2nd National Food Conference

with International Participation

Sofia, March 20th-21st, 2015

New Bulgarian University

Program

CONGRESS ORGANIZERS

NEW BULGARIAN UNIVERSITY

DEPT. NATURAL SCIENCES, BIOLABORATORY

BULGARIAN SOCIETY FOR MICROBIOLOGY (BSM)

BULGARIAN FOOD SAFETY AGENCY

THE STEPHAN ANGELOFF INSTITUTE OF MICROBIOLOGY, BULGARIAN ACADEMY OF SCIENCES

Under the auspices of The Rector of New Bulgarian University and The Central Fund for Strategic Development

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2nd National Food Conference

with International Participation

Sofia

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Organizing Committee

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Conference Sections

FOOD QUALITY (FQ)
FOOD BIOTECHNOLOGY (FB)
FOOD SAFETY (FS)
MICROBIOLOGICAL CONTROL OF FOOD (MCF)
PREBIOTICS AND PROBIOTICS (PP)
REPORTS ON CORRESPONDING TOPICS (VARIA, V)

Social Events

Friday, March 20th-21st, 2015

Conference Cocktail

19:30 RESTAURANT ARTES, NBU

Friday, March 20th, 2015

Registration and Coffee
08:00 – 09:00
Lobby in front of AULA, NBU

Opening Ceremony
09:00 – 09:30
AULA

Welcome address of the Rector of New Bulgarian University – Assoc. Prof. Dr. Plamen Bochkov

Welcome address of the Executive Director of Bulgarian Food Safety Agency - Prof. Dr. Plamen Mollov

Celebration of the 110th Anniversary of *Lactobacillus bulgaricus* Discovery by Dr. Stamen Grigorov
09:30-10:00

Friday, March 20th, 2015
AULA NBU

Session 1: Plenary Lectures
Chair(s): Angel S. Galabov, Galina Satchanska

10:00 V1 **Applications of Nanotechnology and Nanomicrobiology in Food Safety and Health Science**
Vaso Taleski[1], Darko Bosnjakovski[1], Milka Zdravkovska[1], Ilija Nasov[2], Anka Trajkovska-Petkoska[3]
[1]University „Goce Delchev", Faculty of Medical Sciences, Sthip, [2]Center for Plasma Technologies, Skopje, [3]University „St.Climent Ohridski", Bitola, Faculty of Technology and Technical Sciences, Veles, Macedonia.

10:20 V2 **New Strategies and Antibiotics against bad bugs**
Encho Savov
Dept of Military Epidemiology and Hygiene, Laboratory of Microbiology,
Military Medical Academy, Sofia, Bulgaria

10:40 FB1 **Metabolomics: from Basic Concepts to Valuable Applications in Natural Products Research and Biotechnology**
Milen I. Georgiev
Institute of Microbiology, Bulgarian Academy of Sciences

11:00 PP1 **Antibacterial Activity and Probiotic Advantages of Proviotic®**
Kiril Petkov4, Rositsa Tropcheva[1], Svetla Danova[2], Svetlana Saeva-Kondratenko[3], Petko Petkov[4]
[1]Department of Biotechnology, Faculty of Biology, Sofia University St. Kliment Ohridski, [2]The Stephan Angeloff Institute of Microbiology Bulgarian Acad. Sci., [3]Genesis Laboratories LTD, [4]Proviotic LTD,

Round Table
"Education and Career"
With Participants of Business Sector
11:30 – 12:00
Moderator: Dr. Tencho Tenev, BABH

Lunch Break
12:00-13:00

Session 2: Food Quality, Food Safety
Chair(s): Encho Savov, Vaso Taleski

13:00 FQ4 **Genetic Correlation and Cattle Milk Traits**
Hristov P., Neov B., Teofanova D. and G. Radoslavov
Institute of Biodiversity and Ecosystem Research-BAS

13:10 FQ1 **Antioxidant and Antimicrobial Activities from Different Extracts of *in vivo* and *in vitro* Cultivated *Lamium album* L. Plants**
Dimitrova M.[1], Chipeva V.[2], Petrova D.[1], Moncheva P.[2], Dragolova D.[1], Mantovska D.[1], Kapchina-Toteva V.[1]
[1]Department of Plant Physiology, Faculty of Biology, Sofia University "St. Kl. Ohridski, [2]Department of General and Industrial Microbiology, Faculty of Biology, Sofia University "St. Kl. Ohridski

13:20 FS1 **Migration of Melamine and Formaldehyde from the Market Melamine Products**
Svetla Chavdarova, Tania Barakova, Terry Vrabcheva
National Center of Public Health and Analyses Bulgaria

13:30 FS5 **Summary Results of Notified Cases in the Rapid Alert System for Food and Feed Regarding Food Contact Materials in 2013 – 2014**
Snezhana Todorova
Risk Assessment Center, Bulgarian Food Safety Agency

13:40 FS2 **Review of Regulatory Requirements for the Safety Monitoring of Food Supplements, Containing Herbal Extracts**
Svetoslav Stoev, Hristina Lebanova, Ilko Getov
Faculty of Pharmacy, Medical University Sofia, Medical University Pleven

13:50 FS3 **National Monitoring Program for Control of Residues and Retrospective Analysis of Control of Residues of Banned Veterinary Medicinal Products (VMP)**

T. Yankovska, R. Dimitrov, A. Damyanova, N. Stoilova, M. Peycheva, D. Gospodinova, S. Florova
Central Laboratory of VeterinaryControlandEcology (CLVCE), BFSA, Sofia, Bulgaria;

