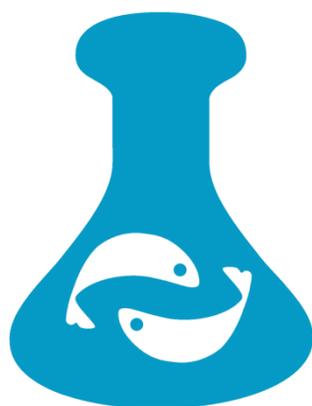


**FOOD-3 INTERNATIONAL
CONFERENCE**

**“THE CHALLENGES FOR QUALITY AND
SAFETY ALONG THE FOOD CHAIN”**



ABSTRACT BOOK

23-25 MARCH 2017

NEW BULGARIAN UNIVERSITY

SOFIA, BULGARIA

ABSTRACTS

Plenary and Oral Presentations

Food 3-1

HISTORY OF THE BULGARIAN SOCIETY FOR MICROBIOLOGY

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Bulgarian Society for Microbiology (BSM) was found in 1927. Since 1946 it has become a member of Union of Scientists in Bulgaria. BSM unites around 200 scientists from all branches of microbiology: general and industrial microbiology, medical microbiology, veterinary microbiology, plant microbiology, soil microbiology, virology and infectious immunology. Since 1965 the society has been organizing the National Congresses with international participation. The last, 13th National congress of microbiology was carried out in the town of Tryavna in 2014. Next, 15th one will be held in 2018. The society organizes also the National congresses in Virology with international participation. The last, eighth Congress was held in 2016 in Sofia.

During 1998 BSM initiated The Balkan Society for Microbiology's foundation. The Balkan Society for Microbiology includes microbiological societies of Greece, Romania, Monte Negro, Albania, Bosnia and Herzegovina, Serbia, Turkey, Republic of Macedonia, Croatia and Bulgaria. Up to now, the Balkan society have organized 9 Balkan congresses "Microbiologica Balkanika", which held biennially. Such congresses were organized twice in Bulgaria: first one –in 1999 in Plovdiv, and in Veliko Tarnovo - in 2013.

BSM is a member of FEMS and participates many of its activities. BSM supports young scientists to obtain grants/specializations in leading laboratories in Europe and USA.

Bulgarian Society for Microbiology supports and is a co-organizer of FOOD-3 International conference "The challenges for quality and safety along the food chain" as well.

Key words: *Bulgarian Society for Microbiology, activities, congresses*

Food 3-2

EFSA -TRUSTED SCIENCE FOR SAFE FOOD

Dr. Donka Popova

National EFSA focal point

EFSA is a European agency funded by the European Union that operates independently of the European legislative and executive institutions (Commission, Council, and Parliament) and EU Member States.

It was set up in 2002 following a series of food crises in the late 1990s to be a source of scientific advice and communication on risks associated with the food chain. The agency was legally established by the EU under the General Food Law - Regulation 178/2002.

The General Food Law created a European food safety system in which responsibility for risk assessment (science) and for risk management (policy) are kept separate. EFSA is responsible for the former area, and also has a duty to communicate its scientific findings to the public.

As risk assessor, EFSA produces scientific opinions and advice that form the basis for European policies and legislation. Its remit covers food and feed safety; nutrition animal health and welfare; plant protection; and plant health. EFSA also considers, through environmental risk assessments, the possible impact of the food chain on the biodiversity of plant and animal habitats.

EFSA also plays an important role in collecting and analysing data to ensure that European risk assessments are supported by the most comprehensive scientific information available. It does this in cooperation with EU Member States.

Communicating on risks associated with the food chain is another key part of EFSA mandate. This means providing appropriate, accurate and timely information on food safety issues to raise awareness and explain the implications of its scientific work.

1. FOOD AND BEVERAGES QUALITY AND SAFETY (FBQS)

Food 3-3

CRITICAL POINTS OF FOOD CONTAMINATION WITH HUMAN NOROVIRUSES

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Human noroviruses are identified as the leading cause of foodborne diseases in many countries (Europe Union, United States, Canada, New Zealand, etc.). Since 2011, in the EU the number of outbreaks caused by viruses has more than doubled, while in the US they cause 5.5 million foodborne illnesses (58%) annually. Children aged less than 5 years

are at particular risk with 4 times higher incidence of norovirus than children aged ≥ 5 years (WHO).

Advantage characteristics of human norovirus are: highly infective, low infectious dose (18-1,015 genome equivalents), persistent and stable in the environment, etc. Noroviruses are typically spread by fecal–oral route and transmitted by contaminated food, water or aerosols, person-to-person contact, cross-contamination from surfaces and contact with fomites. Critical points can be indicated for contamination of foods at risk.

(1) Contamination of water sources with enteric viruses. Water-related diseases are associated with drinking, recreational water, and those used for agricultural practices (crop irrigation, food processing). Norovirus outbreaks associated with fresh produce are primarily linked with the salads and berries. Important vehicle of transmission is bivalve shellfish due to filter-feeding what enables the concentration of norovirus from polluted water in the digestive glands.

(2) Norovirus is highly infectious and could be shed in feces at very high numbers (up to 10^{11} particles/g) for prolonged times (more than 3 weeks) which indicates ease spread by food handlers. Infected food handlers are the source of 53% of norovirus foodborne outbreaks. Foods at risk are those that need extensive handling and ready-to-eat foods that do not undergo further processing.

(3) Human noroviruses are able to attach to inert surfaces and persist for up to 28 days on common food-preparation surfaces. The survival on finger pads is high and their transfer to stainless steel surface and successively to foods surface could occur relatively easily.

Recommendation: Control measures at industrial level: hygienic sewage disposal; treatment of drinking-water; management of wastewater used for irrigation; thermal processing; good hygiene practices during production and processing.

Acknowledgements

The author gratefully acknowledges funding by Ministry of Education, Science and Technological Development (Project III 46009).

Key words: *noroviruses, food contamination, food handler, food contact surfaces*

Food 3-4

WINE - THE EPISODE OF NATURE OR THE CONSEQUENCE OF YEAST SLAVERY

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Grapes and other fruits have been found in human settlements that flourished 8,000 years ago. However it took some time before our ancient ancestors discovered that grape could produce a source of fermented food/beverage. This was the start of new age not only as food but also technologically and culturally. The wine makers craft is one of the oldest in the world. It is not changed much since great Georgian ancestors and their civilizations started wine rise and journey

around the world, but first giving the yeast the chance to make it. During the history wine importantly marked human life and development. It was tied companion of many innovations in technology and nutrition, but also knowledge accumulation. The wine yeast function is just one of milestones well recognised in science. Today we could allocate “wine and wine like products” into many distinct groups, respecting vine varieties, terroirs, technologies, yeasts, gastro-nutritional practices, if we mention a few. In our 20 years long lasting research in the area of wine yeasts we faced many methodological challenges as well as conceptual in the area of wine, and its technologies, which is considered traditional and sometimes even conservative. Domesticated yeast from the species *Saccharomyces cerevisiae* has been known as traditional industrial yeast. This organism has many potent and permanent contacts with humans via food and health products what makes it important in terms of biodiversity, biotechnology but also biosafety, what will be addressed in the lecture.

Key words: *wine, yeast, biodiversity, biotechnology, biosafety*

Food 3-5

PRESERVATION OF FOODS BY COMBINED TECHNOLOGIES USING HIGH PRESSURE PROCESSING: CHALLENGES AND OPPORTUNITIES

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Introduction: High-pressure processing (HPP) is for the food industry a relatively young technology that successfully emerged into one of the most engaging alternatives to conventional thermal processing. Lately there has been a growing consumer demand for minimally processed and chemical additives free foods.

Aim: High hydrostatic pressure with other additional hurdles such as mild thermal treatment, ultrasounds, carbon dioxide, or the use of antimicrobials (essential oils, bacteriocins) and antioxidants (plant phenolic compounds) could provide effective and innovative solutions for new food product design with improved safety. The current industry applications of HPP and a review of the most recent research focus on the multi-hurdle approaches to increase the effectiveness of HPP are introduced and compared.

Results: Specific research results on the combination of thyme extract and HPP to control *Listeria monocytogenes* in cheese; mild thermal treatment and HPP combination applied to fruit puree to prolong its shelf-life and control the presence of microorganisms (total aerobic bacteria,

molds and yeasts); and high pressure combined with ultrasounds to prevent rape honey recrystallization will be discussed in details.

Conclusions: Despite inherent challenges, strategies to develop new products with improved quality based on multi hurdle approach with HPP could prove successful in satisfying consumers' expectations and represent healthier processing alternatives to the current methods in place.

Keywords: *high pressure processing, hurdles, antimicrobials, polyphenols*

Acknowledgements: This work benefited from the infrastructure provided by the RE-SPIA (SMIS code 11377) project REGIO (2007-2013).

1.1 MILK DIARY PRODUCTS AND EGGS (MDE)

Food 3-6

FATTY ACID COMPOSITION OF BULGARIAN MILK

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Introduction: Milk is one of the most consumed foods in Bulgaria. Milk fat contains variety of fatty acids, some of which are included on the nutritional labels of products. Consumers should be informed for fatty acid composition, because specific fatty acids can affect human health.

Aim: Our objective was to survey Bulgarian milk and to determine its fatty acid composition and the eventually addition of non-milk fat.

Material and Methods: For a period of 5 years we analysed 100 samples of cow milk (raw, pasteurized) and 135 samples yogurt from cow milk. Fatty acid composition and purity of milk fat was determined by gas chromatography method (ISO 15885:2002).

Results: Across all the samples saturated fatty acids for cow milk were average 70.1% and for yogurt 71.1%. The quantities of unsaturated fatty acids were respectively 29.9% and 28.9%. We observed small seasonal variation in the fatty acid composition of Bulgarian milk, which was due to differences in concentrations of individual components. Non - milk fat was found in 5 samples yogurt, while all samples of fluid milk were pure, without any adulteration of the fat.

Conclusions: The present study, for first time provides information for fatty acid composition of the milk on Bulgarian market and it is useful for estimation of dietary intake.

Key words: *fatty acid composition, milk*

1.2 MEATFISH AND SEA FOOD (MFSP)

Food 3-7

POULTRY MEAT ON THE BULGARIAN MARKET – ESTIMATION OF ITS QUALITY, ACCORDING REQUIREMENTS OF REGULATION (EC) 543:2008

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Introduction: Why is necessary to control the levels of water in poultry meat which is sold on the market? Do we buy qualified meat or not? The current study answers all these questions. Before freezing, the poultry carcasses should be chilled. During the chilling, the poultry carcasses absorbed technically unavoidable water, which is subject of control. The rules about it are presented in Regulation (EU) 543:2008.

Aim: The study aimed to estimate the absorbed water and respectively the quality of poultry meat produced in Bulgaria and offered to the consumer.

Material and Methods: One hundred sixty five carcasses and 805 poultry parts have been examined for content of proteins (ISO 937) and moisture (ISO 1442), for a period of 5 years. The results were calculated according Regulation (EU) 543:2008.

Results: It was found that 6.7% of analysed carcasses contained water over the limit and 6.8% of poultry parts did not comply requirements of Regulation (EC) 543:2008. For the last 5 years, the other European countries reported 4% – 9% carcasses and 6% -10% poultry parts with water over the limits.

Conclusions: The results of this study lead to conclusion that in Bulgaria is produced and is offered poultry meat of good quality.

Key words: *water content, poultry meat*

1.3 GRAINS (G)

Food 3-8

CONTACT NON-EQUILIBRIUM PLASMA WATER TREATMENT IN SPROUTED BREAD TECHNOLOGY

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Introduction. Bread made without using flour and directly from specially prepared grain may enrich diet by biologically valued components, especially fiber. Sprouted bakery production allows decreasing food waste and losses along food chain. However shortcomings of the technology include a long duration of production cycle, low organoleptic properties and high microbiological risks. Water treatment with using contact non-equilibrium plasma leads to high penetrating ability of water and its antiseptic activity.

Aim. The work aimed studying possible ways to use plasma-chemically activated water in sprouted bread technology.

Material and Methods. Water treatment with nonequilibrium contact plasma was carried out in the discrete-type laboratory unit with the reactor volume of 0,5 dm³ to peroxide compounds concentration of 100–500 mg·L⁻¹. Bread composition excluded refined flour and contained biologically activated soft wheat grain. Determination of changing the technological processes and quality assessment was performed by physical, physicochemical and organoleptic methods.

Results. Activation of physiological and biochemical processes is achieved during grain soaking in plasma-chemically activated water in particular reducing falling number. Simultaneously duration of grain soaking decreases from 24 hours to 16 hours in case of the treatment water previously. Finished bakery products are characterized improved quality properties.

Conclusions. Sprouted bread technology can be developed by using additional water treatment by contact non-equilibrium plasma which allows obtaining functional bakery product having advanced organoleptic characteristics without artificial additives and sufficiently reducing the production cycle duration and prolonging mold-free shelf life.

Key words: sprouted wheat bread; plasma-chemically activated water; quality assessment; safety

1.4 VEGETABLES, FRUITS AND LEGUMES (VFL)

No submissions

1.5 FATS AND OILS (FO)

No submissions

1.6 HERBS, SPICES, COFFEE AND TEA (HSCT)

Food 3-9

PLANT BIOTECHNOLOGY, METABOLOMICS AND LEAD FINDING: PERFECT HOLISTIC MATCH?

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Per definition metabolomics represents a comprehensive holistic approach, comprising of systematic identification and quantification of all metabolites in an organism, at given conditions. The comprehensive analysis of the chemical fingerprints left by metabolic processes started to play a crucial role in the personalized medicine [1].

Since the rise of the omics age several techniques for high throughput analyses of targeted metabolites have been developed. Nuclear magnetic resonance (NMR) appears very suitable and adequate platform to carry out metabolomics analyses, because it allows simultaneous detection of diverse range of abundant (primary and secondary) metabolites, which opens novel avenues to fully explore the total biochemical machinery of plants. A great advantage of ¹H NMR-spectrometry over the other analytical platforms is the possibility for quantification and thus the direct comparison of concentrations of all compounds present in the sample, as the signal intensity is only dependent on the molar concentration of the solutes [2-5].

Some case studies, from author's laboratory, on the application of NMR-based metabolomics concept in natural products research, plant biotechnology and lead finding [3-8] will be presented and discussed.

Acknowledgements

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References

1. Nicholson JK (2010) *Nature* 463:32.
2. Kim HK, Choi YH, Verpoorte R (2010) *Nature Protocols* 5:536-549.
3. Georgiev MI, Radziszewska A, Neumann M, Marchev A, Alipieva K, Ludwig-Müller J (2015) *Plant Cell, Tissue and Organ Culture* 123:349-356.
4. Marchev A, Aneva I, Koycheva I, Georgiev MI (2017) *Phytochemistry Letters*, DOI: 10.1016/j.phytol.2016.12.030.
5. Marchev A, Yordanova Z, Alipieva K, Zahmanov G, Rusinova-Videva S, Kapchina-Toteva V, Simova S, Popova M, Georgiev MI (2016) *Biotechnology Letters* 38:1621-1629.
6. Alipieva K, Korkina L, Erdogan Orhan I, Georgiev MI (2014) *Biotechnology Advances* 32:1065-1076.
7. Vasileva LV, Getova DP, Doncheva ND, Marchev AS, Georgiev MI (2016) *Journal of Ethnopharmacology* 193:586-591.
8. Marchev A, Dimitrova P, Koycheva I, Georgiev MI (2017) *Food and Chemical Toxicology*, DOI: 10.1016/j.fct.2017.02.009.

1.7 HONEY AND BEE PRODUCTS (HBP)

No submission

1.8 MINERAL WATER, NONALCOHOLIC AND ALCOHOLIC BEVERAGES (MWNAB)

Food 3-10

THE QUALITY OF BOTTLED WATER IN TIRANA THE IMPACT OF CLIMATIC CHANGES

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Introduction: During the past few years it was noticed an increase in the usage of bottled water in Tirana. This is not only due to the low quality of drinking-water in the pipelines but also due to social changes and mentality changes.

Aim: To show a full view of what we know and what we do not know about bottled water, its quality, contaminant agents and the impact these agents play on the pollution magnitude of bottled water. Valuation of the connection between the pollution level and seasons, climate changes (during the raining period).

Material and method: This study includes seven production lines of bottled water, bottled water produced by them and sold in Tirana for a period of 6 years. The bacteriological examination of water samples was also performed during rainy weather in order to evaluate the impact of atmospheric changes (rain) in their bacteriological quality. The bacterial load of the examined samples is estimated by using the filtering method and the method of multiple pipes.

Results: Samples taken during the rainy weather (n=220) have a high level of pollution. 90.5% of the examined samples do not fulfill the standard (respectively with 1,2,3,4,5 or 6 pollution indicators). OR=15895%CI (94.8-264.3) p< 0.0001

Conclusions and recommendations: The results of this study suggest the need to establish, improve and spread the surveillance system in all lines of bottled water; the need to improve the HACCP, in order to continuously monitor the water that supplies the production lines and to fulfill all technological processes needed for the production of bottled water.

Key words: *bottled water quality, pollution indicators, treatment technology*

2. FOOD CONTAMINATION AND TOXICOLOGY (FCT)

Food 3 - 11

NATIONAL MONITORING PROGRAM FOR CONTROL OF RESIDUES AND DEVELOPMENT OF ANALYTICAL METHODS

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Introduction: Food safety is an integral part of the Bulgarian policy, by means of the National monitoring program for control of residues (NMPCR), performed by Bulgarian Food Safety Agency. By Order of the Minister of Agriculture and Food, CLVCE is defined as National Reference Laboratory (NRL) and is the only one Laboratory in Bulgaria fulfilling NMPCR.

Aim: Aiming to meet the European requirements for monitoring of residues in food of animal origin, the analytical scope of the methods in CLVCE needs to be extended in accordance with the legislation in this area.

