treatment and internal development stage. Besides, a positive correlation between total phenolic content and browning process was found. Artichokes with the lowest polyphenolic content exhibit the lowest browning after cutting which could increase their use in minimally processed products at market.

*Keywords:* phenolic content, minimally processed artichoke, browning, flower head order

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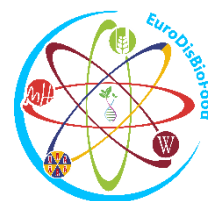
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