14:00 FS4 **Rapid Method for Determination of N-Methyl Carbamates In Liver Using Quechers Methodology**

I.Stoykova[1,2], T. Yankovska-Stafnova[1], L.Yotova[2] , D. Danalev[2]
[1]Central Laboratory of Veterinary Control and Ecology, Bulgarian Food Safety Agency, [2]Department of Biotechnology, University of Chemical Technology and Metallurgy

14:10 MCF2 **Regulation (Ec) № 2073/2005 on the Microbiological Criteria for Foodstuffs**

Maya Makaveeva
Director of Laboratory Activities and Quality Control Directorate Bulgarian Food Safety Agency

14:20 MCF1 **Presence of *Escherichia coli* in Black Sea Mussels**

Gergana Krumova-Valcheva, Eva Gyirova, Yordan Gogov
National Diagnostic and Research Veterinary Institute

Session 3: Prebiotics and Probiotics, Varia

Chair(s): Milen Georgiev, Svetla Danova

14:30 PP2 **Bulgarian Lactobacilli - Promissing Bioprotective and Probiotic Agents**

Svetla Danova
Department of General Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Science

14:40 PP3 **Probiotics for Gut Health**

Katelina Alexandrova, Elena Karaivanova, Nevena Rumian, Plamen Andreev
Kendy LTD

14:50 V3 **Use of the Descriptor Fingerprints for Discrimination between Allergen and Non Allergen Food Proteins**

Ivan Bangov[1], Irini Doychinova[2], Ivan Dimitrov[2]
[1]Konstantin Preslavski Shumen University, Faculty of Natural Sciences, [2]Medical University of Sofia, Faculty of Pharmacy

15:00 V4 **The Opportunities of Small Non-Market Farms to Provide Fresh Fruit**

Antoniy Stoev
Institute of Soil Science, Agrotechnologies and Plant Protection “Nikola Pushkarov”

15:10 V6 **Latest Developments in Biotechnology & Biotechnological Equipment in the Context of International Academic Publishing Practices**

Evgeniya Dimova, Snejana Pavlova
Diagnosis Press EOOD

Coffee Break
15:30-16:00

16:00 V5 **Assess the Level and Structure of Consumed Food Products by Bulgarian Households**

Minka Anastasova[1], Dimitre Nikolov[1], Ivan Boevsky[2]
[1]Institute of Agricultural Economics (IAE), [2]Dept. Business Administration, New Bulgarian University

16:10 FS6 **New Aspects for Labelling of Foodstuffs in Connection with the Entry into Force of Regulation № 1169/2011 on the Provision of Food Information to Consumers**

Rosen Malchev, Stoeva Kr., Rubenova M.
Bulgarian Food Safety Agency

16:20 V10 **Migrating Birds as Carriers of Zoonotic Pathogens**

Najdenski H.[1], Dimova T.[1], Draganova T.[1], Zaharieva M.M.[1], Tsvetkova I.[1], Nikolov B.[2], Petrova-Dinkova G.[2], Dalakchieva S.[2], Popov K.[2], Hristova-Nikolova I.[2], Zehtindjiev P.[2], Peev S.[2], Trifonova A.[3], Martin L.[4], Carniel E.[4], Panferova Y.[5], Tokarevich N.[5]
[1]The Stephan Angeloff Institute for Microbiology, Bulgarian Academy of Sciences, [2] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences,
[3] National Research Station of Game Management, Biology and Pathology Sofia, [4] Institut Pasteur Paris, [5] Pasteur Institute, Saint Petersburg, Russia

16:30 V7 **Protective Activity of *Clinopodium vulgare* L. Leaves on *Saccharomyces Cerevisiae* Nuclear DNA**

Teodora Todorova[1], Daniela Miteva[1], Margarita Pesheva[2], Stephka Chankova[1]
[1]Institute of Biodiversity and Ecosystem Research Bulgarian Academy of Sciences, [2]Sofia University "St. Kliment Ohridski", Faculty of Biology

16:40 V8 **Comparison of Stress Response of Two Closely Related Genotypes to Drought**

Tsveta Angelova[1], Petya Parvanova[1], Zhana Mitrovska[1], Daniela Miteva[1], Diana Svetleva[2], Nadezhda Yurina[3], Stephka Chankova[1]
[1]Institute of Biodiversity and Ecosystem Research BAS Sofia, [2]Agricultural University Plovdiv, [3]A. N. Bach Institute of Biochemistry RAS Moscow

16:50 V9 **Application of Hyperspectral ReflectanceTechnique for Early Detection of Disease on Patatoes**

Dora Krezhova[1], Svetla Maneva[2]
[1]Space Research and Technology Institute
[2]Institute of soil science, agrotechnology and plant protection

17:00 V28 **Analitycal Revew of the price fluctuations of the basic food products**

Vanja Hadjieva
New Bulgarian University

Poster Session
Chair(s): Ekaterina Krumova, Galina Satchanska, Daniela Pilarska
17:30 – 18:30, HALL 310

Awards for Best Poster of Young Scientist
18:30 – 19:00

Cocktail
Restaurant ARTES
19:30

Saturday, March 21st, 2015

Workshop
Biolaboratory, C1/04, NBU

09:00 **ELTA '90M – Methods for GMO assessment in food**
1h

Coffee Break
10:00 – 10:30

10:30 **Bio-Rad - Fluorescent analysis of bacterial cells isolated from food *via* automatic system**
1h

11:30 **DNA visualization of the food pathogens *via* GEL Doc EZ system**
1h

11:30 **Progene - Detection of *Campylobacter jejuni* in food using Real-Time PCR**

ABSTRACTS

Plenary Lectures

V1

APPLICATIONS OF NANOTECHNOLOGY AND NANOMICROBIOLOGY IN FOOD SAFETY AND HEALTH SCIENCE

Vaso Taleski[1], Darko Bosnjakovski[1], Milka Zdravkovska[1], Ilija Nasov[2], Anka Trajkovska-Petkoska[3]

[1]University„GoceDelchev", Faculty of Medical Sciences, Sthip
[2]Center for Plasma Technologies, Skopje
[3]University „St. Climent Ohridski", Bitola, Faculty of Technology and Technical Sciences, Veles, Macedonia.