Materials and Methods: After an appropriate sample preparation, the samples are being analyzed typically by liquid or gas chromatography coupled with mass spectrometry (LC-MS/MS and GC/MS/MS).

Results: For the last year the scope in CLVCE is extended by number of new compounds from different groups, according to Council Directive 96/23/EC as follows: Group A6- nitroimidazoles - 7 new substances in milk samples; Group B2(c) - Carbamates and pyrethroids - 5 new compounds for liver and animal fat; Group B3(a) Organochlorine pesticides and PCBs- 7 new compounds for animal fat.

Conclusions: All the methods have been successfully validated according to the legislation in the food safety area - Document SANTE/11945/2015 for the pesticides and Commission Decision 2002/657 EC for the other compounds. By the validation procedures we prove that method is reliable and we can ensure safe food without any risk to the consumer's health.

Keywords: *monitoring, residues, food safety*

Food 3-12

HEAVY METAL CONTENT IN DANUBE AND BLACK SEA FISH AS ESTIMATED *via* AAS

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Introduction: Cd, Pb and Hg are heavy metals which strongly injures the human health. All three can easily bioaccumulate and biomagnify along the food chain and are monitored widely in food. The consumption of fish is one of the most significant source of ingestion-related heavy metal exposure. Cd damages the respiratory, gastrointestinal, reproductive and nephrous systems. It leads to bone fractures, calcium demineralization and is pronounced cancer inductor. Molecular mechanisms of zinc replacement by cadmium in the enzymes are still unclear. Cd inhibits apoptosis, suppresses DNA-repairation mechanisms and the modification of transmembrane glycoprotein – E-cadherin. Pb is harmful to almost all human physiological functions. Lead affects different organs and systems as gastrointestinal tract, muscles, kidneys, peripheral and central nervous, reproductive, urinary and hematopoietic system. Exposure to lead results in increased oxidative stress and is known to influences the prenatal development as well. Mercury is among the most toxic heavy metals. The crucial targets of mercury toxicity in the cell

are mitochondrial damage, reduced ATP-synthesis, binding to thiol groups (-SH) and free oxygen species generation because of lipid, protein and DNA peroxidation. Hg persists in higher concentrations in carnivorous than the herbivorous fish. Human nervous system is the main target of mercury's action however the molecular mechanisms of its harmful effect are still not elucidated. Additionally, the chronic exposure to inorganic mercury causes stomatitis and tremor.

Aim: To study Cd, Pb and Hg concentration in muscle tissue of most edible fish species.

Materials/Methods: Ten fish species were examined for Cd, Pb and Hg content in their muscle tissue: 4 Danube species - carp (*Cyprinus carpio*), grass carp (*Ctenopharingodon idella*), european catfish (*Siluris glanis*) and pikeperch (*Sander lucioperka*,) and 6 Black sea species: bluefish (*Pomatomus saltarix*) - juvenile and mature, atherina (*Atherina boyeri*), sprat (*Sprattus sprattus*), sardine (*Sardina pichardus*) and horse mackerel (*Trachurus mediterraneus ponticus*). Individuals were collected near towns of Vidin and Sozopol in 2014 and were stored at -20°C prior to analysis. Cd and Pb content was estimated using AAS (Atomic Absorbtion Spectrometry). Hg estimation was conducted *via* CVAAS (Cold Vapor AAS). Data obtained were compared with the Maximum residual limits (MRL) values in fish described in the EC Regulation № 1881/2006.

Results: Our results showed that Cd concentration in Danube fish varied between 0.0004 and 0,0009 mg/kg⁻¹ and in Black sea fish – between 0.007 and 0.0085 mg/kg⁻¹ and does not exceed the Maximum residual limit (0.05 mg/kg⁻¹). We found the values of Pb were 0.006-0.02 for Danube fish and 0.22 to 0.05 mg/kg⁻¹– for marine fish. MRL for Pb is 0.3mg/kg⁻¹ hence, the investigated samples were not Pb contaminated. Regarding Hg, the MRL is 0.5 mg/kg⁻¹. Its concentrations in both freshwater and marine fish were under the MRL – 0.009-0.03 and 0.013-0.017, respectively.

Conclusions: In conclusion, our results revealed the 10 species of fish from Danube and Black sea are free of Cd, Pb and Hg contamination, correspond to the EU regulation and are safe for consumption.

Acknowledgements: This study was funded by Grant № 972/2015 of the Central Fund for Strategic Development (CFSR), New Bulgarian University.

Keywords: *fish, heavy metals, AAS*

3. MICROBIOLOGICAL CONTROL OF FOOD (MCF)

FOODBORNE ZONOSSES – HAZARD AND RISK TO PUBLIC HEALTH

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Foodborne zoonoses are caused after eating of food or drinking water contaminated with pathogenic microorganisms such as bacteria and their toxins, viruses and parasites. The severity of infections in humans ranges from mild gastroenteritis with self-limiting symptoms to life-threatening medical conditions. Many of these microorganisms survive and proliferate both in the environment and in foods of plant and animal origin, regardless of storage conditions (cold temperatures, vacuum packs, salt concentrations, etc.). The most frequent bacterial pathogens in the food chain belong to the genera *Campylobacter*, *Salmonella*, *Yersinia* and the species *Escherichia coli* and *Listeria monocytogenes*. Foodborne zoonoses caused by them are real hazard and widespread global threat to public health, as in the European Union (EU) more than 320,000 cases of infection in humans are annually reported. Their actual number, however, is much higher because many cases are not registered. As it will be shown, a number of animal species are carriers and distributors of food pathogens, representing a constant threat to human health in Europe. Complex and still unknown groups of risk factors and their interactions lead to the emergence of surprisingly severe foodborne infections in humans, as it was the *E. coli* infection in 2011 in Germany and other European countries. Additional epidemiologic data will reveal the importance and potential risk of spreading the zoonotic foodborne infections in Europe. Standard and modern microbiological methods and approaches will contribute to effective monitoring and evaluation of foodstuff and will complement the overall strategy to ensuring food safety and protection the consumer's health.

BRUCELLOSIS - RE-EMERGING ZONOTIC AND FOOD BORNE DISEASE

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Introduction: Zoonotic diseases primarily affects domestic and wild animals than transfer to humans. Depending on the agent, way of spreading may be respiratory, contact, alimentary (food or water) or combination. Brucellosis is a re-emerging zoonotic disease which is spreading by all mentioned ways. Disease exists in R. Macedonia since 1980 with over 12.000 reported and confirmed human cases. Disease is reported in all neighbouring and almost all European countries with significantly different incidence.

Aim: To present current epidemiology situation, ways of spreading of the disease in respect of zoonotic and foodborne disease, and measures for control and prevention of brucellosis in Republic of Macedonia, a small country with endemic areas, long history and experience.

Material and Methods: Review and presentation of official data on epidemiology of brucellosis in past 36 years and situation after implementation of new national strategy based on vaccination of small ruminants.

Results: *Brucella melitensis* biotype 2 was confirmed as etiological agent in R. Macedonia. Recent study based on molecular methods for species typing (AMOS PCR and RT PCR), and genotyping (MLVA-16 and MLVA-8), beside *Brucella melitensis* also confirmed *Brucella abortus* (for the first time in Macedonia). Epidemiological data suggested about 23% of spreading the disease by alimentary way (foodborne disease due to consumption of unpasteurized milk, cheese, and undercooked infected meat), 34% by contact and 43% by combined way of spreading brucellosis. Respiratory way is not often, happens in laboratories or working with infected animals. About 80% of patients lived in rural and 20% in urban areas. Disease in Mediterranean area has seasonal character with maximum in May-June and minimum in winter. Since 2008, in R. Macedonia, national control strategy was completely changed from „test and slaughter” to vaccination of small ruminants (sheep and goats) with Rev 1 vaccine, applied intraocular. Results are significant decreasing of epizooty in animals and human morbidity (287, 167, 107, 94, 47, 35, 20 and 23 in 2009, 2010, 2011, 2012, 2013, 2014, 2015 and 2016 respectively).

Conclusions: Control of Brucellosis is very complicated due large reservoirs in domestic and wild animals. Control of animal brucellosis is imperative for control of human brucellosis. Preventive measures includes effective veterinary and health control of animals (trading and transport and slaughter) and animal products (meat, milk and their products), education of the population, continuously state financial support, institutional cooperation and regional cooperation.

Key words: *brucellosis, control, epidemiology, foodborne.*

Food 3 – 15

FUNGAL AND MYCOTOXINS CONTAMINATION OF FOODS

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The usual concept of mycotoxins is that they are secondary fungal metabolites that are elaborated in food and feed-stuffs contaminated with the producing organisms. Thus they may cause disease in the recipient as a sequela to ingestion of the food. However, in a consideration of all potential manifestations of disease in humans resulting from exposure to these fungal metabolites, other routes must be considered, such as inhalation, contact, and as part of, or a passive exposure resulting from mycotic infection by a toxigenic fungus. The occurrence of mycotoxins in food is often the result of contamination of the commodity by plant pathogenic, toxigenic fungi.

The disease resulting from exposure to a mycotoxin may be manifested as acute to chronic disease ranging from rapid death to tumor formation, but more occult disease may occur where the mycotoxin interferes with the immune processes, rendering the patient more susceptible to infectious diseases. However, mycotoxic event may be overshadowed by the infectious disease and thus not be considered in the overall syndrome. Some mycotoxins are considerably dermonecrotic and some airborne.

Mycotoxins can be identified using techniques that detect mycotoxicogenic moulds or using techniques that directly detect the toxins. Detection of mycotoxins is through chromatographic techniques, mass spectrometry, and immunoassays. Detection of mycotoxins using HPLC coupled with detection of fluorescence (HPLC-FL) has been accepted as the official method in many AOAC protocols. This method has been used to detect and quantify mycotoxins such as OTA, aflatoxins, and citrinin among others. Out of 107 flour samples we measured for total aflatoxins and 14% of them were positive.

THE FATE OF INDIGENOUS MICROBIOTA IN SPONTANEOUSLY FERMENTED GAME MEAT SAUSAGES: SEARCHING FOR MICRO-TREASURES

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Introduction: Spontaneously fermented game meat sausages produced by traditional techniques are complex microbial ecosystems that can be the sources of beneficial or spoilage and pathogenic microorganisms.

Aim: This study aimed to investigate the fate of indigenous microbiota of spontaneously fermented game meat sausages in order to estimate microbiological hazards as well to identify and preserve the microbial pool that could serve as a source of strains relevant for standardization of sausages production.

Material and Methods: Three wild boar and three deer meat sausages, produced by five different manufacturers following traditional techniques, were physicochemically and microbiologically investigated at different time points of their production.

Results: Total of 917 isolates were collected from different media used for cultivation of lactic acid bacteria (LAB) and identified to species and strain level by PCR tools. Although commonly used for the isolation of specific groups of LAB, most of the applied media exhibited poor selectivity and supported the growth of diverse LAB or even non-LAB species. In total, *Leuconostocmesenteroides* was identified as the most frequently isolated species (28.24%), followed by *Lactobacillus sakei* (20.72%) and *Enterococcus casseliflavus* (11.55%), however, strong differences were observed in distribution profiles of LAB among investigated sausages. Cluster analysis revealed strong sausage-specific clustering of strains and remarkable diversity with 280 genotypes detected. High count of spoilage and/or pathogenic bacteria was noted for two sausages, making them potentially hazardous for human health.

Conclusions: The vast differences between sausages in prevailing microbiota indicated the need for exploring the possibilities of product standardization, likely to select starter/bioprotective cultures from indigenous microbiota.

Keywords: *spontaneous fermentation, game meat sausages, lactic acid bacteria, rep-PCR, media selectivity*

Acknowledgements: This project was funded by Croatian Science Foundation, OeAD and Croatian Ministry of Science, Education and Sport.

Food 3-17

FLUORESCENCE *IN-SITU* HYBRIDIZATION - CULTURE-INDEPENDENT METHOD FOR PSEUDOMONAS DETECTION PERSPECTIVE FOR FOOD APPLICATION

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Introduction The bacteria belonging to genus *Pseudomonas* are known as pathogens and opportunistic pathogens in humans (*Ps. aeruginosa*, *Ps. alcaligenes*) or vegetables (*Ps. Syringae*, *Ps. cichorii*) and as food spoilers (*Ps. fluorescence*). Thus the control of its presence in food and vegetables is of significant importance.

Aim The aim of this study was to demonstrate the application of the molecular technique fluorescence *in-situ* hybridization (FISH) as fast, accurate and culture-independent method for control of microorganisms from genus *Pseudomonas*.

Material and Methods Model biofilm degrading the food colorant Amaranth (E 123) was used. The azo-dye was applied in concentrations from 10 mg/l up to 55 mg/l. The model process had duration of 623 h. The group of bacteria belonging to the genus *Pseudomonas* was studied by FISH using the oligonucleotide probe *Ps* (5'- GCT GGC CTA GCC TTC -3'). The results were compared with the results obtained by plate count technique. Also digital image processing was used for establish the relative abundance of microorganisms of the genus *Pseudomonas* studied by FISH.

Results The results from the study showed clear and bright fluorescent signal from the target microorganisms in the dense biofilm structure. Quantity of the bacteria of genus *Pseudomonas* increased during the process, but their relative part of the community remained around 30 %. Plate count techniques showed decreased number of *Pseudomonas sp.*

Conclusions Fluorescence *in-situ* hybridization with the probe *Ps* is suitable method for detection of microorganisms of genus *Pseudomonas* in food because it shows the target bacteria *in-situ* in the sample; it is faster and more accurate compared with the standard culture-dependent techniques.

Key words *Pseudomonas spp.*, Fluorescence *in-situ* hybridization, Amaranth, microbiological control

COLONIZATION OF VEGETABLE PLANTS BY *HERBASPIRILLUM FRISINGENSE*

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Introduction: The fresh vegetables could be colonized by different kind of bacteria. There are bacterial endophytes which are able to colonize inner plant tissues. Normally, endophytes do not cause any changes or disease symptoms in the plants. Some of them could stimulate plant growth or they could be used as biocontrol agents. The one of the predominant endophyte bacteria in soil and rizosphere include genera *Herbaspirillum*. The *Herbaspirillum* species are mostly diazotrophic, have potential for colonization plants and have ability for nitrogen fixation.

Aim: The aim of this work was to investigate the ability of *Herbaspirillum frisingense* for surface and endophytic colonization on different vegetable plants.

Material and Methods: Bacterial strain used in this experiment was *Herbaspirillum frisingense* (GSF30BA28). The model plants were: lettuce, spinach, parsley, carrot, celery, tomato and sweet corn. The bacterial suspension applied for inoculation seeds was $\approx 10^8$ CFU/ml PBS. The inoculated plants were grown in monoxenic model for 3 weeks. The strain *Herbaspirillum frisingense* (GSF30BA28) was Gfp-transformed before inoculation. The colonization of plant roots by bacteria were investigated using 3D confocal laser scanning microscopy and Zeiss LSM Image Browser software.

Results: The highest degree of colonization by this strain was detected at surface and in inner tissue of lettuce root (1.71×10^6 cells/mm³ and 3.90×10^5 cells/mm³ of absolutely dry root). The lower colonization degree but not much smaller was detected at other plants,

Conclusions: *Herbaspirillum frisingense* (GSF30BA28) are able to surface and endophytic colonize all investigated plants. The bacterial cells were visible in intercellular spaces very close to plant root xylem.

Key words: *endophytic colonization plants, Herbaspirillum frisingense, confocal laser scanning microscopy, Gfp transformed bacteria.*

MICROBIOLOGY OF SOIL IN PROTECTIVE BELT OF GRUŽA RESERVOIR LAKE (SERBIA)

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Introduction: Presence of certain microorganisms in the soil is an indicator of bacterial safety of food and drinkable water in the given area or region. Results of microbiological analyses of soil in the area of the Gruža reservoir lake (Serbia) point to a varying presence of certain microorganisms, depending on the type of farming land (meadows, plough-fields, wheat fields and vegetable fields).

Results: In the humus accumulative horizon (0-30cm), the largest counts of faecal and coliform bacteria were established in plough fields. The average number of *E.coli* ranged from 5.95 in plough fields, to 23.14 MPN/gSM in soils under meadows, with individual values reaching 62 MPN/gSM. The counts of the total bacteria were at the lowest levels in fields under stubble, whereas the largest total bacteria counts were recorded in the soils of wheat fields.

Conclusions: Control of pathogens in the agro-ecosystem was determined by the application of sanitary protection measures, whereas prevention and control of pathogens ought to be conducted in the protection zone, as well as in the whole production chain, in order to sustain the balance of the ecosystem, as well as the safety of drinking water and food.

Key words: *Gruža Lake, Coliform bacteria, E.coli, Total bacteria.*

Food 3 -20

PLASMA BASED BIO-DECONTAMINATION OF LIQUIDS AND SURFACES: POTENTIAL FOR USE IN FOOD INDUSTRY

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Introduction: The need of new advanced technologies for bio-decontamination and effective microbial control are critical for modern development of food industry and safety. The high quality requirements for starting substances, food-processing water and bio-compatible package materials determine the inapplicability of standard steam, chemical and dry heat sterilization. In

these cases the innovative technologies based on plasma processes are considered as promising and effective “clean” tool for bio-decontamination.

Aim:The aim of this study is to assess the bactericidal effect of plasma in liquids and surfaces, contaminated with Gram-negative and Gram-positive spore-forming bacteria. On the base of this effect, the potential of plasma technologies for application in food industry was discussed.

Material and Methods:The used plasma source was surface-wave-sustained discharge (SWD) operating at 2.45 GHz in Argon at atmospheric pressure (plasma torch) produced by an electromagnetic wave launcher surfatron type. The plasma antimicrobial effect was studied by direct contact treatment of bacteria-contaminated liquids and agar plates. The bacteria of *Pseudomonassp.* AP-9 and *Brevibacillus laterosporus* BT-271 were treated at different cell density ($10^7\div 10^9$ cells/ml), exposure time, volume and wave power.

Results: The results show that the SWD plasma is able to efficiently inactivate bacteria at short exposure time < 60 sec. The clear sterilization zones on treated surfaces with diameter depending on exposure time and initial bacterial density were obtained. In bacteria-contaminated liquids the partial disinfection was observed at least. The SEM pictures of treated cells show serious damages on cell surface.

Conclusions:The potential of plasma based technologies for bio-decontamination of liquids and surfaces is high and can be used for various purposes related to microbial control and food safety.

Acknowledgements: This work was supported by Bulgarian Fund for Scientific Research under Grant No DCOST 01/3/2016.

Key words: *Plasma; bio-decontamination; treatment of bacteria-contaminated liquids*

Food 3 -21

MIGRATION OF HAZARDOUS POLLUTANTS IN FOOD THROUGH TROPHIC CHAINS

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Introduction: One of the mechanisms of contamination of foods with vegetable and animal origin by toxic substances (organic xenobiotics), heavy metals or pathogens is via water and river sediments. The accumulation of these contaminants in trophic chains in many cases reflected in their final storage in plant biomass and raw materials of animal origin which are a resource for food production. The way the penetration of these contaminants in food is as follows: untreated or bad purified water from wastewater treatment plants-accumulation in sediments and waters of the

river-passing in the agricultural and livestock production-passing in food. Thus heavy metals diagnosed in sediments can have a final destination - milk, dairy products, meat.

Aim: The aim of this study is to follow the accumulation of heavy metals and pathogenic microorganisms in the sediments and water of the Iskar River as the preferred matrix for their accumulation on the one hand and the source of their distribution in agricultural raw material production on the other.

Material and Methods: In this study the sediments and water from dams in hydro-plant cascade "Middle Iskar" were investigated. The concentration of heavy metals in sediments were analyzed by ICP. The number of total coliforms in surface water and sediments from the dams of the cascade was determinate.

Results: The high concentrations of indicators of faecal contamination (group of coliforms) were detected in the waters of dams. High levels of coliforms were reported in dam Tserovo which is situated immediately after the town of Svoge (town without wastewater treatment plant). The results for heavy metals showed critical high values for Cd and Zn in the sediments of a dam Tserovo.

Conclusions: The results revealed that in the area of cascade "Middle Iskar" there is potential risk associated with the transport of pollutants from water and sediments in the trophic chain, with final destination - food products.

Key words *Sediments; heavy metals; total coliforms; trophic chains*

Food 3 -22

THE EFFECT OF NINE ANTIMICROBIAL AGENTS AGAINST MICROORGANISM ISOLATED FROM FOOD

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Introduction: Microorganisms are widespread in the environment. Most of them are not dangerous to human health, but some of them produce toxins which are toxic, carcinogenic, mutagenic or teratogenic for humans and animals. Their fast way of reproduction leads to contamination on food and damage to material goods.

Aim: Within this experimental research it is determined the effects of nine laboratory obtained antimicrobial agents with different chemical composition and concentration.

Material and Methods: As test microorganisms we use: *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633 и *Staphylococcus aureus* 6538, isolate FM2 (yeast from pickled peppers), isolate FM20 (yeast from pickle), isolate FM3 (mold from bread), isolate FM4 (mold from jam), isolate FM18 (mold from tangerine) and isolate FM15 (mold from meat).

The experiment was based on macrodilution method. Microorganisms were exposed to different compositions and different concentrations of components of antimicrobial agents (designated as No1-No9), and it was determined a minimum inhibitory concentration.

Results: The results showed that all antimicrobial agents possess antimicrobial activity against tested microorganisms, depend of the type of microorganisms (procaryots or eucaryots) and the compositions of antimicrobial agents. Anyway, the lowest MIC for both, procaryots and eucaryots, showed antimicrobial agent we designated as No2, with strength of 3,125%; the antimicrobial agent with no or some weak activity for eucaryots was No4, and for procaryots No7 and No8.

Conclusion: Each of tested agents has a different potential impact on the growth and multiplication of the test organisms. These differences are result of the composition of laboratory obtained antimicrobials and the resistance of test microorganisms towards them.

Keywords: *macrodilution method, microbial growth, inhibition, antimicrobial activity.*

Food 3 -23

LABORATORY IDENTIFICATIONS OF MICROORGANISMS IN FOOD PRODUCTS IN TIRANA

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Introduction: Microbiological hazards are the most significant food safety hazards. Foodborne diseases are caused by the consumption of foods that are contaminated with bacteria, protozoa, viruses, mold and toxin produced by them.

Aim: The aim of the study is identification of pathogenic microorganisms in food products in day care centers and markets of Tirana, one of the main problems with impact on public health.

Materials and Methods: This study was performed during November 2010 till March 2013. We collected in total 2145 samples from food products and environmental samples from 14 markets and 24 day care centers in Tirana.

Results: In day care centers the organism that had the highest frequency was found Enterobacteriace (30.2%) followed by mesophilic aerobic bacteria (21.3%) and mold (20.8%). In

the markets the prevalence of mesophilic aerobic bacteria(36.8%) was the highest, followed by Enterobacteriace (30.2%), mold (24.9%) and coliform bacteria (24.7%). The most polluted among the group products were dairy products group (in both day care centers and markets). They showed significance compared to other products group $\chi^2 = 99.3$ for the value of $p < 0.01$. Bottled water samples resulted out of norm 77 (17.3%) of them. We found mycotic pollution in dried fruits/snack and cereals in 87 (26%) samples. We concluded that mesophilic aerobic bacteria. Coliform bacteria and yeasts dominated in the market, compared with day care centers, with significant difference of $p < 0.01$.

Conclusion: These results shows to us how important are the hygiene preventive measures to the day care centers and markets.

Key words: *microbial pollution, food products, day care centers and markets, Tirana.*

4. PREBIOTICS AND PROBIOTICS (PP)

Food 3 – 24

TRADITIONAL BULGARIAN DAIRY PRODUCTS, AS PROMISING SOURCE OF NEW BENEFICIAL LACTIC ACID BACTERIA

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Introduction: Fermented products possess various nutritional and therapeutic properties. They are widely accepted as functional foods, with long history of safety use. Lactic acid bacteria (LAB) play a major role in determining the organoleptic properties and healthy effects of these products. However, little is known on the biodiversity and beneficial role of autochthonous lactic microbiota of artisanal milk products.

Aim of research: The aim of our study is a complex study of LAB microbiota of traditional Bulgarian yogurt, cheese and “katak”. Two major axes were followed: LAB biodiversity and possible beneficial effects on the consumers’ health.

Materials and Methods: Several microbiological and molecular methods were applied. The widely accepted criteria for functionality, safety and technological relevance of LAB were respected, in order to select promising strains.

Results: A high vitality and presence of LAB species with GRAS/QPS status has been shown in collected artisanal samples of dairy products, made in different rural regions of Bulgaria. A collection of 89 newly isolated LAB was created. The strains with broad spectrum of activity were identified according to the modern polyphasic taxonomy. In addition, estimation of functionality and technological relevance of active LAB were applied. They were estimated as bio-preservative/probiotic adjuncts to Bulgarian dairy products with a favorable influence on organoleptic properties and shelf-life. The consumers could benefit from their metabolic and antioxidant activity, ability to compete the pathogenic/food-spoilage bacteria, preventing also biofilm formation.

Conclusion: The obtained results seemed promising for the development of new approaches for design of functional foods and to tackle a variety of new specific consumers' demands.

Key words: *cheese, yogurt, katak, Lactobacillus, probiotics*

Food 3 – 25

NEW GENERATION OF PREBIOTIC OLIGOSACCHARIDES – OPTIMIZATION OF ENZYME REACTIONS FOR PRODUCTION

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Prebiotics are defined by Gibson and Roberfroid (1995) as a non-degradable carbohydrates, selectively stimulating the growth and metabolic activity of one or a limited number of bacterial species in the colon, thus improving the overall health state.

The main purpose of all scientific studies concerning the enzymatic synthesis of oligosaccharides, is to obtain structures of OS, giving them high prebiotic potential. The type of linkages in OS and their degree of polymerization (DP) are the main factors determining their prebiotic potential. The controlled enzymatic synthesis of glucooligosaccharides by glucosyltransferases, in the presence of sucrose and suitable acceptors is a perspective method with fundamental significance for their preparation. It was found that glucooligosaccharides containing α -(1→2) and/or α -(1→3) glycosidic bonds have specific prebiotic properties.

Levan-type fructooligosaccharides are of increasing interest because of their potential health benefits to selectively support the intestinal health. Levansucrase (EC 2.4.1.10), which belongs to the glycoside hydrolase family 68 (GH68), catalyzes the synthesis of different types of fructooligosaccharides using sucrose as a donor and different monosaccharides and disaccharides as acceptors. *Leuconostoc mesenteroides* Lm 17 produces levansucrase of about 120 kDa and dextransucrase of about 180 kDa. The encoding levansucrase gene from this strain was cloned and expressed in *Escherichia coli* BL21. The recombinant enzyme, called L17, was shown to be 1022 amino acids-long protein with high homology to levansucrase LevS from *L. mesenteroides* NRRL B-512F strain. *In situ* analysis showed an active band of 120 kDa, similar to the one produced by native strain. The optimal conditions for action of enzyme were determined at pH 5.5 and 35 °C. K_m and V_{max} of L17 were at 64 mM of sucrose and 12 U/mg of enzyme.

The synthesized fructan fraction was identified as levan, and the synthesized oligosaccharides are fructooligosaccharide type.

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Keywords: *oligosaccharides, prebiotics, glucansucrases, levansucrases*

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5. ALLERGENS IN FOOD (AF) -

Food 3-26

ORAL HYPOSENSITIZATION IN NICKEL DERMATITIS

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Nickel found in several foods is an essential element in the diet, and is the second cause of allergic dermatitis throughout the world. Its daily ingestion from food varies from 150 to 500 µg, and vegetables are the main source. The amount of nickel absorption depends on the concurrent intake of other food stuffs such as alcohol and proteins. Good sources with high nickel content include whole-grain flour, oats, soya, chocolate, tea, red kidney beans, peanuts, dried peas, beans, hazelnuts, gelatin, baking powder, sunflower seeds, and dried fruits. Nickel allergy can result in both cutaneous and systemic manifestations, and can be aggravated by various other

food items and drinks such as beer, red wine, tomato, carrot, onion, herring, mackerel, tuna, and citrus fruits. The nickel dermatitis is easy to be recognized.

It is recommended patients with nickel dermatitis to undergo a complete allergological evaluation including prick tests with a panel of food allergens and patch tests with the European standard series. The complete nickel avoidance is extremely difficult and, if prolonged, may have nutritional consequences. Excellent clinical results can be achieved by using increasing oral doses of nickel sulfate associated to an elimination diet. This therapeutic protocol has been effective in inducing clinical tolerance to nickel-containing foods, with a low incidence of side effects.

Key words: *nickel dermatitis, food allergens, prick tests, patch tests, oral hyposensitization*

6. FOOD ADDITIVES (FA) -

No submissions

7. ORGANIC FOOD (OF)

No submissions

8. GMO (GMO)

No submissions

9. FOOD AND HUMAN HEALTH (FHH)

Food 3 -27

THE GLOBAL THREAT OF ANTIMICROBIAL RESISTANCE TO CARBAPENEMS AND COLISTIN: THE FOOD CHAIN CONTRIBUTION

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The emergence of carbapenem-resistant gram-negative pathogens poses a serious threat to public health. Carbapenem resistance is mainly attributed to the production of various carbapenemases. For many years carbapenemase-producing *Enterobacteriaceae* (CPE) strains were restricted to humans and human environment (hospitals). After the first isolation of a VIM-1-producing *Escherichia coli* in a pig farm in Germany during 2012, CPE strains are increasingly detected among food-producing animals, their environment (farms), food (squids, vegetables and chicken meat) and domestic animals. Several factors have contributed to the dissemination of carbapenemase genes in the food chain, such as the intrinsic presence of these genes in environmental bacteria, the isolation of CPE from sewage and waste water derived from hospitals, the use of carbapenems to treat resistant infections in companion animals and the fact that wild birds may act as reservoir. Colistin is also a last resort antimicrobial in the battle of multidrug-resistant gram-negative pathogens and the food chain has a great contribution to the dissemination of colistin resistance. Lately, the emergence of plasmid-mediated *mcr-1* colistin-resistant gene has been increasingly documented in the food chain. The clonal transmission from animal to human has been documented (the same ST, virulence and PFGE patterns) in China. There are also several reports documenting *mcr-1* in *Enterobacteriaceae* from rivers and imported vegetables as well as from sewage waters, which was attributed to the heavy use of colistin in veterinary medicine. Thus, the presence of carbapenem- and/or colistin-resistant microorganisms in the food supply and in ready-to-eat food is alarming. Appropriate measures such as the improvement of agricultural practices and water quality need to be taken. In addition, improved laboratory methods for colistin resistance and *mcr-1* detection are required. There is also an imperative need to reduce colistin usage in food-producing animals worldwide as was done in Europe. Finally, cleaning and disinfection between meat production cycles and prevention of contamination of humans following direct contact with animals or meat should be considered as important interventions.

Food 3 –28

GUT MICROBIOME: PROBABLE RELATIONS TO HUMAN HEALTH

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The Human Microbiome Project (HMP) reflects that we are supraorganisms composed of human and microbial components. The gut microbiota, which hosts up to 1000 bacterial species

that encode about 5 million genes, protects against enteropathogens, extracts nutrients and energy from our diets, and contributes to normal immune functions. Disturbance of the normal balance between the gut microbiota and the human host have been associated with obesity and metabolic syndrome, malnutrition, autoimmune diseases, inflammatory bowel disease, neurological and probably psychological disorders, and colorectal cancer.

The normal gut microbiota is most diverse, can exhibit variations over time, and varies between individuals due to age, genetics, lifestyle and diet. Of these, diet is easiest to modify and presents the simplest route for therapeutic interventions. The more diverse the diet, the more diverse the microbiota and the more adaptable it will be to disturbances. Richness of the gastro-intestinal microbiota and microbiome is well correlated with good health. In this sense, food may be a new form of personalised medicine for some of the chronic diseases.

Keywords: *gut microbiota, gut microbiome, health, disease*

Food 3 -29

THE ROLE OF *STREPTOCOCCUS AGALACTIAE* IN HUMAN GUT MICROBIOME

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Introduction: *Streptococcus agalactiae* (group B streptococci- GBS) was firstly recognized as a causative agent of bovine mastitis. Its importance as a human pathogen was confirmed during 60s in last century. Nowadays, it is known that GBS colonize 20-40% of the population of young adults and 20-25% in elderly persons. The preferential locations for human colonization are human gut, perianal region and vagina.

One of the interesting investigation subject in GBS is about transmission routes. It can be transferred sexually, but it is not considered as sexually transmitted disease. Also it can be transferred vertically from mother to newborn - ~45% of neonates born to colonized mothers. The important dilemma is about the likelihood of GBS transmission between animals and humans.

Another intriguing question is how relatively friendly bacterium from normal microflora suddenly becomes invasive, mostly among infected newborns, but also among some adults. Although this happens rarely it is worthy to know whether this role plays the same bacterium.

Aim: That is why we decided to follow the distribution of GBS capsular serotypes. Serotyping was carried out in groups of invasive and not invasive isolates. Determination of certain serotypes was explored by capsular typing – multiplex PCR and *hvg A* gene detection - PCR .

Results: Dominating serotypes among invasive serotypes were Ia and III, and among noninvasive serotypes - III and V. Interesting point is that their antibiotic susceptibility was different. Based of this notifications it would be very important to test the same characteristics of animal isolates.

Food 3 -30

CLINICAL IMPACT OF ANTIBIOTIC-RESISTANT GRAM-NEGATIVE BACTERIA

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Introduction: The spread of multidrug-resistant /MDR/ - Gram-negative bacteria in the hospital setting is a worldwide problem. Accurate estimates of their clinical impact are vital for justifying interventions directed towards preventing or managing infections caused by these pathogens.

Aim: In this study we present data about the resistance to antimicrobials of some problematic for hospital infectious pathology bacteria – *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* on the model of a multi profile hospital.

Results: There has been an increase in *ESBLs*-producing *E.coli* and *K.pneumoniae* strains, isolated especially in some so-called “risky units” at the Military Medical Academy /MMA/ for the last years. There were also registered the first strains *E. coli*, producing *metallo-beta-lactamase NDM1*. The data show specific association between the *blaNDM-1* and *rrmB* genes conferring high-level resistance to all aminoglycosides in these strains.

Conclusions: Non-fermenting *A.baumannii* and *P.aeruginosa* strains isolated usually are multiresistant with high-level resistance to carbapenems and other beta-lactams and quinolones, and the resistance to carbapenems in *A.baumannii* strains is associated with the production of *Oxa 23*, *Oxa 58* and *Oxa 72* carbapenemases, but not metallo-beta-lactamases.

Keywords: *gram-negative bacteria, resistance to antimicrobials, ESBLs, carbapenemases*

Food 3 -31

ALTERNATIVE DIETS AND HEALTH RISK EFFECTS

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Introduction: Based on studies and literature data, the most spread alternative to the healthy nutrition diets and the subsequent health risk effects are discussed.

Aim: To find the relationship between different kinds of diets on people`s health using our own studies.

Material and Methods: Own experimental studies are presented, showing that in reduced or irregular diets, the deficiency of nutrients or their irregular intake activates the systems of absorption of energetic components of food dominating on the absorption of the plastic ones. This can explain the so called ”yo-yo effect” after turning to normal diet.