Nanomaterials are chemically or physically different to their macro-scale counterparts (due higher surface/volume ratio, colour, solubility, diffusivity, material strength, toxicity, thermodynamic, magnetic, optical and other properties) and may have unique health and environmental impacts. Extremelly small size (1-100 nm) enables nanoparticles to enter the human body through usual or unusual routes, pass through cell membranes, or cross the blood-brain barrier. Alimentary exposure is considered most typical food safety concerns.

At present nanotechnology is being used in a range of applications, with potential to be applied at any stage in food industry: food production, processing, packaging, labelling, transporting, tracing, keeping the quality of food product and extend the product shelf-life, leadingto less food waste, water filtration, removal of undesirable tastes, flavours or allergens from food products, nano(bio)sensors for food safety.

Due to the low infectious doses, based on nano(bio)senzors, rapid and sensitive detection methods are developed for food-borne pathogens *E. coli* strains, especially *E. coli* O157:H7, *S. aureus, S. typhimurium, C. jejuni, E. cloacae, B. subtilis*, *L. monocytogenes* and yeast (*Saccharomyces cerevisiae*). Also, sensors to detect biofilm formation by bacteria on surfaces are developing.

Food preservation, detection and inhibition of bacterial biofilm formations are fast developing area based on advantage of metal nanomaterials that possess unique antimicrobial activities (such as silver, gold, copper, titanium, zink, magnesium, cadmium, alumina).

Multy drug resistant microorganisms (MDR) present one of the most serious and increasing global, public health threat. New strategies to combat MDR are urgently needed and nanomaterials are very promising approach.

Risks of nanotechnology are still unknown and unpredictable. Initial scientific studies showed negative effects on living organisms and a potential for serious threat to human health.

Authorities of the most developed countries, have set up, updating, guidance documentsand procedures for nanoenabled products based on existing regulations, appropriate *in vitro* and *in vivo* ADME studies (absorbtion, distribution, metabolism and excretion) and requirements for standardised and harmonised analytical test methods for proper risk assesments, clear identificationand characterization of nano-hazards.

V2

NEW STRATEGIES AND ANTIBIOTICS AGAINST BAD BUGS

Encho Savov

Dept of Military Epidemiology and Hygiene, Laboratory of Microbiology,
Military Medical Academy, Sofia, Bulgaria

Bacterial resistance to antibiotics is growing up day by day in both community and hospital setting, with a significant impact on the mortality and morbidity rates and the financial burden that is associated. / Baseti,M. et al. 2013/. Especially attention deserve so called multidrug resistant /MDR/ or pandrug-resistant bacteria like *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus (*MRSA/, Multi-drug resistant extended-spectrum beta-lactamases /ESBL/ and carbapenemases producing *Klebsiella* species and *Escherichia coli* and others. However, despite this very bad scenario, since 2000 only three new classes of antibiotics have been introduced to the market for human use. Antibiotic use in any setting, including animal husbandry, promotes the development of resistance, as well. In this sense, four core actions, proposed by CDC, need to be taken to prevent antimicrobial resistance were emphasized together with new antibiotic stewardship program and the program 10x20, proposed and developed by Infectious Disease Society of America / IDSA / were estimated as very important measures for infection control prevention and antibiotic use improving in the human medicine.

Keywords: multidrug-resistant bacteria, antibiotic stewardship programs, treatment and cooperation against resistant bacteria

FB1

METABOLOMICS: FROM BASIC CONCEPTS TO VALUABLE APPLICATIONS IN NATURAL PRODUCTS RESEARCH AND BIOTECHNOLOGY

Milen I. Georgiev

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139 Ruski Blvd., 4000 Plovdiv, Bulgaria, e-mail: .gbg.bg

Per definition metabolomics represents a comprehensive holistic approach, defined as 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 term 'metabolome' has been introduced, several platforms and techniques for high throughput analyses of targeted metabolites have been developed (mainly mass spectrometry and nuclear magnetic resonance spectroscopy). 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 ^{1}H NMR-spectrometry over the other analytical platforms (MS in particular) is the possibility for (relative) 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, 3].

Some case studies of the application of NMR-based metabolomics concept in natural products research, biotechnology and lead finding [3-6] will be presented and thoroughly discussed.

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PP1

ANTIBACTERIAL ACTIVITY AND PROBIOTIC ADVANTAGES OF PROVIOTIC®

Rositsa Tropcheva[1], Svetla Danova[2], Svetlana Saeva-Kondratenko[3], Petko Petkov[4], Kiril Petkov[4]

[1]Department of Biotechnology, Faculty of Biology, Sofia University St. Kliment Ohridski, 8, Dragan Tsankov Blvd., 1164, Sofia, Bulgaria.
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[3]Genesis Laboratories LTD, Sofia, Bulgaria.
[4]Proviotic LTD, Sofia, Bulgaria.