Results: It is established by the authors “phenomenon of satiety”, characterised with suppression of the systems for hydrolysis and transport of a given nutrient during period of intensive and prolonged intake. At the same time the deficient intake of a nutrient stimulates the systems for its absorption. In relation to this the diets with increased intake of nutrient and decreased of another are discussed. The different options of vegetarian diets are analysed, the accent is on the risk health effects of the diets with permanent exclusion of animal products. The scientific view on “separated nutrition” is discussed and some principles of its use are explained.

Conclusions: Diverse unbalanced diets can cause serious metabolic disorders with harmful health consequences which may increase disability and higher morbidity and mortality among the population.

Key words:*alternative diets, nutrient, health risk*

10. HUMAN NUTRITION AND DIETETICS, GUT MICROBIOME **(HNDGM)**

No submissions

11. FOOD BIOTECHNOLOGY (FB)

No submissions

12. NANOTECHNOLOGY IN FOOD (NTF)

No submissions

13. FOOD LABELING AND PACKAGING (FLP)

No submissions

14. RISK ASSESSMENT AND FOOD AUDIT (RAFO)

No submissions

15. FREE TOPICS (FT)

Food 3-32

GENOTYPE'S SUSCEPTIBILITY AND ADAPTIVE POTENTIAL OF *PHASEOLUS VULGARIS* L TO DROUGHT STRESS

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Introduction: In the recent year's drought stress becoming more harmful to worldwide agro ecosystems because of increasingly dynamic global warming. During the evolution plants have develop different adaptive mechanisms to cope with drought. The mechanisms of sensitivity/tolerance of crop plants to drought are still not completely known. Therefore clarification is needed for their better understanding.

Aim: To compare susceptibility and adaptive potential of three genetically closely related genotypes of *Phaseolus vulgaris* L to drought stress.

Materials and Methods: Seed germination of *Phaseolus vulgaris* L. genotypes was performed in growth chamber at standard conditions. To mimic drought stress, 16% PEG - MW 10 000 for 24h was applied. Two experimental schemes were used: 1) PEG treatment on the third-leaf phase; 2) PEG treatment at cotyledon phase. Photosynthetic pigments and reducing sugars were chosen as endpoints. Measurements were carried out immediately after the treatment and ten days after the treatment.

Results: Our data show different genotype response concerning content of total chlorophylls, chlorophyll *a*, chlorophyll *b* and total carotenoids measured just after PEG treatment. Ten days

after the removal of PEG genotypes respond in a similar way. Increased reducing sugars content was obtained immediately after the treatment and similar to control ten days after. No change in chlorophyll *a/b* ratio just after the treatment and ten days after the treatment was found.

Conclusions: Our data illustrate that genotypes having a different response to drought have a similar adaptive potential. This fact would play significance in a further study of prolonged genetic events.

Acknowledgements: This study was funded by the projects: DDVU_02/87 “Complex morphometric, physiological, biochemical and molecular assessment of drought tolerance in Bulgarian common bean genotypes (*Phaseolus vulgaris* L.)” and „Ecological and genetic risk: methods and strategies for overcoming“– BAS.

Key words: *Phaseolus vulgaris* L., polyethylene glycol, genetically closely related genotypes, photosynthetic pigments, reducing sugars

Food 3-33

CURRENT PERSPECTIVES OF FUTURE PROFESSIONAL FOOD HANDLERS TOWARDS FOOD SAFETY AND THEIR FOOD SAFETY PRACTICES

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Introduction: To decrease the number of foodborne outbreaks annually, it is essential for food handlers to use safe food handling and preparation practices at each step of food production, processing, distribution, and serving.

Aim: The purpose of the current study was therefore *i)* to gain insight into various food safety aspects of future professional food handlers representing different professions in the food supply chain and *ii)* systematically investigate future food handlers' practices during practical lessons close to the end of their formal vocational education.

Materials and Methods: A total of 90 students divided into six observation sessions in the field of catering and six observation sessions in the field of food processing (bakery, confectionery, butchery) were observed for 40 hours. Additionally, 15 focus group discussions about the comprehension of food safety, the responsibility for food safety, the barriers hindering food safety practices, and the food safety influence of other people were conducted.

Results: The results of observation demonstrate that student behaviour in food production process significantly depends on hygienic-technical conditions in the training classroom and on teachers' actions during the practical lessons being as an example. Results of focus groups additionally demonstrate the diversity of interpretations of food safety, perception of responsibility in relation to the position of future profession in the food supply chain, barriers related to the working

environment and personal factors and decrease of parental influence due to the greater impact of teachers of practical classes and instructors in food enterprises.

Conclusions: The outcomes indicate the weaknesses in the formal vocational education of future professional food handlers and consequently suggest modifications of existing approaches. The results also suggest that once formal education is completed, on-going training that focuses only on improving knowledge may not assist in overcoming barriers identified.

Key words: *food safety, food handler, catering, food processing, qualitative research, vocational education, observation*

Food 3-34

BIO-INDICATIVE CONTROL SYSTEM OF THE TECHNOLOGIES FOR BIOGAS PRODUCTION FROM FOOD BIO-WASTE

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Introduction

According to the principles of the circular economy and the food waste pyramid if not possible to reduce the food waste, it could be used to feed starving people or livestock or the waste should be composted/treated in technologies for production of 100% renewable energy. The most convenient way to recycle the municipal and domestic food bio-waste is their treatment in the widely distributed technologies for biogas production.

Aim: To outline the critical moments in the technologies for biogas production and development of a bio-indicative control system of these technologies.

Materials and methods: Data about the functioning of the technology for food bio-waste treatment at Sofia Municipal Enterprise for waste treatment and private installations for biogas production in Bulgaria have been analyzed. The following approaches have been used – total quality management, SWOT and PEST analysis, ranking of the critical problems.

Results: The critical problems in these technologies are: 1/ a lack of adequate and express control; 2/ an unsolved task is the increase of methane in biogas. After large-scale analysis we have proposed two innovations for solving the identified problems – fluorescent system for bio-indication of methane producing processes as well as ultrasonic pretreatment of the substrate in order to increase the percent of methane in the biogas.

Conclusions: The bio-control system uses reliable express indicators of the functioning of these technologies presenting the accurate conditions of the biological system. It could allow adapting the microbiological consortium and lead to increase of methane quantity in the biogas.

Key words: *food waste, biogas, control, methane*

Food 3-35

ADVANCED TECHNOLOGY FOR WASTEWATER TREATMENT FROM DAIRY INDUSTRY

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Introduction: The amount of processed milk in Bulgaria decreased with about 30% and dairies with around 6% ten years after our country accession in EU. One of the prerequisites for these trends are increased requirements to raw milk, milk products and technologies; the introduction of standards for quality management and food safety management. Another reason is that many enterprises can not meet the requirements and emission standards according to environmental legislation about purified wastewater quality. The existing wastewater treatment plants in the dairies are based on aerobic sequencing batch reactors (SBR). At the same time, COD in influent is more than 1500 mgO₂/l, which is too high for an aerobic treatment process. The introduction of an anaerobic bioreactor for preliminary treatment and for significant reduction of the initial COD is one of the possible solutions for problem solving.

Aim: The aim is to determine whether the anaerobic bioreactor with immobilized biomass is useful as a preliminary step for dairy wastewater treatment as well as for sufficient reduction of COD in effluent for subsequent aerobic treatment.

Material and Methods: Research was conducted in model bioreactors and whey was used as model pollutant. COD of model wastewater was 4000 mgO₂/l. COD, inorganic nitrogen and phosphorus were analyzed by standard methods.

Results: COD in effluent was below 600 mgO₂/l. The concentration of the phosphate phosphorus was less than 3 mg/l and the ammonium nitrogen was above 100 mg/l in effluent. These values allow discharge of treated water in municipal sewage in accordance to Regulation 7/2000, but not in the water receivers in accordance to Regulation 6/2000.

Conclusions: The proposed anaerobic biofilter is suitable module for pre-treatment (before wastewater treatment in aerobic SBR). Its introduction in the local wastewater treatment plants of dairies will ensure compliance with the emissions standards according to the environmental legislation, will reduce regular penalties and closure of dairies due to water receivers pollution.

Key words: *anaerobic-aerobic treatment system, biofilm, dairy wastewater*

FAST QPCR METHOD FOR PATHOGEN IDENTIFICATION AND QUANTIFICATION

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Real-time PCR (qPCR) became a routine technique for food safety control due to its low detection limits, high specificity and ease of use. It is used mainly for food identification, GMO screening and quantitation, pathogen detection and quantitation, allergen detection.

Specialists can choose from variety of instruments, reagents and consumables offered by different vendors. The key factors for purchasing include instrument configuration and quality, overall budget, price per sample, technical service.

We shall present an affordable qPCR instrument from Primer Design (UK) for fast qPCR detection and quantitation of food pathogens.

Key words: real-time PCR, food pathogens, instruments

POSTERS

1. FOOD AND BEVERAGES QUALITY AND SAFETY (FBQS)

Food 3-37

ANTIVIRAL EFFECTS OF POMEGRANATE PEEL EXTRACTS ON HUMAN NOROVIRUS GII

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Introduction: Requirements for novel natural antimicrobial substances have brought in the focus pomegranates (*Punica granatum* L.) extracts against diverse pathogens. It is worth mentioning that the human noroviruses (hNoV) are the leading cause of viral gastroenteritis worldwide, with the occurrence of the new highly virulent strains every two-three years.

Aim: Among different parts of the pomegranate plant, it has been shown that peel extracts possess broadest antimicrobial activity. The aim of this study was to investigate the effect of different concentrations of pomegranate peel extracts on hNoV.

Material and Methods: The peel from wild growing pomegranate fruit was air-dried and grounded. Extraction was done with 70% MeOH by maceration. The extracts were filtered and solvent was evaporated under low pressure. Dry extracts were dissolved in DMSO in concentration range 0.2 - 5 mg/mL. Human NoV GII suspension was prepared using previously positively identified human fecal sample. Experiments were conducted mixing the same volume of hNoV and extracts suspensions; with two contact times (0 and 1h) and temperatures (25 and 37°C). Norovirus RNA was isolated by Kit Ribo Virus (Sacace, Biotechnologies). For removal of inhibitory compounds One Step™ PCR Inhibitor Removal Kit (Zymo Research, USA) was used. Number of viral RNA genomic copies, after treatments, was quantified with PrimerDesign™ Ltd advanced kit (genesig) RT-qPCR.

Results: The number of hNoV GII particles was reduced by 2 and 2.5 log₁₀ genomic copies after 1 h treatment with pomegranate extract concentrations of 5 mg/mL, at 37°C and 25°C, respectively.

Conclusions: Pomegranate peel extracts showed antiviral effect against human norovirus GII particles with significant genomic copies reduction. The potential benefits of pomegranate extracts are existent and could be suitable in inhibiting noroviruses transmitted by food.

Acknowledgements: The research was funded by Ministry of Education, Science and Technological Development (Projects III 46009, III 46013).

Key words *Norovirus, pomegranate extracts, antiviral effect*

Food 3-38

MICROBIOLOGICAL ANALYSIS OF WATERS OF RIVER MORAVA BINQËS DURING SUMMER SEASON 2004

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Introduction: Dumping of waste in rivers has become a global problem. So the need for clean water also has become a global problem. With a trend of extreme pollution, the world will be gripped a "dryness". Even though there is abundance of water, it is contaminated one.

Aim: The main objective was to investigate microbiologically the waters of river Morava Binqës (Kosovo).

Material and Methods: The water samples were collected from different stations of river Morava Binqës, during the summer season 2004.

Results: The isolates were characterized and identified as: heterotrophic bacteria, total coliform, *Streptococcus* bacteria, *Salmonella* and *Shigella*, and fungi. The study therefore, stresses on the need to control the fecal pollution of water bodies.

Conclusion: The results obtained led us to conclude: the waters of river "Morava Binqës" are moderately polluted by bacteria at all localities. High number of all microorganism was registered at all localities.

Key words: *microbiology, analysis, river, Morava Binqës*

Food 3-39

NUTRITION, EMBRYONIC DEVELOPMENT AND EPIGENETIC REPROGRAMMING

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The proper functioning of human body requires a fine regulation of gene expression in the various cells and tissues. It is well known the gene activity depends on chromatin epigenetic modifications affecting DNA and histones. A number of explorations on experimental animals and isolated human populations show that the lifestyle of the fathers as well as of the mothers could influence the epigenetic modifications of the future generations. In the cases of wrong epigenetic reprogramming in embryos implantation and giving birth of healthy children are common phenomena. Even though, such children usually are vulnerable to certain diseases in their adulthood.

One of the critical periods in which the maternal organism is supposed to assure optimal content of nutrients for the embryo is the pre-implantation stage. Two or three days before implantation the DNA of the embryo is completely demethylated after which de novo methylation occurs. The correct carrying out of these two steps is defined to a great degree by the environmental conditions and it is crucial for the gene activity in the developing individual.

This on its part raises questions about the optimal content of nutrients in the in vitro culture media and its impact on later human health.

Keywords: *epigenetics, nutrition, embryo development*

Food 3-40

STRESS RESPONSE OF *RHODOTORULA* SPP. 74 UPON HIGH VOLTAGE ELECTRICAL DISCHARGE PLASMA TREATMENT

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Introduction: High voltage electrical discharge is a new technology used for inactivation of pathogen microorganisms. *Rhodotorula* can cause digestive diseases in humans.

Aim: The aim of this study was to examine whether the high voltage electrical discharge plasma treatment has an influence on yeast reduction and degree of recovery in pure culture.

Material and Methods: Sample volume was 200 mL. Treatment time (5 /10 min), frequency (60/90 Hz) and injected gas (air/argon 99.99%) were changed. Medical stainless steel needle was used as high voltage electrode (negative polarity) through which air or argon was injected at the gas flow of 0.6 L/min. Experimental design and statistical analyses were obtained by Statgraphics Centurion software.

Results and Conclusions: The highest inactivation of 1.42 log₁₀ cycles was after 10 min of treatment, frequency of 90 Hz and injected argon. There was no significant difference in inactivation rate comparing treatments with injected air or argon. With a longer treatment time the inactivation rate increased. After the 18 h of recovery all treated samples had recovered at 70-100%. Therefore the further optimization of method is needed to understand the mechanism of yeasts inactivation and cells recovery.

Acknowledgements: The authors would like to acknowledge the support by the Croatian Science Foundation (HRZZ-IP-11-2013).

Key words: *plasma, Rhodotorula, inactivation, recovery*

QUALITY OF FRUIT PUREE OBTAINED BY THERMAL, HIGH-PRESSURE AND HIGH-PRESSURE-MILD-THERMAL TREATMENTS - A COMPARATIVE STUDY

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Introduction: Fruit purees are consumed as such or as ingredients in many ready-to-eat healthy and nutritious deserts. A mixture of peach pulp and strawberry with a 1:1 ratio was selected.

Aim: The objective was to evaluate the quality of fruit puree produced by three different treatments: thermal (70°C/15 min)-TT, high-pressure (600 MPa/10 min)-HPP and high-pressure-mild-thermal treatment (600MPa/50°C/10min)-HPMT, stored 14 days.

Materials and Methods: Samples of fruit puree packed in commercially available packaging material were treated by HPP and HPMT. The treatments were performed in a multivessel (4x100 mL) high-pressure equipment (Resato). A non-treated sample of fruit puree represented the control. After the treatment SPME GC/MS, sensorial and rheological analysis were performed.

Results: The untargeted metabolomic analysis of the GC/MS fingerprint coupled with the sensorial analysis and rheology (SAOS) results showed that although more flavour compounds (esters, polyphenols, carbonyl compounds, etc.) and a better taste was registered for the HPP and HPMT samples, a better texture was registered for the TT sample. During storage the differences in the elastic behavior of the thermally and high pressure treated samples were significantly reduced.

Conclusions: This study indicated that all HPT samples demonstrated better quality compared to the other samples.

Keywords: *peaches, strawberries, high pressure, thermal treatment*

Acknowledgements: This work benefited from the infrastructure provided by the RE-SPIA (SMIS code 11377) project REGIO (2007-2013).

POLYPHENOLS IN DOMESTIC AND IMPORTED OLIVE OILS IN ALBANIA

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Introduction: True extra virgin olive oils contain higher levels of beneficial compounds including antioxidant phenolic compounds. Specific mechanisms have been identified in which phenolic compounds in the olive oil matrix exhibit antioxidant activity contributing to the prevention of cancer and cardiovascular disease, among other health benefits.

Aim of this study was to analyze the concentration of total polyphenols in olive oil samples with different varieties, to orientate the consumers to choose the right sample of olive oil which contains high level of poly phenols and to know the health benefits of these polyphenols.

Material and Methods: In this study we analyzed 19 samples of domestic and imported olive oil. The used method for determining the total polyphenols is Folin-Ciocalteu. Measurements were performed in UV-VIS spectrophotometer in 725nm wavelength. The calibration curve was constructed by caffeic acid.

Results: It was observed that the concentration of total polyphenols varied from 11.1mg/Kg to 313.13mg/ Kg. The results of the chemical analysis supported that olive oils vary greatly in the concentration of phenolic compounds. The samples of olive oil of high quality contain high level of total polyphenols.

Conclusions: The amount of polyphenols contained in olive oil depends on the type of cultivar, the period of collation of olives, the process of extraction and storage conditions.

Keywords: *polyphenols, olive oil, caffeic acid, etc.*

Food 3-43

PYTHIUM APHANIDERMATUM* SUPPRESSION BY ANTAGONISTIC ACTION OF *TRICHODERMA LONGIBRACHIATUM

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Introduction: Excessive use of pesticides represents a growing problem in agriculture sustainability and food safety. Application of antagonistic fungal interactions represent a promising approach to achieve reduced pesticide input. Beneficial saprophytic fungi from genus *Trichoderma* can express different mechanisms of antagonistic activity and effectively suppress plant pathogens.

Aim: The aim of the present research was to consider the potential application of antagonistic effect of *T. longibrachiatum* 10/5 against plant pathogen *Pythium aphanidermatum*.

Material and Methods: Antagonistic activity of *T. longibrachiatum* 10/5 against *P. aphanidermatum* was tested *in vitro* by dual culture test. Glasshouse trial was conducted with cucumber plants grown on commercial substrate. Ten plants per container were inoculated in cotyledon phenophase with *P. aphanidermatum* mycelia. Experiment was performed in five replications. A number of inoculated plants without symptoms was compared between plants treated with sterile liquid culture filtrate (SLCF) of *T. longibrachiatum* 10/5, and plants treated with propamocarb hydrochloride.

Results: *P. aphanidermatum* showed asymmetrical growth in the confrontation test, and colony diameter was reduced for 77% in comparison to control. SLCF of *T. longibrachiatum* 10/5 showed 50% efficiency in suppression of cucumber damping-off caused by *P. aphanidermatum*.

Conclusions: Metabolites produced by *T. longibrachiatum* 10/5 showed suppressive effect on *P. aphanidermatum* damping-off which has a potential in application in sustainable agriculture.

Acknowledgements: This research is supported by Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. TR 31080.

Key words: *Trichoderma longibrachiatum*, *Pythium aphanidermatum*, antagonism, disease suppression

Food 3-44

MICROBIAL QUALITY OF FRESH VEGETABLES AND IRRIGATION WATERS IN CENTRAL SERBIA

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Introduction: Utilization of poor quality water in agriculture presents potential health risk, especially in the irrigation of fruits and vegetables that are consumed fresh. The risk of disease transmission from irrigation water is influenced by the level of contamination, the persistence of pathogens in water, in soil, and on crops.

Aim: The aim of the present research was determining microbial quality of irrigation water and fresh vegetables.

Material and Methods: Detection and enumeration of coliform bacteria was performed by standard dilution methods and selective differential microbiological media. The samples of vegetables (tomato, red pepper, cabbage, potato, and carrot) were taken from different agricultural locations in the central Serbia. In the same locations, samples of irrigation water were taken for microbial analyses including total and faecal coliform bacteria, presence of *Escherichia coli* and *Enterococcae*.

Results: Results showed that highest number of coliform bacteria was determined in the combined crops of potato and carrot, and lowest in red pepper. On the other hand, number of *E. coli* in tomato and red pepper was higher compared with cabbage. Low presence of *Enterococcae* was present in all vegetables. Irrigation water quality of the most of the water samples was satisfactory.

Conclusions: The results indicate the importance of standard implementation in microbial quality of irrigation water in legislation of Serbia.

Acknowledgements: This research is supported by Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. TR 31080.

Key words: *quality water, coliform bacteria, vegetables, contamination.*

Food 3-45

THE EVALUATION OF THE MICROBIAL CONSIGNMENT IN THE ICE CREAM SAMPLES DURING THE PERIOD BETWEEN 2010 AND 2014 IN THE CITY OF TIRANA.

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Introduction: Ice creams are by-products of milk, which along nutritional values also represent a very suitable ground for the microorganisms development.

Aim: The purpose of this research is connected with the evaluation of the contamination grade of the Ice-Cream samples and also the determination of the microbial consignment which is above their allowed values (limits).

Material and methods: This research is carried out in the Laboratory of Food Microbiology in the Hygiene Sector at the Directory of Public Health Tirana. Six hundredeighty six Ice-Cream samples have been analyzed during the 2010-2014 period. The isolated bacteria have been expressed in CFU/g. The Ice-Creams have been cultivated in the relevant fields: Blood agar, Endo, Agar base, Capek, Bile lactose. Depending on the morphology of the colonies, the identification has been done through biochemical tests.

Results: 97.9 % of the samples analyzed are brought from the network (national samples), while 2.1% are imported samples. From the total number of the samples, only 61.34% of them have been evaluated within the standard, whereas 38.66% have been evaluated with high microbial consignment. The most common isolates are the Mesophyll Bacteria 92.30%, Coliform Bacteria 48.35%, *Staphylococcus aureus* 13.18%, *Streptococcus faecalis* 12.08%, *Escherichia coli* 43.9% and Mycotic consignment 4.39%.

Conclusions: Constant control is needed of the Ice-Cream samples from the relevant Institutions in order to improve their quality.

Key words: *Ice-Cream, contamination, bacteria, quality.*

Food 3-46

A STUDY OF SALT CONTENT OF BREAD IN SOME MARKETS IN TIRANA, ALBANIA

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Introduction: Many diseases, including cardiovascular diseases, cancer, diabetes or chronic lung diseases are the leading cause of premature death in the 21st century. Dietary factors as high salt intake constitute the main risk factors. Bread is considered as one of the most important sources of dietary salt.

Aim: The aim of this study was to evaluate the range of salt content and investigate potential factors contributing to differences in salt content of unwrapped craft bread in Tirana, Albania. We have taken 40 samples in different markets in Tirana.

Materials and methods: All samples were collected fresh, at room temperature, within 12 hours of supply by the bakeries. All samples were sealed in plastic bags in order to preserve the loaves'

moisture and stored in a refrigerator at 4°C until analyzed. Titration is the most common method of analysis in in-house laboratories for determining salt in foods. Titrimetric methods have been adopted as the reference method by organizations such as the Association of the Official Analytical Chemists (AOAC) for a variety of food matrices, which include cheeses, meats, and vegetables

Results and Conclusion: The average salt content of the analyzed bread samples was 1.36 ± 0.18 g salt/100g of fresh bread. The present study shows that 10% of total bread samples available in the different markets of Tirana possess salt content ≥ 2.0 g salt/100g fresh bread and 20% of total bread samples have salt content ≤ 1.0 g salt/100g. A wide range of salt content in bread was observed in this study. The great variability in bread making recipes indicates potential challenges in salt reduction and highlights the importance of accelerating and intensifying efforts towards reducing salt in all types of bread by all bakers. The outcome of this project provides baseline information for monitoring the progress and evaluating the success of any efforts made by the bakery sector regarding salt reduction.

Keywords: *bread, Tirana, salt, titration*

Food 3-47

PRELIMINARY DATA FOR THE PRESENCE OF *GIARDIA LAMBLIA* IN WATER SAMPLE FROM WELLS IN TIRANA DISTRICT

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Introductions: Giardiasis is a diarrheal illness caused by a parasite, *Giardia intestinalis* (also known as *Giardia lamblia* or *Giardia duodenalis*). During the past 30 years, *Giardia* infection has become recognized as a common cause of waterborne disease in humans. Wells may be more vulnerable to such contamination after flooding, particularly shallow wells, have been dug or bored, or have been submerged by floodwater for long periods of time.

Aim: This study is the first attempt to detect protozoan parasites in water samples such as *Giardia lamblia*.

Material and Methods: A total of 57 water samples from different wells were collected between March and October 2015. Each sample was transported to a laboratory within 24 h of collection and filtered to use membrane filtration method. The water samples were processed in accordance with the ISO methods 15553:2006 and after microscopical observation.

Results: In recent years, Tirana district has seen an exponential increase in the number of residents. The growing population has meant that there is an urgent need for clean water for daily usage, however this is rather limited in some places especially in rural areas. Analysis of water samples taken in wells from these areas have resulted in the presence of cysts of *G. lamblia* was detected in 9 water samples from the 57 wells that means 15,7 % of all analysed sample.

Conclusion: The presence of cysts of *G. lamblia* in the water samples means that there is potential risk for zoonotic disease transmission in wells. This requires attention to implement the necessary preventive measures.

Keywords: *Giardia intestinalis, wells, water.*

1.1. MILK DIARY PRODUCTS AND EGGS (MDE)

Food 3-48

FACTORS AFFECTING FRESH MILK, COW MILK FOR INDUSTRIAL PROCESSING

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Aim: This research was aimed at evaluating the quality of milk and hygiene from the farmer to the industrial processing.

Material and Methods: As material for research 132 samples were collected in period November-December 2015 to July 2016. Among them 60 samples were taken from farmers in villages in the municipality of Gjilan and Ferizaj, 25 samples from collection points, 15 farmers with the milking equipment and preservation of milk, 15 in the processing industries "Kabi" Gjilan.

Results: During the research evaluation of the quality of milk, the number of bacterial colonies per ml [NKB / ml], and somatic cell/ml, [QS/ml], and the physicochemical composition according to the results of milk was 75% of grade II and III, while 20% of the I class, and 5% of

EXSTRA class, according to EU legislation [BE / 92/46] Food Safety and Harmonization of RKS legislation and Legal Framework for Food Security Kosovo [Ma. No.20 / 2006.].

Food 3-49

PROTEOLYTIC AND TEXTURAL CHANGES DURING RIPENING OF CHEESE IN A SACK

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Introduction: Cheese in a sack is Croatian autochthonous hard ewe's milk cheese. Its main specificity is ripening in a sack made of a lamb skin. As casein forms the base of cheese structure in which other compounds are incorporated, proteolysis is considered as the most important process which contributes to the formation of characteristic cheese texture. Moreover, proteolysis is involved in the formation of desirable cheese flavour.

Aim: The aim of this research was to determine physicochemical, proteolytic and textural changes over 30 days of cheese ripening, and correlations between these groups of parameters.

Material and Methods: Research was conducted on the 10 batches of cheese which were manufactured at the small scale dairy farm. From each batch, samples of curd and cheese at 15 and 30 days of ripening were taken. Physicochemical analyses of cheese included determination of pH value, total solids, fat, protein and salt content. Proteolytic changes in cheese was monitored by determining the content of α_{s1} -casein, α_{s1} -I-casein, β -casein, γ -casein, water-soluble nitrogen fraction (WSN %TN) and 12%-trichloroacetic acid soluble nitrogen fraction (TCA-SN %TN) in the total nitrogen, and ripening indexes (alpha and beta). The cheese texture was determined by the compression test.

Results: The time of ripening had a significant effect on the observed physicochemical, proteolytic and textural parameters ($P < 0.05$, $P < 0.01$). The content of protein, TCA-SN %TN, index alpha ($P < 0.05$), α_{s1} -casein, β -casein, γ -casein and index beta correlated significantly ($P < 0.01$) with the cheese texture.

Conclusions: Proteolysis is essential for achieving desirable sensorial quality of cheese (proper appearance, flavour, colour, odour and texture).

Key words: *Proteolysis, texture, sensorial quality, cheese in a sack, traditional cheeses*

QUALITY CHARACTERISTICS OF EWE'S BEATEN CHEESE

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Introduction: The beaten cheese with characteristic yellowish color and salty taste is traditional product of the Balkans. The interest in production of beaten cheese industrially with same characteristics as the traditionally produced one has increased.

Aim: The aim was to evaluate the influence of bioprotective culture, Fresh Q, on the beaten cheese quality.

Material and Methods: The cheese was produced by standard production procedure by using ewe's milk, the heterofermentative culture strains, chymosin, and bioprotective cultures supplied by Chr. Hansen (Denmark). After treatment with hot water (80 °C), the curd was stored at 37 and 45 °C for 3 days, followed by brine salting. The referent samples were produced in the same procedure without Fresh Q. In the beaten cheese, surface color, texture and salt content were determined.

Results: The influence of Fresh Q on the color is insignificant, whereas the higher temperature of curd storage (45 °C) decreased the lightness of the final product. The Fresh Q culture had significant influence on the firmness. The cheese with Fresh Q treated at 37 °C showed firmness of 19 N. In the curd cheese samples stored at 37 °C higher salt content (4 %) was determined than samples at 45 °C (2.8 %).

Conclusions: The temperature of curd treatment is important factor on the eye formation. The smallest sizes and uniform eye distribution were determined in the beaten cheese produced from curd treated at 37°C. The Fresh Q improved the sensory characteristics in terms of flavor and taste and prolonged the shelf life of the beaten cheese.

Keywords: *beaten cheese, quality, salting, surface color, texture, Fresh Q*

QUALITY CONTROL OF YOGURT AND SOUR CREAM DURING TRANSPORT AND MARKET STORAGE

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Introduction: The quality of milk and dairy products during production and market storage is of high importance.

Aim: The aim was to study the influence of transport and storage conditions on the stability and quality of yogurt and sour cream.

Material and Methods: Pasteurized cow's milk was used in yogurt (3.2 % fat) and sour cream (15% fat) production. The stability of yogurt and sour cream stored at 4 and 9°C was evaluated through pH values, fat and dry matter without fats. The ambient temperature in the warehouse was 4°C. In the transport vehicles, the temperature varied from 2 to 8°C due to the loading and unloading.

Results: The pH values of yogurt samples stored at 4°C ranged from 4.45 to 4.25. The samples previously transported from producer's warehouse to the market were stored at 9°C and exhibited pH values decrease from 4.41 to 4.12, which intensified after 8 days of storage. In the sour cream stored at 4 and 9°C, pH was 4.50 and 4.23. It decreased negligibly until the 15th day, but afterwards dropped more intensively in samples stored at 9°C. The changes in fat content in yogurt (3.2% ±0.05) and sour cream (15% ±0.11), as well as in dry matter without fats (3.2% ±0.05) were insignificant. The white yellowish color, characteristic odor and thick liquid consistency were retained in both analyzed products, stored at 4 and 9°C. The pleasant sour taste was characteristic for the samples stored at 4 °C, while for the samples at 9°C more pronounced sour taste was detected.

Key words: *dairy products, quality, storage, pH value, fats, dry matter*

1.2 MEAT FISH AND SEA FOOD (MFSP)

MERCURY CONTENT INCULTIVATED MUSSELS FROM BULGARIAN BLACK SEA FARMS

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Introduction: Lately the consumption of cultivated mussels in Bulgaria has increased and it has become commercially important. Mussels are source of nutrients, but as a result of filter-feeding, they can be potentially toxic because can accumulate in their soft tissue metals as Hg. It is very hazardous for consumers, as the concentrations of accumulated metals often are higher than presented in the water.

Aim: Determination of the total mercury content in cultivated mussels from Bulgarian Black Sea and assessment of the risk for consumers.

Material and Methods: Sampling was conducted from 2013 to 2014 year. The analyzed 57 samples cultured mussels were from 23 farms on the Bulgarian coast. The mussels belonged to the genus *Mitylus galloprovincialis*. A direct mercury analyzer Hydra C was used for the determination of mercury in soft tissue of mussels. The apparatus provided correct results without prior sample preparation.

Results: Total mercury concentrations in the edible part of mussels ranged from 0.07 mg.kg⁻¹ to 0.16 mg.kg⁻¹, which was below the legal limit (0.5 mg.kg⁻¹) set in Commission Regulation (EO) 1881/2006.

Conclusions: The study on the content of total mercury in the edible part of mussels, cultivated in Bulgarian Black Sea farms, suggest that there is no human health risk, associated with the consumption of mussels.

Key words: *Black Sea, mussel, mercury*

DETERMINATION OF HYDROXYPROLINE AND RATIO COLLAGEN/PROTEIN IN MINCED MEAT

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Introduction: Hydroxyproline is a non-essential amino acid, which is the main component of the collagen. The determination of hydroxyproline in meat and meat products is a criterion to assess the content of collagen. Some producers replace the meat with nutritive value with the cheap collagen. To protect consumer interests and quality of minced meat, the Regulation (EU) 1169/2011 has laid down the requirements for the ratio collagen/protein and this information should be declared correctly in the label of the product.

Aim: The objective of the paper was to study the content of hydroxyproline, protein and fat in minced meat from Bulgarian market and to determine ratio collagen/protein.

Material and Methods: Twenty five samples of 5 types of minced meat were analysed for hydroxyproline through method ISO 3496, for protein with BS 9374 and for fat with BS 8549.

Results: The high quantities of hydroxyproline (26.2% and 22%) and respectively high ratio collagen/protein were found for minced pork meat and minced beef meat. The minced pork flank did not meet the requirement in Regulation (EU) 1169/2011 due to high quantity of fat (40.6%).

Conclusions: The presence of high amounts of hydroxyproline and fat in minced meat indicated that some producers offer minced meat with high content of connective tissue and less nutritional value.

Key words: *hydroxyproline, collagen, minced meat*

1.4 VEGETABLES, FRUITS AND LEGUMES (VFL)

EFFECT OF TOMATO SPOTTED WILT VIRUS (TSWV) ON PEPPER PLANTS AND AGRICULTURAL PRODUCTION

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Introduction:The genus *Tospovirus* contains viruses, which are very unstable, especially at pH values below 5.5. TSWV is the only known member of this genus that infects pepper. The virus is known to cause chlorosis, necrosis and different chlorotic and necrotic rings and patterns on pepper leaves and fruits. It is transmitted persistently by thrips. The virus causes severe damage on pepper production.

Aim: Our aim was to identify TSWV virus infection in pepper plants and to reduce the spread of the infection in the pepper crops using healthy seedlings and resistant cultivars.

Material and Methods: DAS-ELISA to identify virus infection in the pepper plants was used.

Results: TSWV was identified in several pepper cultivars. Most of the pepper cultivars were susceptible to TSWV infection, but we found some tolerant cultivars that didn't express any symptom on pepper fruits.

Conclusions: TSWV causes severe damages on susceptible pepper cultivars for agricultural production. To reduce these damages we use virus free seedlings and tolerant pepper cultivars.

Key words: *TSWV, pepper, virus, DAS-ELISA*

APPLICATION OF LEAF SPECTRAL REFLECTANCE FOR EARLY DETECTION OF VIRAL INFECTIONS OF POTATO

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Introduction: Potato is on fourth position worldwide after wheat, rice and maize as food crop. Potato tubers constitute a highly nutritious food. It provides carbohydrates, vitamin C, minerals, high quality protein and dietary fibre. More than 40 viruses cause diseases of potato that affect significant its yield. Potato Virus Y (PVY) and Potato leaf roll virus (PLRV) are the most important viruses infecting potatoes. Symptoms vary depending upon the strain of the viruses and the variety of potato. Serology and nucleic acid detection techniques are usually used to diagnose and characterize suspected viral diseases.