Object of Research: Probiotics are health promoting bacteria which, when consumed in adequate amounts, confer a benefit on the host. The great public necessity of such products, determines the intensive developments. Proviotic® is a probiotic, containing the strain *Lactobacillus bulgaricus* GLB44 (property of Genesis Laboratories LTD). GLB44 was isolated from the leaves of *Galanthus nivalis* in Bulgaria, becoming the only known strain of this subspecies that has vegan origin available as a commercial probiotic. GLB44 is grown in vegetable juice, therefore Proviotic® does not contain any of the seven major allergens for which the U.S. Food and Drug Administration requires additional labeling. This is especially important for the lactose and gluten sensitive population. The antibacterial activity of probiotics is among their essential features and with this aim, the inhibitory activity of GLB44 was studied.
Materials and Methods: *Lactobacillus bulgaricus* GLB44 was screened for antibacterial activity against *(i)* different pathogenic species – *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Acinetobacter baumanii*, *Enterococcus faecalis*, *Psuedomonas aeruginosa* and *(ii)* 16 lactic acid bacteria strains and 2 commercially available probiotics using plate counting. All counts were recorded as CFU/mL.
Results: The study has revealed Proviotic®'s strength and effectiveness against bacterial pathogens. *L. bulgaricus* GLB44 demonstrated 100% inhibition of 48h pathogenic levels at 37° C of *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Acinetobacter baumanii*, *Psuedomonas aeruginosa* and 99.86% inhibition of *Enterococcus faecalis*. With regard to the lactic acid bacteria strains and commercial probiotics, no inhibitory activity of Proviotic® was observed.
Conclusions: The study confirms that GLB44 is a specific strain of the *L. bulgaricus* and that its inhibitory power against pathogens surpasses other *L. bulgaricus* strains. GLB44 is an example of an effective probiotic with suitable scientific substantiation of health benefits.

Oral Presentations

FOOD QUALITY

FQ1

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES FROM DIFFERENT EXTRACTS OF IN VIVO AND IN VITRO CULTIVATED *LAMIUM ALBUM L.* PLANTS

Dimitrova M.[1], Chipeva V.[2], Petrova D.[1], Moncheva P.[2], Dragolova D.[1], Mantovska D.[1], Kapchina-Toteva V.[1]

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[2]Department of General and Industrial Microbiology, Faculty of Biology, Sofia University "St. Kl. Ohridski, 8 Dragan Tsankov

Object of Research: *Lamium album L.* (white dead nettle) is valuable medicinal plant which possesses astringent, antispasmodic, anti-inflammatory, antibiotic and bacteriostatic properties.
Materials and Methods: In the present work antioxidant and antimicrobial activities from different extracts (chloroform, methanol, ethanol and water) obtained by thermostat and Soxhlet extractions of *in vivo* and *in vitro* cultivated *L. album L.* was performed. Three different assays (DPPH, ABTS and total antioxidant activity) for screening of antioxidant capacity were applied. Antimicrobial activity analysis was carried out using the well diffusion method.
Results: Methanol extracts, obtained from *in vivoL. album L.* plants demonstrated the highest DPPH- and ABTS - scavenging potentials. All used extracts demonstrated antibacterial activity on the tested bacteria strains (*Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Proteus hauseri, Pseudomonas aeruginosa, Salmonella enterica, Staphylococcus aureus, Staphylococcus epide rmidis*) with minimum inhibitory concentration (MIC) ranging from 10 to 0.313 mg.ml-1. The Gram-positive bacteria were more sensitive than the Gram-negative bacteria. Only the water extracts from in vivo plants possessed antifungal activity against *Candida albicans* and *Candida glabrata*.
Conclusions: The methanol and ethanol extracts obtained from the *in vitro* propagated plants had a broader spectrum of antibacterial activity than those from *in vivo* plants, while the opposite tendency was observed for the chloroform extracts. The obtained results showed that *L. album L.* could be of interest for biotechnology since it can be used as a natural source of antimicrobial and antioxidant substances.

FQ3

GENETIC CORRELATION AND CATTLE MILK TRAITS

Hristov P., Neov B., Teofanova D. and G. Radoslavov

Institute of Biodiversity and Ecosystem Research-BAS

Object of Research: The present study aimed to compare the size of casein micelle in cow milk sample in function of kappa casein (CSN3) genetic polymorphism.
Materials and Methods: Sixteen cows from Bulgarian Rhodopean cattle breed were genotyped by PCR-RFLP analysis. Milk samples from the three found CSN3 genotypes (AB, AA and BB) were employed for the determination of casein micelles size by Dynamic Light Scattering (DLS).
Results: The results showed differences in the size and polydispersity of the casein micelles between the milks of cows with different genotypes. Hydrodynamic radii of micelles at a scattering angle of 90 °C varied from 80 to 120 nm and polydispersity varied from 0.15 to 0.37. In conclusion casein micelle size of CSN3 AA cows (~ 120 nm) exceed with about 60% cows with AB (~ 80 nm) and BB genotype (~ 70 nm).
Conclusions: This study reveals for the first time the correlation between κ-CN genotypes and casein micelle size in individual milk samples. CSN3 AB genotype showing distinct variations of micelle size. DLS data suggest that there is a correspondence with CSN3 genotype e.g. AA genotype shows bigger size of casein micelle. In contrast, protein and fat content in milk cannot be correlated to casein micelles size.

FOOD SAFETY

FS1

MIGRATION OF MELAMINE AND FORMALDEHYDE FROM THE MARKET MELAMINE PRODUCTS

Svetla Chavdarova, Tania Barakova, Terry Vrabcheva

National Center of Public Health and Analyses
Bulgaria, 1431 Sofia, 15, Akad. Ivan Geshov blvd.