Aim: In this study non-destructive remote sensing method based on hyperspectral leaf reflectance measurements was used for detecting the presence of viral infections on potato plants.

Material and Methods: For the investigations young potato plants cultivar Arnova, Crone, Armada, and Agata from the potato collection of Institute of soil sciences, agrotechnologies and plant protection (ISSAPP) were used. Reflectance data were collected by means of a portable fibre-optics spectrometer in the visible and near infrared (NIR) spectral ranges.

Results: The differences between the spectral data of control and infected plants were assessed by means of Student's t-test, first derivative analyses, and calculation of some vegetation indices in green, red, red edge and NIR spectral ranges. Significant changes in spectral characteristics of the leaves from infected plants were observed in spectral range 450-850 nm.

Conclusions: Strong relationship was found with the results from serological test DAS-ELISA for presence and the degree of the infections.

Key words: *remote sensing, leaf spectral reflectance, DAS-ELISA, PVY, PLRV, potato cultivars*

Food 3-56

REACTION OF PEAR CULTIVARS TO THE PATHOGEN *GYMNOSPORANGIUM SABINAE* OF PEAR RUST DISEASE

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Introduction: The paper presents results of a screening performed during the period between 2014 – 2016 year in the natural conditions of Sofia field. The phytosanitary status of pear trees grown in orchards was evaluated.

Aim: Identifying of cultivars resistant to the pathogen *Gymnosporangium sabinae* of pear rust disease.

Material and Methods The investigation includes: itinerary observations, founding of pear trees manifesting symptoms of pear rust and evaluation of injuries caused by *Gymnosporangium sabinae*. The spread of the disease and rate of attack was traced out on the leaves of pear trees belonging to different cultivars. The injuries on the leaf lamina were determined by means of HP Scanjet 300 desktop scanner. Measuring the area of digital image was done by Adobe Photoshop.

Results: The trees of inspected cultivars: Beurré Giffard, Conference, Hardenponte Winterbutterbirne (Schinkenbirne), Popska (Pastornice, Pastorenbirne, Curé), Passe Crassane and Williams grown in the outskirts of the north slopes of mountain Vitosha were strongly affected by *G. sabinae*. There is a constant infectious background due to the presence of *Juniperus* species which are natural host plants of *G. sabinae*.

Conclusions: The choice of place for planting of new pear orchard is one of the important conditions for successful pear growing. The lack of immunity of pear cultivars to *G. sabinae* could impose using of against the pathogen. The necessity of fungicide treatment is a limiting condition for organic fruit growing.

Key words: *pear rust, Gymnosporangium sabinae, Rostellia cancellata*

1.6 HERBS, SPICES, COFFEE AND TEA (HSCT)

THE SPECIES OF GENUS *SATUREJA* L. IN BULGARIAN FLORA AND POSSIBILITIES OF THEIR USE AS MEDICINAL AND AROMATIC PLANTS

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Genus *Satureja* L. includes approximately 200 species, 12 of them occurring naturally in Europe. Bulgarian flora contains 5 species growing in dry and sunny habitats. Two species are endemics – *S. rumelica* Velen. is restricted only to Bulgaria and *S. pilosa* Velen. – to Balkan Peninsula. The other three species are more common and can be found in different parts of the country - *S. montana* L. mostly in the western and northern regions, *S. cuneifolia* Ten. – in the mountain regions up to 1500 m and *S. coerulea* Janka – predominantly in the eastern part up to 1000 m a.s.l. The populations are stable and no considerable anthropogenic pressure exist, which is due to wide cultivation of *S. hortensis* L. Regulations of herb collection are provided only for *S. montana* ssp. *kitaibelii* (Wierzb.) Ball., which is element of petrophyllous steppe habitats. The species are considered important both as medicinal plants and spices. Aromatic qualities of the species are due to their essential oil whose composition varies among the different species but the main constituents are phenols, carvacrol and thymol. Therefore an important issue is the study of qualitative and quantitative variation among the species and among populations within the species.

Acknowledgements: The authors are grateful to the financial support provided by the Bulgarian National Science Fund (Project M 06/7).

Key words: *Satureja*, conservation, distribution

***MICROMERIA DALMATICA* BENTH. - VALUABLE MEDICINAL AND SPICY PLANT SPECIES**

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Introduction: *Micromeria* species are traditionally used as medicinal plants and spices. Numerous studies identified antimicrobial, antioxidant, gastroprotective, hepatoprotective, cytotoxic, anti-inflammatory, and analgesic activity of different *Micromeria* species. Out of 4 species occurring naturally in Bulgaria, *Micromeria dalmatica* Benth. is the most widespread one and with the largest size. Therefore it is most collected one and used as a spice.

Aim: The aim of present study was to characterize chemical composition and antioxidant potential of *Micromeria dalmatica* extracts in view of their use in the culinary and herbal medicine.

Material and Methods: Plant material was collected in natural populations in Bulgaria. Chemical composition from aerial parts of *M. dalmatica* was analyzed by GC/MS and HPTLC. Free radical scavenging activity of the species was evaluated using DPPH assay.

Results: A wide variety of fatty acids was found. Hexadecanoic acid (palmitic acid, C16:0) was the main fatty acid. Omega-3 fatty acid: octadecatrienoic acid (α -linolenic acid C18:3) present in significant amount too. Other identified fatty acids were linoleic acid (18:2), oleic acid (C18:1), stearic acid (C18:0), lignoceric acid (C24:0), behenic acid (C22:0), eicosenoic acid (20:1), margaric acid (C17:0), myristic acid (C14:0). Gamma--amyrin (triterpene) was found in significant amount. Phenolic and organic acids, flavonoids, carbohydrates have been detected too.

Significant free radical scavenging activity was established.

Conclusions: The results showed that *M. dalmatica* is rich in important nutrients as fatty acids (saturated and unsaturated - omega-3), triperpens, flavonoids, phenolic and organic acids which make it a very valuable spice and medicinal plant.

Acknowledgements: Program for career development of young scientists, BAS Grant № DFNP-67_A1

Key words: *fatty acids, flavonoids, terpens, phenolic acids*

BIOLOGICALLY ACTIVE SUBSTANCES IN INFUSIONS AND EXTRACTS FROM MEDICINAL PLANTS

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Introduction: Medicinal plants present a rich source of bioactive compounds and antioxidants with significant importance in human healthy nutrition. In Bulgarian folk medicine rosehip, peppermint, thyme, coltsfoot and elecampane are traditionally used for curing respiratory disorders. Peppermint and dandelion roots enhance digestion. The content of bioactive compounds in extracts depends on technique, solvents, time and temperature of extraction. The most commonly used herbal extracts for oral intake are herbal infusion. Nowadays, people frequently apply microwave irradiation for preparation of herbal extracts.

Aim: The aim of the current study was to evaluate the bioactive compounds in extracts from six medicinal plants rosehip fruits, elecampane roots, dandelion roots, thyme, coltsfoot, peppermint obtained by infusion and microwave irradiation.

Material and Methods: Herbal extracts were obtained by infusion and microwave-assisted extraction. Total phenolic, flavonoids and fructans content and antioxidant activity of these extracts were determined by Folin-Ciocalteu reagent, AlCl₃ reagent, resorcinol-thiourea and DPPH assays.

Results: The highest content of biologically active substances and antioxidant activity were found in all extracts obtained by microwave-assisted extraction. Coltsfoot and thyme showed the highest antioxidant activity (334 mM TE/g and 287 mM TE/g dw, respectively). Total phenolic and total flavonoid contents were the highest in coltsfoot extracts. Fructans were found in coltsfoot leaves (4.6%), dandelion and elecampane roots - 34 and 44% dw.

Conclusions: Extracts and infusion from coltsfoot and thyme were evaluated as herbs with the highest antioxidant activity, while elecampane and dandelion roots present natural sources of prebiotic inulin.

Key words: *herbs, phenolic compounds, DPPH, inulin*

1.8 MINERAL WATER, NONALCOHOLIC AND ALCOHOLIC BEVERAGES (MWNAB)

MICROBIOLOGICAL INDICATORS OF BOTTLED MINERAL WATER IN ALBANIA

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Introduction: Bottled water is generally perceived as clean, of good quality and protected. So, the consumption of bottled mineral water in Albania has increased largely. There have been reported waterborne diseases associated with consumption of bottled water.

Aim: was the assessment of quality of mineral bottled waters and the impact on health public.

Material and Methods: During 2016 we analyzed 126 mineral water samples (formats 0,25 -10 liters) taken in the random way, representing almost all mineral waters distributing in our country. We analyzed our sample for four microbiological indicators regarding to our new National regulation Nr-379 date 25.5.2016 (Colony count of microorganisms in 37 °C, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*). They indicate the possible presence of microbial pathogenic agents. The methods were respectively ISO: 6222-1999, ISO: 9308-2:1990, ISO: 7899 – 1 and ISO 16266).

Results: In total we found that *Enterococcus faecalis* was present in 5% of samples. *Pseudomonas aeruginosa* was present in 15% of samples. *Escherichia coli* was present in 21, 5% of samples. Colony count of microorganisms identified over limited in 39 % of samples. The highest level of bacteriological contamination was found in the 5-10 litre format and the lowest level was found in the 0, 25-0, 75 litre format. We found that all formats have higher level of bacteriological contamination compared with other studies we have done.

Conclusions: The results revealed in a high rate not a good quality of bottled water and the use of large quantities of contaminated water increases the risk of waterborne diseases.

Key words: *bottled water, quality of mineral water, microbiological indicators.*

2. FOOD CONTAMINATION AND TOXICOLOGY (FCT)

INVESTIGATION OF EXTRACTS DERIVED FROM THE PLANT *GEUM URBANUM* L. FOR ANTIMICROBIAL AND CYTOTOXIC POTENTIAL

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Introduction: The genus *Geum* consists of about 70 species, rich in monoterpenoids, sesquiterpenes, triterpenoids, flavonoids, ellagitannins, gallotannins, phenylpropanoids and phenolic acids. *Geum urbanum* L. is a medicinal plant which has been used from ancient times in the Bulgarian folk medicine for the treatment of inflammatory and infectious diseases of the gastro-intestinal tract.

Aim: Our study aimed to investigate the antimicrobial, anti-quorum sensing, and cytotoxic potential of extracts from *Geum urbanum*.

Materials and Methods: Roots and stems were used to obtain petroleum ether, ethyl acetate and *n*-butanol extracts. Minimal inhibitory and bactericidal concentrations (MIC/MBC) were estimated by broth microdilution method. Bacterial growth rate was determined by time kill assay. Swarming motility and inhibitory potential of synthesis of pyocyanin were investigated on *P. aeruginosa* (PA01). The cytotoxicity was tested on normal and tumor cell lines by MTT assay. IC₅₀ values were calculated with GraphPadPrizm.

Results: All extracts exhibited antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus cereus*. The ethyl acetate extract showed highest activity. The IC₅₀ on cell lines corresponded to MIC and MBC depending on the extract type. Both, ethyl acetate and *n*-butanol extracts inhibited the swarming motility and synthesis of pyocyanin by PA01.

Conclusions: *Geum urbanum* L. is perspective new source of bioactive compounds. Antibacterial features of investigated extracts reveal the possible potential for treatment of certain food infections. The established corresponding cytotoxicity supposes activity against bladder cancer with favorable antibacterial effect.

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Key words: *Geumurbanum*, plant extract, antibacterial activity, cytotoxicity

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DETACHMENT OF *PSEUDOMONAS AERUGINOSA* PRE-FORMED BIOFILM BY HYBRID POLYMER SYSTEMS

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Introduction: Bacterial biofilm contamination of various surfaces in hospitals and food industry represents a serious health risk. A major concern is their intrinsic resistance to routinely applied antibacterials and disinfectants. This requires the development of novel approaches to biofilm detachment.

Aim: The aim of the study is to evaluate the effectiveness of hybrid polymer systems against a pre-formed bacterial biofilm.

Materials and Methods: Hybrid polymer systems based on different polycations were used. Some of them were loaded with gold nanoparticles. The systems were characterised by dynamic light scattering (DLS) and transmission electron microscopy (TEM) after negative staining. *P. aeruginosa* PAO1, previously shown to be a good biofilm former, was used as a model microorganism. The bacterial biofilm was cultivated for 24h on 96-well plates. Then the medium was removed and the biofilm was treated with the nanoparticles for 2 or 4 h respectively.

Results: The hydrodynamic radius of hybrid polymer systems ranged from 35 to 120 nm depending on polycation used and the presence of gold nanoparticles. Their morphology was visualized by TEM. Very good correlation between the sizes determined from DLS and TEM was observed. The ability of hybrid polymer systems to interact with a pre-formed biofilm, loosen its structure and facilitate its detachment was evaluated. It was observed that all systems reduced the biofilm biomass 3 to 4-fold compared with the control (treatment of the biofilm with water alone).

Conclusions: The results indicate the applicability of the tested hybrid polymer systems for disinfection of biofilm-contaminated surfaces.

Acknowledgements: This work was funded by Project DFNP 12 in the frame of the support program for Young Scientists from Bulgarian Academy of Sciences.

Key words: *polymer nanoparticles, golden nanoparticles, biofilm detachment.*

Food 3-63

Cd, Pb AND Hg CONTENT IN MUSCLES AND CAVIAR OF NORTHERN PIKE (*Esox lucius*)

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Introduction: Among heavy metals cadmium, lead, manganese and arsenic are the main threat for human health that is why they are continuously monitored by WHO in all kind of food. Cd is known as carcinogenic one, lead is harmful to all human organs and systems, injuring strongly blood and reproductive systems. Children are under outlined risk because of their intensive lead gastrointestinal uptake and permeability of blood-brain barrier. Methyl-mercury is a highly toxic to humans and usually is associated with high fish consumption. Pregnant women should avoid freshwater fish consumption, collected from polluted waters. Wild Northern pike (*Esox lucius*) is an edible predatory fish known as “freshwater shark”.

Aim: To assess the content of Cd, Pb and Hg in both muscles and caviar of Northern pike (*Esox lucius*).

Materials and Methods: Northern pike was collected in Danube river in December 2016 near the town Svishtoff and stored at -20°C prior to analysis. Heavy metals were analyzed in fish muscles and caviar. Cd and Pb content was estimated using AAS (Atomic Absorbtion Spectrometry). Hg assessment was conducted *via* CVAAS (Cold Vapor AAS). Data obtained were compared with the Maximum residual limits (MRL) values in fish described in the EC Regulation № 1881/2006. Data obtained were compared with the Maximum residual limits (MRL) of fish contaminants described in the EC Regulation № 1881/2006.

Results: Our results revealed that the heavy metal contents in muscles and caviar (in mg/kg wet weight) were 0.0029 and 0.0027 for Cd, 0.035 and 0.048 for Pb, and 0.161 and 0.011 for Hg. Analysis demonstrated no heavy metal contamination in both muscles and caviar of examined fish. However, an interesting finding of the study was the higher Hg accumulation in fish muscles than in caviar. This result evinces a protective mechanism averting Hg uptake in fish eggs saving offspring from Hg toxicity.

Conclusions: Northern pike, collected in Danube river in 2016 was not polluted with Cd, Pb and Hg, meets the food safety standards and is save for consumption.

Key words: *Cd, Pb, Hg, bioaccumulation, freshwater fish*

3. MICROBIOLOGICAL CONTROL OF FOOD (MCF)

Food 3-64

THE SURVEY ON THE PREVALENCE OF *SALMONELLA* AND ANTIMICROBIAL RESISTANCE ON BROILER CARCASSES

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Introduction: Antimicrobial Resistance (AMR) is a significant public health problem that is receiving increasing attention worldwide. Any antibiotic used in people, animals or plants can promote the development and spread of antibiotic resistance, and thus prudent use is necessary. Antibiotic resistance does not respect geographical or biological borders, and can be readily disseminated. The usage of antibiotics in one sector, setting, or country affect the spread of resistance in others. Resistance to antibiotics is also a food safety concern considering the use of antibiotic in food animals for treatment, diseases prevention, and growth promotion.

Aim: The main objective of the study was to evaluate the prevalence and antimicrobial resistance (AMR) of *Salmonella* isolated from broiler carcasses in the farms of Albania.

Material and Methods: The survey was conducted during 2009-2010. The survey included 150 intestinal samples from healthy broiler chicken. We analyzed samples from broiler chicken to isolates and identify *Salmonella* according to ISO protocols. All the strains of *E.coli* and *Salmonella.spp* were performed for AST (Antibiotic Susceptibility Test) against to 11 antibiotics tested.

Results: Resistance of 84 isolates of *E.coli* and *Salmonella* spp. against 11 tested antibiotics, resulted in values from 36,4% (Lincospectine per *E.coli*) until 100% (Tetracycline and Furazolidon for *Salmonella* spp.). The highest levels of resistance for *Salmonella* spp. during study period (2009-2010) was seen for Tetracycline (100%) and Furazolidon (100 %). For *E.coli* isolates the highest level of resistance were noticed against Oxytetracycline (98, 1%), Tetracycline (97, 2%) and Doxycyclin (90, 2%).

Conclusion: *Salmonella* spp. seem to be important causes of diarrhoeal diseases in Albania and level of AMR was high for broiler chicken and the results reflected the usage of antimicrobial agents in the poultry in Albania.

Key words: AMR, *Salmonella* spp., poultry.

Food 3-65

STUDY OF GENETIC DETERMINANTS AT BACTERIOCIN HELVETICIN IN *L. crispatus* STRAINS

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Introduction: Bacteriocins are diverse group of ribosomally synthesized antimicrobial peptides produced by bacteria and archaea. To date, more of five hundred bacteriocins have been identified and classified. Recent discoveries have shown that bacteriocins are highly diverse and widely distributed among bacterial species. Bacteriocins produced by Gram-positive bacteria, including Lactic Acid Bacteria (LAB), are under focus as safe natural biopreservatives and as therapeutic alternatives to antibiotics.