Object of Research: Melamine formaldehyde resins are used in the manufacturing of kitchen products that come into contact with food. Melamine is heat-resistant and that allows it to be used for storage of hot food or in microwave ovens.
As a result of technological problems, and when heated, there is a possibility of migration of melamine and formaldehyde in the food. Our preliminary studies in 2013 showed migration of melamine in 69% of samples analyzed. In a subsequent study in 2014 on children's melamine products melamine was found in 33% of samples, and there was no evidence of formaldehyde migration.
The aim of this study was to examine the quality of the China-imported melamine kitchen products for migration of melamine and formaldehyde again, in compliance with the provisions of Regulation № 284/2011.
Materials and Methods: 19 units of kitchen products were purchased from the market, a total of 55 samples. Test conditions for some of the units were consistent with the guidance of the European reference laboratory. The other products were treated at the recommended temperature and time (2 hours at 70°C). 3% aqueous acetic acid solution was used as a simulant for all samples. Formaldehyde was determined spectrophotometrically, and melamine – with high performance liquid chromatography.
Results: Migration of melamine above the limit (2,5 mg/kg) was found in 29% of the samples (16 samples), and migration of formaldehyde above the limit (15 mg/kg) – in 27% (15 samples). The maximum level of migration of melamine reached 6,49 mg/kg, and for formaldehyde - 66,64 mg/kg.
Conclusions: It is clear from the study that the import of poor quality melamine kitchen tableware continues to be a problem for the Bulgarian market.

FS2

REVIEW OF REGULATORY REQUIREMENTS FOR THE SAFETY MONITORING OF FOOD SUPPLEMENTS, CONTAINING HERBAL EXTRACTS

Svetoslav Stoev, Hristina Lebanova, Ilko Getov

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Object of Research: The study aims to investigate the current approaches, methods and good practices for safety monitoring of food supplements, containing herbal extracts. We plan to analyze the legislative requirements, current problems and future challenges for safety monitoring of herbal products.

Materials and Methods: A literature review of good safety surveillance practices for herbal supplements in Bulgaria, EU and US, and a systematic review of current legislation requirements for collection, systematization and analysis of data collected for adverse events of food additives, containing herbal extracts were performed.

Results: The problems of the existing safety monitoring system are associated with the specifications of plant products and food additives: their different regulatory status in the country of origin and the different requirements for them; complex and heterogeneous in chemical composition; problems with the classification and identification of the botanical origin of the plants; tendency of growing number of patients who use herbal products and consume energy drinks; very low rate of reporting of side effects and adverse events. Among the challenges for the system of safety monitoring of medicines and food supplements containing plant extracts are solving the problems associated with the nomenclature used in herbal substances, ensuring quality and control; issues related to consumers; issues related to the identification of side effects and adverse events.

Conclusions: The growing use of energy drinks and various food supplements will inevitably lead to the need for brief and strictly regulated monitoring methodology for safety surveillance of plant products and risk reducing measures, regardless of their status as medicinal products or food supplements.

FS3

NATIONAL MONITORING PROGRAM FOR CONTROL OF RESIDUES AND RETROSPECTIVE ANALYSIS OF CONTROL OF RESIDUES OF BANNED VETERINARY MEDICINAL PRODUCTS (VMP)

T. Yankovska, R. Dimitrov, A. Damyanova, N. Stoilova, M. Peycheva, D. Gospodinova, S. Florova

Central Laboratory of Veterinary Control and Ecology (CLVCE), BFSA, Sofia, Bulgaria; . @clvce.eu

One of the basic programs to implement the policy in the field of agriculture is "Safety of the Food Chain". Main priority of CLVCE is implementing the National monitoring program for control of residues of veterinary medicine sand environmental contaminants (NMPCR) in live animals and food stuffs of animal origin. The program is realized in compliance with the requirements of Council Directive 96/23/EC, which was introduced in Bulgarian legislation through Ordinance № 119 (SG. 6 of 2007) on measures to monitor certain substances and residues in live animals, raw materials and food stuffs of animal origin intended for human consumption. NMPCR is carried out in order to establish an efficient and uniform system for monitoring of the use of illegal substances, monitoring a number of residues of pharmacologically active substances and environmental contaminants, review and disclosure of there as ons for the presence of these residues in the raw materials and products from animal origin. Restrictions on the use of substances with hormonal, anabolic or thyrostatic action and beta-agonists in food producing animals are introduced in Bulgaria by Ordinance № 14 of May 8, 2009 (SG. 37 19 May 2009), in fulfillment of the requirements of Council Directive (EC) 96/22/EC, Directive 2003/74/EC of the European Parliament and of the Council and Directive 2008/97/EC of the European Parliament and the Council. Other prohibited substances are listed in Annex 2 of Regulation (EC) № 37/2010 of the Commission. To implement the Directives in CLVCE in the last 15 years have been developed, validated and revalidated appreciated number of methods for determining residues of prohibited VMP in biological fluids, raw materials and food stuffs of animal origin. The validation strictly applies to the requirements of Commission Decision № 2002/657/EC. For the analysis ELISA, gas chromatography with mass spectrometry (GC/MS/MS) and liquid chromatography coupled with mass spectrometry (LC-MS/MS) are used. By Order of the Minister of Agriculture and Food CLVCE is defined as National Reference Laboratory (NRL) for these groups of substances and is specialized as the only one Laboratory in Bulgaria fulfilling NMPCR.