Aim: Our work presents analyze of the genes encoding bacteriocin helveticin in *Lactobacillus* isolates with different origin.

Materials and Methods: We used PCR, sequencing analysis, NCBI GenBank database, agar spot test and well-diffusion test.

Results: New primer pairs, targeting helveticin operon were designed. The helveticin genes in genomes of studied strains were detected by PCR and identified by sequencing analysis. Antibacterial activity of our isolates were determined by two tests against 20 Gram (+) and Gram (-) pathogenic and nonpathogenic bacteria.

Conclusions: This study provides a quick method to screening the helveticin-producing strain. A further advancement to the bacteriocin research is the bioengineering of structural genes to develop mutants having an enhanced activity, a wide antimicrobial spectrum, and an increased efficacy in a complex food environment.

Key Words: *bacteriocin genes, helveticin, Lactobacillus*

Food 3-66

DETECTION OF *YERSINIA ENTEROCOLITICA* IN PIG FAECES AND TONSILS BY LAMP

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Introduction: *Yersinia enterocolitica* is the third most commonly reported zoonotic pathogen in the European Union. Natural reservoirs of this enteric pathogen are the pigs. Its detection is related to time consuming and laborious bacteriological methods, therefore new protocols are needed for faster identification in food samples.

Aim: To establish fast, specific, sensitive and inexpensive loop mediated DNA amplification (LAMP) based methodology for detection of *Y. enterocolitica* in pig samples.

Materials and methods: The ISO standard method for *Y. enterocolitica* identification was compared to LAMP after direct DNA isolation in 75 pig faeces and tonsils samples. The chosen LAMP primer set was published before¹. Its selectivity was tested on 10 non-pathogenic, 6 pathogenic (serotypes: O:3, O:5, O:8, O:9) *Y. enterocolitica* strains, and 16 other Gram (-) and Gram (+) bacterial species. Its sensitivity was compared to that of TaqMan-qPCR after artificial contamination of pig faeces. The efficacy of several commercially available DNA isolation kits was tested too. The LAMP-product visualisation was performed with hydroxynaphthol blue and DNA electrophoresis.

Results: The primers were strongly selective for pathogenic *Y. enterocolitica* strains. The sensitivity of the LAMP assay corresponded to that of TaqMan-qPCR and strongly depended on the DNA isolation protocol. The hydroxynaphthol blue visualisation showed the same efficacy as the DNA gel electrophoresis.

Conclusions: The LAMP method is reliable, fast, selective and sensitive. Determining factor for its sensitivity is the DNA isolation protocol.

Acknowledgements: The authors are thankful to ILVO for the two months fellowship granted to Dr. Maya Zaharieva.

Key words: *Yersinia enterocolitica*, pig tonsils and faeces, LAMP

Food 3-67

MICROBIOLOGICAL ESTIMATION OF WATER OF RIVER ZHEGRA, DURING AUTUMN SEASON IN 2005

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Introduction: Habit to throw the wastewater to the rivers, it has become the dominant habits of many countries, but in Kosovo it is more pronounced habit.

Aim: The main objective of this study was to assess the quality of water, of the river Zhegra during autumn season, 2005 year, through the microbiological analysis.

Materials and Methods: River Zhegra located in south - east part of Kosovo, which passes through the village Zhegra, nearby the city Gjlani. Samples for microbiological analyses were collected in four localities along the river.

Results: Based on achieving results led us to conclude: the water of river "Zhegra" is moderately polluted by bacteria at all localities.

Conclusions: High number of all microorganisms was registered in all localities. On base of coliform bacteria according to Tumbling system the waters of "Zhegra" river belongs at second to third class of pollution.

Key words: *microbiological, analysis, water, river, Zhegra*

Food 3-68

MICROBIOLOGICAL ESTIMATION OF THE LAKE BADOVC WATER DURING AUTUMN SEASON 2005

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Introduction: The presence of pathogens in water for drinking and swimming purposes is of public health significance considering the possibility of the presence of other bacteria, protozoa and enteric viruses that are implicated in gastro-intestinal water borne diseases and the low infectious dose for these water borne pathogens.

Aim: Bacteriological analyses were carried out on lake water used for drinking and swimming purposes in lake Badovc, nearby Prishtina city.

Materials and Methods: The results obtained were compared with WHO and EPA standards for drinking and recreational water. Physicochemical parameters analysed are: Turbidity, pH, Color, Total solids, Total dissolved solids (TDS), conductivity, temperature.

Results: The obtained results (Bacteriological and Physicochemical) did not comply with standards (for drinking and recreational water) of WHO and EPA.

Key words: *microbiology, analysis, lake, water, badovc*

Food 3-69

FUNGI FOUND IN DRIED FRUITS AND CEREALS-BASED PRODUCTS

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Introduction: Recently healthy diets promoted dried fruits and cereals-based products as significant portion. The presence of yeasts and molds on that can be a public health risk from exposure to fungal growth and mycotoxin production.

Aim: The aims of this study were to determine the mold contaminations of dried fruits.

Materials and Methods: In total 330 samples were collected from different type of cereals and dried fruits. From them 190 samples were packed products and 140 were npacked. Analyzed samples were selected randomly. Each sample (250 gram) was put in a sterile bag, sealed and transported immediately to the laboratory. Fungi were isolated using diluting plate method. The developing mould were counted and identified microscopically.

Result: Our samples resulted in a wide range of mold contamination. We have found in the same sample more than one isolated mould species. *Aspergillus* spp. was the most frequently isolated genus, counting 34% (114) of samples. *Penicillium* spp. was the second genus in 29 % (95) from dried apricots, figs, prunes and raisins. From *Aspergillus* spp. genus was isolated: *A. niger* (60

samples), *A. flavus* (26 samples) followed by *A. fumigatus* (18 samples) and *A. versicolor* (10 samples). *Zygomycetes* were isolated in 14.5 % of peanut samples. *Rhizopus* spp. was another common fungus isolated in 8.8 % of the samples. Unknown mould/Unidentified was isolated in 13.4% of samples.

Conclusion: This study showed that the *Penicillium* spp. and *Aspergillus niger* were found in most of the samples. Unpacked products were more contaminated than packaged products.

Key words: *mold, contamination, dried fruits.*

Food 3-70

FREQUENCY AND PATTERN OF RESISTANCE OF *CAMPYLOBACTER* IN "MOTHER TERESA" HOSPITAL IN ALBANIA

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Introduction: Studies have emphasized the impact of veterinary antibiotic use and the crossing of antibiotic-resistant bacteria between animal and human population.

Aim: Evaluation of *Campylobacter* infection and pattern of resistance

Methods: 373 fecal samples submitted to the Laboratory of Microbiology at University Hospital Center of Tirana, were tested. Samples were taken during October – December 2016. Samples were processed according to our laboratory protocol using ISO methods and were characterized biochemically using API 20E and Vitek. Susceptibility tests were performed by disk diffusion test according to EUCAST and ECDC recommendation. We used the following antibiotics: Ciprofloxacin, Gentamicin, Erythromycin and Tetracycline.

Results: Forty (10,72 %) bacterial isolates from 373 samples were positive for *Campylobacter* and were tested for resistance to antibiotics. Resistance rates were: Ciprofloxacin 21%, Erythromycin 47%, Tetracycline 52%, Gentamicin 67%

Conclusion: Our study showed a higher frequency of *Campylobacter* in human samples than in those of other countries, where the frequency range (20% -80%) and the pattern of resistance is the same. A pilot antibiotic resistance survey encompassed 6 Albanian poultry farms in 3-month

period December 2013 - February 2014. Compared to this study, has resulted that the frequency of *Campylobacter* found in chickens was 40% (180 chickens, 72 positive) and sensitivity test was in the same trend as in humans: Ciprofloxacin 21 %, Erythromycin 81%, Tetracyclin 52% and Ampicillin 17%.

4. PREBIOTICS AND PROBIOTICS (PP)

Food 3-71

BACTERIOCIINOGENIC *LACTOBACILLUS CURVATUS* STRAINS ISOLATED FROM SMOKED SALMON – TO BE OR NOT TO BE APPLIED AS BENEFICIAL CULTURE?

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Introduction: *Lactobacillus curvatus* is a LAB naturally presented in different natural environment and its potential in producing bacteriocins represents an important opportunity for exploration it as tools for food biopreservation. Safety assessment for presence of virulence and antibiotic resistance genes in LAB is an important task to be evaluated in order to be selecting these strains as commercial beneficial cultures.

Aim: The aim of this study was to explore safety aspects of bacteriocinogenic *Lb. curvatus* strains based on presence and expression of genes related to the virulence factors, production of biogenic amines and antibiotic resistance.

Materials and Methods: *Lb. curvatus* ET06, ET30 and ET31 were isolated from smoked salmon, identified based on their biochemical and genetic characteristics including PCR with species-specific primers, and characterized as bacteriocin producers against some food spoilage microorganisms and food borne pathogens. The strains were subjected to molecular and phenotypical tests to assess the presence of more than 50 genes related to virulence factors, production of biogenic amines and antibiotic resistance.

Results: *Lb. curvatus* ET06, ET30 and ET31 produce class IIa bacteriocins with 2.8, 3.1 and 4.5 kDa, respectively, with bactericidal activity against *Staphylococcus* spp., *Enterococcus* spp. and *Listeria* spp., including *L. monocytogenes* from various serological groups. *Lb. curvatus* ET06,

ET30 and ET31 presented also low virulence profile, indicated by the presence of few genes related to antibiotic resistance and surface proteins, based on genetic and physiological tests. Besides all beneficial properties studied for various LAB, most considered as GRAS, a special attention need to be pay on the possible presence of virulence factors, production of biogenic amines and antibiotic resistance. Horizontal gene transfer of virulence factors between pathogenic and LAB, including probiotics is a highly possible scenario in case of uncontrolled application of probiotics or starter cultures.

Acknowledgements: CNPq, CAPES, FAPEMIG, FAPESP

Key words: *Lactic acid bacteria, bacteriocins, probioitcs, virulence factors, antibiotic resistance*

Food 3-72

IDENTIFICATION OF YOGURT LACTOBACILLI USING SCANNING ELECTRON MICROSCOPY

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Introduction: Lactic acid bacteria (LAB) are found naturally in the body, usually in the mouth, intestinal and reproductive system incl. man. They have long been consumed by people in raw milk and dairy products such as cheeses, yoghurts and fermented milks. Today, LAB provoke a great interest among scientists and consumers with their specific *probiotic properties*. They express strong antifungal and bactericidal activity including growth inhibition of *Helicobacter pillory*. Production of many vitamins leads to beneficial to human health effect improving the immune system. Thus, it is of significant importance to check and compare the present quality status and probiotic potential of the most popular Bulgarian yoghurts on the market.

Aim: To study morphology and identify lactobacilli isolated from commercial yogurt samples.

Materials and Methods: Six top brand Bulgarian 3.6% cow yogurt were purchased from an international food retailer store - “Vereya”, “Na baba”, “Elena”, “LB”, “Parshevitza” and “Rodopeya”. Samples were initially enriched with skim milk, 100 µl plated on MRS plates and further cultivated at 42 °C for 48 h. Totally 6 LAB isolates, one per yogurt sample, were grown in liquid MRS media, washed and centrifuged triple with 0.9 % NaCl. Prior to Scanning Electron microscopy bacteria were consequently dehydrated in 60-70-80-90 % ethanol. Analysis was performed using JEOL JSM-5510 (Jeol Ltd., Tokyo, Japan) with gold plating of the sample for 45 sec. Bacteria were triple beamed 10 kV for 10 sec. Magnification used was x 1 000, x 10 000, x

15 000 and x 20 000. Results were discussed along with data of API-50CH (Biomerieux, France) biochemical analysis of fermentative capability of LAB isolates to 50 different metabolites.

Results: The isolated strains formed rough, pale white-yellow, non-pigmented colonies, 1-2 mm in diameter. Scanning Electron Analysis showed typical for lactobacilli morphology - rods up to 10 µm length, rounded ends and singly occurred some. Some of them were found to curl. API-50 CH biochemical analysis showed conspicuous results: the isolated lactobacilli degrade just 4 out of 50 metabolites: D-glucose, D-fructose, D-mannose and D-lactose and belong. Both, SEM and API-50CH test were firm and in concordance, our LAB isolates belong to *Lactobacillus bulgaricus* ssp. *delbrueckii*.

Conclusions: LAB, isolated from 6 top-brand bulgarian yogurt samples belong to *Lactobacillus bulgaricus* ssp. *delbrueckii* and possess probiotic capability.

Keywords: LAB isolates, commercial yogurt, probiotics

Acknowledgements: This research was funded by Contract of Faculty of Bachelors, NBU

6. FOOD ADDITIVES (FA)

Food 3-73

COMPARISON OF THE BIOCHEMICAL PROFILE OF GREEN AND RED ALGAE IN RELATION TO THEIR POTENTIAL APPLICATION AS FOOD ADDITIVES

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Introduction: With the improvements in the cultivation of microalgae, it became possible to add algal biomass and/or metabolites in foods in order to consume balanced and health-food.

Aim: For this investigation two different algal species – the green alga *Scenedesmus* sp. and the red alga *Porphyridium cruentum* are studied for potential source of food additives.

Material and Methods: Experiments are conducted in order to determine their growth and biochemical composition (lipids, carbohydrates and proteins).

Results: It turns out that in *Scenedesmus sp. BGP* the most abundant component are the proteins, followed by carbohydrates and lipids, which makes it an excellent unconventional protein source. As opposed to it, the biochemical composition of *Porphyridium cruentum* is dominated by carbohydrates, then proteins and lipids, but there is also a high content of poly unsaturated fatty acids (20:4) and (20:50), which are essential for humans

Conclusions: Considering the fact that microalgae are poorly explored and their cultivation can be independent of external conditions, the production of a wide range of substances makes these organisms present a really remarkable source of biomass and certain compounds. As the results show, both microalgal strains proved to be important sources of functional ingredients that could be successfully used as food additives together and separately.

Key words: *Scenedesmus sp.*, *Porphyridium cruentum*, *biochemical component*, *food additives*

7. ORGANIC FOOD (OF)

No submissions

9. FOOD AND HUMAN HEALTH (FHH)

Food 3-74

ANTIMICROBIAL RESISTANCE AND IMPACT OF INTENSIVE FARMING ON HUMAN HEALTH

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Introduction: Antimicrobial resistance (AMR) is not a recent phenomenon, but it is a critical health issue today. The world's public-health experts agreed that drug-resistant bacteria are created in farm animals by antibiotic use and are transmitted to people in food and then spread by person-to-person transmission.

Aim: The aim of this study is to analyze the contemporary data of how antibiotics used in animals can cause resistance to drugs used in people. To present data of increasing of difficult to treat due to the associated multidrug-resistance profiles bacterial isolates both in out and inpatients in

Bulgarian University hospital.

Materials and methods: Twenty six papers and international programme documents were analyzed. The identification and antimicrobial susceptibility tests were provided by VITEK 2, *BioMerieux, France* and conventional methods. The EUCAST interpretation system applied. Modified Hodge test, colorimetric carbapenem hydrolysis assay and DDST (IMP/MER-EDTA) and multiplex PCR were used for carbapenemases detection.

Results: Between 2014 and 2016 was registered increasing of MRSA isolates totally in hospital from 12,9%-14,9% ; in ICU-40%. Regarding MLS resistance: *S. aureus* MLS from 32,3%-76,1%; *S. epidermidis* 67%-86%; *S. pneumoniae* 50%-73.5%. *Enterococcus* spp. with HLAR -84,3% in ICU. Persisting high level of ESBL producing *K. pneumoniae*-75,7% ; quinolone R- 62%; *E. coli*-37% and QNR 36,4%.; carbapenem R *A.baumannii*-97,2%. Twenty two CRE *Klebsiella pneumoniae* isolates were registered. Clone A showed modified M-22 and defective in both major porins OmpK35 ; OmpK36, likely explaining carbapenem resistance. Clone B -positive PCR for NDM, identified as NDM-1 via sequencing.

Conclusion: When animals are administered an antibiotic that, closely related to an antibiotic used in human medicine, cross-resistance occurs and disease-causing bacteria become difficult to treat. The consensus of veterinary and medical experts is important for AMR surveillance, strong antibiotic stewardship, guidelines and restriction measures concerning over and unnecessary antibiotic use in farms to prevent the spread of these major resistance threats.

Key words: antimicrobial resistance, animals, MRSA, NDM-1

Food 3-75

ANTIOXIDANT ACTIVITY AND ANTIFUNGAL EFFECT OF WILD BERRY FRUIT EXTRACTS

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Introduction: The Bulgarian wild berry fruits contain many bioactive compounds, such as anthocyanins and flavonoids and possess various bioactivities and health benefits, such as free radical scavenging, anti-inflammatory, antimicrobial, and anticancer activity.

Aim: The aim of the present study was to evaluate the antioxidant activity of total methanol extracts and their anthocyanin and non-anthocyanin fractions from the wild berries naturally growing in Bulgaria (bilberry, strawberry, raspberry and cranberry). As well, *in vitro* antifungal effect against selected pathogenic fungi was determined.

Material and Methods: The antioxidant potential of the methanol extracts and their fractions was determined by superoxide-anion scavenging assay. The influence of superoxide dismutase on non-enzymatic reduction of the nitro blue tetrazolium was evaluated. *In vitro* antifungal activities were examined by the agar disk diffusion method against *Aspergillus niger* and *A. fumigatus*.

Results: The results demonstrated strong antioxidant activities of the total methanol extracts from wild small berry fruits. The order of scavenging effectiveness was: cranberry \geq bilberry Ch \geq strawberry \geq bilberry Bg \geq raspberry. Similar data were obtained for both nonanthocyanin and anthocyanins fractions. Nonanthocyanin component of cranberry showed the highest radical scavenging activity. Significant antioxidant activity of anthocyanin fraction from raspberry, strawberry and cranberry was measured as compare to other fractions. The highest antifungal effect showed bilberry extract.