Key words: monitoring, residues, banned VMP, methods for analyses

FS4

RAPID METHOD FOR DETERMINATION OF N-METHYL CARBAMATES IN LIVER USING QUECHERS METHODOLOGY

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Object of Research: N-Methylcarbamates insecticides are widely used in homes, gardens, and agriculture. They inhibit cholinesterase enzymes and therefore share similar symptomatology as organophosphates during acute and chronic exposures. Unfortunately, traces of them can further fall into animal tissues, milk, honey, eggs, etc. Therefore, food safety is an integral part of the EU policy for protection of consumer's health and maximum residue levels for pesticides are defined in specific Regulations.
Materials and Methods: Analysis of pesticide residues in foods from animal origin includes many different methods for extraction and clean-up steps, followed by a final measurement analysis, typically with chromatography equipment. Extraction of pesticides in liver samples is much more complicated than the other animal origin samples, because of the phospholipid content. A Quick Easy Cheap Effective Rugged and Safe (QuEchERS) sample preparation was used for the determination of 4 N-methyl carbametes residues in samples of liver - Aldicarb, Methomyl, Propoxur and Carbofuran. The original method involved extraction with acetonitrile, liquid–liquid partition with addition of $MgSO_4$ and NaCl followed by dispersive SPE cleanup with PSA sorbent and the analyses were carried out with a HPLC-Fl equipment.
Results: The method was validated using liver samples, spiked at 50%, 100% and 150% from the Maximum residue levels for every compound and the average recovery by the method varied from 75.6% to 101.4% with RSDs < 18%. The method showed good linearity and the LODs for the pesticides studied ranged from 0.0007 to 0.01 mg/kg.
Conclusions: A modified QuEchERS extraction was developed and followed by selective analysis using a high performance liquid chromatography with fluorescence detector for the quantification of 4 N-methyl carbamates. This method was successfully validated for liver samples. The presence of target pesticides was found in the range of 0.002 to 0,05 mg/kg.

Key words: QuEchERS, pesticides, N-methyl carbamates, HPLC

FS5

SUMMARY RESULTS OF NOTIFIED CASES IN THE RAPID ALERT SYSTEM FOR FOOD AND FEED REGARDING FOOD CONTACT MATERIALS IN 2013 – 2014

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Object of Research: The European Union (EU) has one of the highest food safety standards in the world - largely thanks to the solid set of EU legislation in place, which ensures that food is safe for consumers. A key tool to ensure the cross-border follow of information to swiftly react when risks to public health are detected in the food chain is RASFF – the Rapid Alert System for Food and Feed.

The Rapid Alert System for Food and Feed, maintained by the European Commission and including the member-states and the European Food Safety Authority has been established according to Regulation (EU) №178/2002. Its main purpose is to provide the competent authorities for food and feed control with effective information exchange regarding risks and on the measures taken for their management.

Materials and Methods: The RASFF portal features an interactive searchable online RASFF database. It gives public access to summary information about the most recently transmitted RASFF notifications as well as search for information on any notification issued in the past.

For this work the Risk Assessment Center has been used online RASFF database for food contact materials:

https://webgate.ec.europa.eu/rasffwindow/portal/index.cfm?event=searchForm&cleanSearch=1

Results: This paper analyses data from RASFF notifications concerning food contact materials for the time period 2013 – 2014. It is found out that the highest number of notifications submitted is regarding migration of heavy metals, phthalates, formaldehyde, primary aromatic amines. The total number of notifications is 402.

Conclusions: Base on this research can draw the following conclusions: over the years a significant number of notifications in respect of the overall migration; a significant number of notifications for metals (chromium, manganese, nickel, etc.); melamine and formaldehyde.

FS6

NEW ASPECTS FOR LABELLING OF FOODSTUFFS IN CONNECTION WITH THE ENTRY INTO FORCE OF REGULATION № 1169/2011 ON THE PROVISION OF FOOD INFORMATION TO CONSUMERS

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Free movement of safe and whole some food is an essential aspect of the common European market and considerably contributes for health and welfare of citizens and their social and economic interests. In order to achieve high level of protection of consumer‘s health and guaranteeing the irright for information, they should be properly informed for the food stuffs they consume. For reaching the objectives for awareness, besides requirements for general labeling several specific requirements are being adopted applicable for all food stuffs under certain circumstances or for some categories of food stuffs. For that reason part of the acting legislative acts were repealed and amended with single Regulation to guarantee security of consumer sand other stakeholders and to reduce the administrative burden. Regulation (EC) № 1169/2011 serves for the interest of the internal market simplifying the legislation as well as for the interest of citizens envisaging distinct, clear and readable labelling of foodstuffs. The Regulation prohibits the use of misleading for the consumer information, especially regarding the characteristics of the food, its influence or features or information transferring medicinal characteristic of the food. In order to be effective, the prohibition should be implemented regarding advertisement and presenting of food as well. The Regulation introduces several new aspects in the requirements for labelling of food stuffs: The responsibilities of business operators for presenting information for foods tuffs; more severe requirements for objective information for the consumers; distinctive indication on the background of the label of substances provoking allergies and intolerances; criteria for legibility, size of the print and contrast of the background; presenting of nutritive information; distance selling, etc. In order food business operators to adapt labelling of their products to the new requirements, introduced with the Regulation, relevant derogation periods are envisaged regarding its implementation. In order to enforce observing the requirements of Regulation (EC) № 1169/2011, Member States will exercise official control in conformity with Regulation № 882/2004 on official controls.