Conclusions: The obtained results show that bioactive molecules extracted from Bulgarian wild berry fruits can be used as antifungal and antioxidant additives in the food, cosmetic and pharmaceutical industry.

Key words: *wild berry fruits, antioxidant activity, antifungal effect*

Food 3-76

YOGURT FORTIFIED WITH OMEGA-3 FATTY ACIDS AND AUTOCHTHONOUS POTENTIAL PROBIOTIC LACTOBACILLI

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Introduction: Adding of probiotic bacteria and polyunsaturated omega-3 fatty acids (omega-3 PUFAs) in food production can achieve many positive effects on human health.

Aim: The objective was to investigate the survival of autochthonous potential probiotic bacteria *Lactobacillus plantarum* 564, *Lactobacillus paracasei* Z8 and commercial *Lactobacillus acidophilus* LA 05 and the influence of omega-3 PUFA, on the sensory quality of yoghurt.

Materials and methods: Experimental yogurt variants were produced using starter culture *Lactobacillus delbrueckii ssp. bulgaricus*, *Streptococcus thermophilus* (Chr. Hansen) and potential probiotic: *Lb. plantarum* 564 and *Lb. paracasei* Z8 (Culture Collection of the Department for Food Microbiology, Faculty of Agriculture, University of Belgrade) and commercial *Lb. acidophilus* LA 05 (Chr. Hansen). For each probiotic strain three variants of yogurt were produced 1. without omega-3 PUFAs 2. with 100mg/L omega-3 PUFAs; 3. with 200mg/L omega-3 PUFAs. Viability of probiotics and sensory evaluation were observed for 3 weeks.

Results: The amount of *Lb. plantarum* 564 and *Lactobacillus paracasei* Z8 were 10^8 - 10^9 cfu/ml during storage. Cell counts of *Lb. acidophilus* LA 05 were maintained at the level 10^7 - 10^8 cfu/ml. Addition of both concentrations of omega-3 PUFA did not significantly affect the sensory quality of yoghurt. All variants of yoghurt evaluated with 80-99% of maximal sensory quality through examined storage period.

Conclusions: Results indicate that autochthonous potential probiotic strains and omega-3 fatty acids can be successfully used in the production of yoghurt as a new functional dairy product.

Key words: *potential probiotic bacteria, yoghurt, polyunsaturated omega-3 fatty acids, sensory quality*

Acknowledgments: This work was supported by Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. 046009 and 046010).

11. FOOD BIOTECHNOLOGY (FB)

A BRIEF LOOK AT THE BIOTECHNOLOGICAL POTENTIAL OF A LOCAL MICROALGAL STRAIN *COELASTRELLA* SP.

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Introduction: The demand of modern food industry for cheaper and healthier products is constantly increasing. Microalgae are an enormous biological resource of new products with a broad range of applications. Unlike *Haematococcus* and *Dunaliella*, the green microalga *Coelastrella* is much less studied and a promising producer of carotenoids.

Aim: The aim of this study was a preliminary evaluation of the biotechnological potential of a local Bulgarian strain – *Coelastrella* sp. BGV (Chlorophyceae) on the basis of induced carotenoid synthesis.

Material and Methods: Carotenoid accumulation was induced by applying the following stress conditions: absence of CO₂; addition of 2% NaCl in the medium and high light intensity (35000 Lx) for a period of 240 h. Algal biomass was harvested and analyzed for protein, carbohydrate, pigment and lipid contents.

Results: The best growth and the highest carbohydrate content were achieved at high light intensity. Protein accumulation was stimulated by the absence of CO₂. Lipids showed minimal differences. Carotenoid accumulation was stimulated to the highest extent by the salt stress (2% NaCl). The results from microscopic observation of the cultures were also noteworthy.

Conclusions: High productivity of *Coelastrella* sp. BGV at high light intensity is a big advantage of the strain in respect to mass outdoor cultivation. Increase in carotenoid accumulation in the biomass was achieved but we consider these results insufficient. Further experiments with intense pulsed light and light with different wavelengths will be conducted.

Acknowledgements: This work was financially assured by "Program for career development of young scientists, BAS, 2016".

Key words: *carotenoids, Coelastrella, biotechnology, stress*

Food 3-78

PHYTOCHEMICAL VARIATIONS OF *RHODIOLA ROSEA* L. AND ITS EFFECT ON T CELLS

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Introduction: *Rhodiola rosea* L. is among the most popular plants, acting as adaptogens. Its pharmacological properties are tightly associated with the phenylethanoids and phenylpropanoids, accumulated and stored in the rhizomes and roots of the plant.

Aim: The study aimed to investigate the metabolic differentiations in rhizomes, roots and aerial parts from *R. rosea*. Furthermore, the rhizomes and its marker compounds were assessed for their effect on T cells activation and apoptosis.

Material and Methods: *R. rosea* plants were collected in October 2014 from Pirin National park, Bulgaria. The metabolites were identified by 1D and 2D NMR-based metabolomics and quantified by HPLC. To evaluate the apoptotic effect Jurkat cell line was used. Surface markers expression and phosphorylation of ERK were determined in CD3 T cells.

Results: The chemical structures of salidroside, rosarin, rosavin and rosin were identified according to their NMR spectra. These metabolites were absent in the aerial parts and presented in highest concentrations in the rhizomes (2.67%, 0.37%, 1.97% and 0.04%). The rhizomes extracts, rosavin and rosarin showed the strongest apoptotic activity towards Jurkat T cells. Only rosavin inhibited TNF-related apoptosis-inducing ligand (TRAIL) expression, while rosin had the opposite effect.

Conclusions: NMR-based metabolomics has been successfully applied to determine the phytochemical variations in different morphological parts of *R. rosea*. The effects of rosarin and rosavin on TRAIL expression could involve distinct action on ERK signaling and hence highlighted their potential to manipulate TRAIL as a tool to rescue the resistance to apoptosis in autoimmune diseases and cancer.

Acknowledgements: This study was supported by Program for career development of young scientists, Bulgarian Academy of Sciences, Project number DFNP-58.

Key words: *Golden root, salidroside, rosavins, NMR, T cells, TRAIL, ERK*

12. NANOTECHNOLOGY IN FOOD (NTF)

GREENER SYNTHESIS OF SILVER NANOPARTICLES USING *ROSA DAMASCENA* WASTES

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Introduction: Green methods for nanoparticles synthesis were widely used lately due to the milder conditions and eco-friendly procedures. Nevertheless few investigations rely on plant wastes. Their utilization will contribute for greener methods for metal nanoparticles synthesis and applications.

Aim: The aim of this work was to explore the possibility for utilization and valorization of rose flowers wastes as substrate for green synthesis of silver nanoparticles (AgNPs) and their possible application.

Material and Methods: Ten mL extract from wastes (EKOMAAT-2016) and 30 mL 10⁻²M AgNO₃ were mixed (final volume-50ml). Images were acquired with HR-TEM JEOL-JEM2100 (200kV). Diffraction patterns were collected (5.3÷80°2θ; constant step 0.02°2θ) on Bruker-D8 (Cu-Kα radiation, LynxEye detector). The electrode was from spectroscopic graphite (5.6mm, 25mm²-RWO, Germany) and was modified in AgNPs solution (2h, 25°C).

Results: Mixing the extract and AgNO₃ turned the initial yellowish color to brown. The AgNPs formation was confirmed by TEM and X-ray. The average AgNPs size was 25.8nm. AgNPs crystallize in a face centered cubic space group Fm-3m with unit cell parameter a=4.085(2)Å and mean coherent domain size 13.2(5) nm. The assembled electrode showed excellent potential for reduction of H₂O₂.

Conclusions: The present work showed the possibility to use wastes from rose oil industry as reduction agent in green synthesis of AgNPs with potential applications in different industries.

Acknowledgements (if needed): This work was supported by project 6/14-H “Investigation on the combined valorization of waste rose flowers” (University of Food Technologies-Plovdiv).

Key words: *silver nanoparticles; Rosa damascene; green synthesis; waste valorization; hydrogen peroxide reduction.*

14. RISK ASSESSMENT AND FOOD AUDIT (RAFO)

Food 3-80

SOIL AND PLANT CONTAMINATION BY PSEUDOMONAS AERUGINOSA IN THE REPUBLIC OF SERBIA

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Introduction: *Pseudomonas aeruginosa* is bacterium well known as hardly eliminated with different disinfectants, including antibiotics. Antibiotic resistance has traditionally been viewed as a clinical problem, but recently non-clinical environments have been highlighted as an important factor in the dissemination of antibiotic resistance genes. It is considered that a large number of antibiotic-resistant *P. aeruginosa* are continuously discharge into environment and circle through water, soil and plant and can have significant impact to human health.

Aim: The aim of this paper was to present the distribution of *P. aeruginosa* in agricultural land and vegetable in Serbia, and also to present the results of the antibiotic susceptibility of isolated *P. aeruginosa*.

Material and Methods: *Pseudomonas aeruginosa* isolates were collected during 2015 from several locations in Serbia. Isolation and identification was done following standard procedures. Antibiotic susceptibility was tested by Kirby-Bauer or disc diffusion test (Bauer et al., 1996) by using seven different antibiotics: Gentamicin, Neomycin, Erythromycin, Chloramphenicol, Tetracycline, Kanamycin and Ampicillin. According to size of inhibitory area around the disc, isolates were classified as: sensitive, intermediary or resistant.

Results: In this work it is isolated 24 *P. aeruginosa*, 17 from soil and 7 from vegetable. All isolates were resistant at, at least, one tested antibiotic. All tested isolates were sensitive to Gentamicin, about 85% were resistant to Ampicillin and Erythromycin, and 68% to Kanamycin. About 20% of isolates were resistant to Neomycin, Chloramphenicol and Tetracycline.

Conclusions: The results from this study suggest that agricultural land and vegetable contain antibiotic resistant isolates of opportunistic pathogen bacterium. Further, presented results support the opinion about the wide distribution of antibiotic resistant bacteria.

Acknowledgements: This research was partially supported by the Ministry of Education and Science of Republic of Serbia, grant No. TR 31080.

Key words: *Antibiotic susceptibility, Pseudomonas aeruginosa, soil, vegetable*

15. FREE TOPICS (FT)

Food 3-81

ENHANCED STRUCTURAL STABILITY OF OXIDIZED HEMOCYANIN

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Introduction: Hemocyanins (Hcs) are large glycoproteins present in the blood of some mollusks and arthropods, whose biological function is mainly related with the oxygen transport to the tissues. In addition, molluscan Hcs have shown promising properties in the development of various medicinal products including antiviral agents, conjugate vaccines and immunotherapy of cancer. Achieving structural stabilization in proteins having therapeutic application is an important task.

Aim: The aim of the present study is to enhance the structural stability of representatives of Hcs from molluscan species by chemical oxidation of their sugar moieties.

Material and Methods: Molluscan Hcs, used in this study, were purified from marine snails *Rapana thomasi* (RtH) and garden snails *Helix aspersa* maxima (HaH). Chemical oxidation with sodium periodate method was utilized to modify the proteins. Oxidized Hcs were analyzed by SDS-PAGE, proteolytic treatment and spectroscopic methods. Thermal stability of native and oxidized Hcs was evaluated by microcalorimetry.

Results: To stabilize the structure of investigated Hcs, their carbohydrates were oxidized with sodium periodate to generate Schiff bases between the free amines and the reactive aldehydes, formed by the oxidation procedure. SDS-PAGE analysis showed differences in the mobility pattern between the native and the periodate-treated Hcs. Trypsin was not able to digest oxidized Hcs. These differences were attributed to internal cross-linking within Hc molecules as a result of the periodate treatment. Study by microcalorimetry demonstrated an increased thermal stability of modified proteins.

Conclusions: This study reveals that the oxidation of sugar moieties in Hc molecules with sodium periodate leads to enhance of the structural and conformational stability of these oxygen-transport proteins.

Acknowledgements: This work was supported by research grant DFNP-152/12.05.2016 from the Program for career development of young scientists, BAS, Bulgaria.

Key words: *hemocyanin, mollusk, Rapana thomasiana, Helix aspersa maxima, periodate oxidation*

Food 3-82

MORPHOLOGICAL DIVERSITY OF SECRETORY STRUCTURES AMONGST PLANTS USED AS FOOD

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Introduction: The human interest towards edible plants is defined by his desire to know, use and grow them, as well as his necessity to be informed about the substances that they synthesise. Least known to us are the organization and diversity of the secretory structures, which are to be found mostly in leaves – the vegetative organs, used for the production of food.

Aim: The object of the current research is the different types of families with a notable place in everyday human activities.

Material and Methods: A research on five kinds of leaves was conducted.

Results: The histological organization of the leaf, as well as the morphological diversity of the inner and outer secretory structures, were anatomically observed for the aforementioned species. The results are documented on Olympus optical microscope and are illustrated with images. They have a substantial meaning for the quantitative as well as the qualitative characteristic of the produced secondary metabolites.

Keywords: *plants, secretory structures, food*

Food 3-83

OPTIMIZATION OF FACTORS ENHANCING THE PRODUCTION OF LIGNINOLYTIC ENZYMES BY WHITE ROT FUNGUS *TRAMETES TROGII*

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Introduction: Over the last few years, white rot fungi from Phylum *Basidiomycota* have proven to be quite promising in different aspects of biotechnological processes. In regard to the most important ligninolytic enzymes produced by these species, the possibilities for optimization of their synthesis are of particular importance.

Aim: The aim of this study was to optimize various factors which would increase the production of ligninolytic complex (laccase, lignin peroxidase, Mn-peroxidase) in the model strain. We examined the influence of the following parameters: quantity of seed discs of mycelium, submerged cultivation conditions (static and under agitation) and composition of the cultural media (PDM, ME and AN 3).

Materials and methods: The model fungal strain *Trametes trogii* was isolated from Bulgarian soils. All of the analyses were made after a treatment period of 4, 7 and 10 days. The biomass development and the activity of the main ligninolytic enzymes (laccase, Mn-peroxidase and lignin peroxidase) were determined after each treatment.

Results: The present work revealed that, in terms of ligninolytic enzymes production, *Tr. trogii* is most effective when cultivated under static conditions and using 3 plugs of 5 mm diameter of fresh mycelium. Out of the three media used, PDM appeared to be optimal, both for the growth of the strain and the activities of its ligninolytic enzymes.

Conclusions: The results would be used as a basis for further stimulation of the ligninolytic potential of *Trametes trogii* in a combination with other important factors.

Key words: *phylum Basidiomycota, white rot fungi, ligninolytic enzymes, enzymes activity optimization*

Acknowledgments: This work was supported by the Bulgarian Ministry of Education and Science and Bulgarian Academy of Sciences (grant № 301/18.02.2016), which is greatly acknowledged.

Food 3-84

ANTIMICROBIAL ACTIVITY OF THREE COMERCIAL ESSENTIAL OILS FROM *EUCALYPTUS*

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Introduction: Genus *Eucalyptus* includes plants that have been found to possess antimicrobial activity. Essential oils of these plants can slow the growth of pathogens microorganisms or completely destroy them, without harmful effects on the human body.

Aim: In this research we determined antimicrobial activity of essential oil of eucalyptus on several types of microorganisms.

Material and Methods: For this purpose we have used three commercially obtained essential oils (1-Robertet, 1999; 2-Mane, 2000; 3-Fitofarm, 2012) that are varied on date of manufacture. The examined cultures belong to the collection owned by the Department of Microbiology and Microbial Biotechnology, Faculty of Natural Sciences and Mathematics, Skopje. The following test microorganisms were used: gram positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538), gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027), yeasts (*Candida albicans* ATCC 10231, *Candida albicans* clinical isolate) and mold (*Aspergillus niger* ATCC 16404). The experiment was based on microdilution method. This method allows determination of the minimum inhibitory concentration of eucalyptus essential oils against various microorganisms.

Results: The results showed that all three essential oils possess antimicrobial activity against microorganisms and date of manufacture affects their activity. The first and second essential oil showed more effective against tested microorganisms, with MICs between 0.078-1.25%, while the third essential oil (the newest) show lowest antimicrobial activity against all tested microorganisms.

Conclusions: Lowest antimicrobial activity against the tested microorganisms possess the essential oil with the newest date of manufacturing. This is because the active components in the essential oils after extraction need to stay in a dry and dark place for some period to become more active.

Keywords: *Eucalyptus*, essential oil, microdilution method, minimal inhibitory concentration, antimicrobial activity.

Food 3-85

IN VITRO INVESTIGATION OF ANTIOXIDANT PROPERTIES OF CANCER PAGURUS HEMOCYANIN

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Introduction: Hemocyanins are copper-containing proteins in the hemolymph of many arthropods and mollusks, whose biological function is mainly related with the oxygen transport to the tissues. As a component of the marine food, that traditionally is associated with a healthy diet, they are an interesting object for examination of their biological activity and pharmacological potential.

Aim: In the present study, the hemocyanin from marine crab *Cancer pagurus* (CpH) was isolated and purified, and its antioxidant properties were investigated in prooxidant systems in vitro.

Material and Methods: Three radicals were used – superoxide, hydroxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH). The protection by CpH against oxidative damages of two model lipid membranes - rat liver supernatant and liposomal suspension in conditions of iron-induced lipid peroxidation was tested.

Results: CpH exhibited good DPPH and hydroxyl radicals scavenging activities in a concentration-dependent manner and a lower ability to capture superoxide radicals at

physiological value of pH. CpH showed very good capacity to inhibit Fe²⁺- induced lipid peroxidation in applied systems, and chelating activity toward iron ions.

Conclusions: This study reveals that CpH has the ability to act as iron chelating protein, as well it could provide protection against oxidative stress and in this manner to decline the risk of destruction of biomolecules, caused by initiation of harmful free radicals - mediated chain reactions.

Key words: *hemocyanin, antioxidant activity, Cancer pagurus*

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