MICROBIOLOGICAL CONTROL OF FOOD

MCF1

PRESENCE OF *ESCHERICHIA COLI* IN BLACK SEA MUSSELS

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Object of Research: The mussels are filter-feeding organisms. Their feeding is associated adoption of a huge amount of sea water, which increases the risk of ingesting any pathogens. For this reason, the European Community include in its legislation more stringent measures for the admission of live mussels on the market. Regulation (EO) № 2073/2005 determined rate of 230 MPN *E. coli* per 100 g of meat and intravalvular liquid from mussels. In 2002, the Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) concluded that should be carefully reference to samples with high levels of *E. coli* and considering them as dangerous to the health of consumers. This requires systematic monitoring of bacterial contaminants in live molluscs.

Materials and Methods: Studies have been performed on 38 samples of live mussels (*Mytillus galloprovincialis*) originating from Bulgarian Black Sea. Studies carried out conditional in two seasons: warm (May to September) and cold (October to April). To determine the most probable number (MPN) of *E. coli* per 100 g of meat and intravalvular liquid has been used ISO/TS 16649-3: 2003.

Results: It has been found that the amount of *E. coli* during the cold period ranging from less than 2 x 10^1 MPN/100 g to 1,1 x 10^5 MPN/100 g. During the warm sea son have been detected values of less than 2 x 10^1 MPN/100 g to 9,2 x 10^6 MPN/100 g. In 88% of the samples tested in the warm season, the amount of *E. coli* (MPN/100 g) exceeded the limit values, laid down in Regulation (EO) №2073/2005. Studied during the cold season samples demonstrate discrepancies only in 19%.

Conclusions: The highest levels of contamination have been the live mussels tested in the warm season - in 15 of 17 test samples. Significantly better the hygienic status of live molluscs during the cold period - 4 out of 21 samples. These results demonstrate that contamination of shellfish is related to higher faecal contamination of the sea in the summer.

MCF2

REGULATION (EC) № 2073/2005 ON THE MICROBIOLOGICAL CRITERIA FOR FOODSTUFFS

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Object of Research: Regulation (EO) № 2073/2005, concerning microbiological criteria for foodstuffs, is the first of its kind normative document in the field of microbiological norming of foods. The Regulation gives not only the microbiological criteria, the standarts for safety and the evaluation of production hygiene, but is also a document defining the basic concepts in this field, the terms and definitions and the general methodology of the microbiological control.

Materials and Methods: The safety criteria are monitored by the Control Authorities and the deviation from these criteria, i.e. proof of pathogenic microorganisms is a direct indication of food unfit for human consumption.

The criteria for processes hygiene are of particular importance for business operators in the production process, as they apply in the various steps in the food chain.

As a rule, food safety is ensured by implementing preventive measures such as Good Hygiene Practices and HACCP. The application of microbiological criteria is inseparable from the implementation of HACCP procedures and other measures for hygiene control.

Results: This paper provides information on safety criteria and the criteria for processes hygiene of various types of food, as attention is paid to another important problem as well – sampling. Regulation (EO) № 2073/2005 indicates specific rules for taking samples from carcasses. These are both destructive and non-destructive methods or principles of swabs done by different tools.

The reference methods stated in the Regulation are classical microbiological ISO methods which ensures full comparability of the results, generated in different laboratories.

Conclusions: Knowledge of the Regulation and its proper application are a guarantee for achieving a high protection level for the consumer health.

PREBIOTICS AND PROBIOTICS

PP2

BULGARIAN LACTIC ACID BACTERIA - PROMISSING BIOPROTECTIVE AND PROBIOTIC AGENTS

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Keywords: *Lactobacillus;* Probiotics, Beneficial effects

Object of research: Lactic acid bacteria (LAB) are used in the production of fermented products from centuries and are well-recognized health-promoting agents.

The aim of this study was to assess the impact of new isolated Bulgarian lactobacilli as probiotic and/or bioprotective adjuncts for new functional foods.

Materials and methods: Several microbiological and molecular methods were used in different model systems, designed for *in vitro* laboratory simulation of *in vivo* and *in situ* conditions. The widely accepted criteria for functionality, safety and technological relevance of LAB were applied in order to select promising strains.

Results: A rich collection of newly identified LAB was created, as a result of more than 10 years intensive studies on lactic acid microflora of fermented products, traditional for the Balkans. The bacteriocinogenic lactobacilli with a broad spectrum of activity, including different foods-associated/spoilage bacteria, Gram (+) and Gram(-) clinical pathogens, and micromycetes or viruses, contribute positively to the shelf-life and sensory properties of experimental samples of yogurts and cream-cheese. In addition, a strain-specific high transit tolerance, combined with immuno-modulatory activity will contribute to the stable gut colonization with beneficial effect on the healthy balance of consumers. Several laboratory tests (milk coagulation, acidifying and enzymatic activity) improved the technological relevance of pre-selected candidate probiotic strains, belonging to the species *L. plantarum* and *L. brevis.*

Conclusion: *In vitro* tests establish high bio-protective activity of several Bulgarian strains, which could be promising starters/additives for naturally preserved and functional foods and for reducing the risk of food-borne pathogens. Additional *in vivo* evaluation is also needed in order to include these strains in new probiotics for therapy

PP3

PROBIOTICS FOR GUT HEALTH

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Object of Research: The gut flora plays a key role in human health and disease. The healthy gut is dominated by lactic acid bacteria (LAB). As a major protective compound they promotes normal gastrointestinal function, combat with pathogens, regulate metabolism and comprise more than 75% of immune signals. Deregulated gut is linked to several diseases and the probiotics (PRO-For and BIO-life) are a promising guarantee for healthy balance in the gastrointestinal tract. With this aim several LAB strains were isolated from ecologically preserved Bulgarian natural sources and were characterized as beneficial probiotic cultures.

Materials and Methods: Different microbiological and genetics methods were applied, according to the criteria of FAO/WHO Working Group and International Scientific Association for Probiotics and Prebiotics for probiotics' evaluation.

Results: A rich collection of newly isolated LAB was subjected firstly to *in vitro* estimation. A group of 12 strains belonging to the species *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus helveticus* and *Bifidobacterium* spp. have been selected. They well-tolerated stomach acidity and bile and contribute significantly to the regulation of the intestinal bacterial ecosystem. Moreover, a multibacterial formula of 6 strains exhibits profound immunostimulatory effect, while other combination with *Saccharomyces bulardii* reduced significantly the frequency of diarhorea in following clinical study. The promising therapeutic results and a positive synergic effect with prebiotics have been confirmed in human trials, including patients with chronic enteritis and syndrome of small intestinal bacterial overgrowth.

Conclusions: The careful selection and the complex *in vitro* and *in vivo* evaluation of natural LAB strains, allow design of new effective probiotics Lactoflor with scientifically proven positive effect on healthy balance in gut.

REPORTS ON CORRESPONDING TOPICS (VARIA)

V3

USE OF THE DESCRIPTOR FINGERPRINTS FOR DISCRIMINATION BETWEEN ALLERGEN AND NON ALLERGEN FOOD PROTEINS.

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Object of Research: The present research is an application of a novel approach, that of the descriptor fingerprints. It has been applied here for discrimination between allergen and non allergen food proteins.
Materials and Methods: Structural fingerprints have been widely used in structural similarity search procedures. Recently, we have proposed a novel type of fingerprints, which we call descriptor fingerprints. They can characterize physical and user properties of different objects, from molecules to other materials, in our case food proteins. Additionally, we developed a new type of descriptor fingerprints, fuzzy descriptor fingerprints which have real values. Each fuzzy descriptor fingerprint uniquely describes a cluster having certain properties. The descriptors of a set of 1400 food proteins, allergens and non allergens have been created and the similarity of each query protein (of unknown type) is compared with the proteins from this set by using Tanimoto criterion T:

$$T=C/(A+B-C)$$

The Tanimoto index has values in the range from 0.0 to 1.0. The higher is the value the more similar is the object.
Results: Our results show that this method discriminates well between allergen and non allergen food proteins (with highest Tanimoto indices). Additionally, the fuzzy fingerprint method clusters the food proteins into allergen and non allergen clusters.
Conclusions: Our descriptor fingerprint method produces good results and can be applied to other properties including quality of foods provided the proper descriptors being created on the basis of appropriate analyses.
Acknowledgements: This work was supported by the National Science Fund Bulgaria NFSI IO1/7 and 01-1/2009 for this work is acknowledged.

V4

THE OPPORTUNITIES OF SMALL NON-MARKET FARMS TO PROVIDE FRESH FRUIT

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Object of Research: The research targeted the opportunities of small non-market farms to provide fresh fruit for personal and family consumption.
Materials **and Methods:** Two non-market farms in Stolichna (Sofia City) and Kostinbrod municipalities were monitored in the period of December 2013 – December 2014. The farms had fruit gardens with apple as the prevailing fruit species. The apple cultivars were identified and the growing practices and labor and money investment monitored. The yield was reported in terms of quantity, quality and possible retail price. The indexes of quality and price were compared to the products on the market, i.e. the Kaufland retail chain and the Sofia Central Farmer's Market.
Results: The production of the monitored farms was more reliable in terms of cultivar belonging and information concerning pesticides used for plant protection. It was inferior in terms of appearance but still completely suitable for fresh consumption with preserved taste in consumption maturity.
Conclusions: The small non-market farms are a reserve of fresh food and raw material for processing. They are a place, where old cultivars can be found, which is important for maintaining the variety of the apple gene pool. They provide opportunities for market pressure against the solicitation between wholesale and retail merchants that are harmful to the consumer in terms of food quality and safety.

V5

ASSESS THE LEVEL AND STRUCTURE OF CONSUMED FOOD PRODUCTS BY BULGARIAN HOUSEHOLDS

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Object of Research: The report presents the results of research on changes in the level and structure of food set consumption by Bulgarian households during 2000-2012. The analysis of these changes is presented in comparative terms between households in villages and towns. The meal patterns of people living in the villages is related to the improving the quality of life on rural households as one of the objectives the RDP in 2014-2020. Therefore, additionally a forecast on the expected level of basic food products and beverages consumption and their respective caloric structure on rural households by 2020 is made.
Materials and Methods: The methods on comparative statistical analysis and predictive approach on ARIMA modeling are attached. The comparative analysis was performed between households of residence and based on a comparison of real consumption with scientifically based standards for rational nutrition. Data from NSI are used. Time series covering the period from 1992 to 2012 were used to obtain sufficiently reliable forecast values.
Results: The most important conclusions from the results obtained are the following: Rural residents are irrational nutritional model in the a greater extent than those living in urban areas; Consumption of basic foodstuffs (meat and meat products, milk, yogurt, eggs, dairy oils, fresh and frozen fruits and vegetables) are far below the required norm; The intake of bread and bakery products is still significantly greater than the rational standards of nutrition; Irrational pattern of eating has an imbalance projection on the caloric content structure of consumed basic foodstuffs;
Conclusions: The current unfavorable structure of the caloric content will be preserved in the near future.

V6

LATEST DEVELOPMENTS IN BIOTECHNOLOGY & BIOTECHNOLOGICAL EQUIPMENT IN THE CONTEXT OF INTERNATIONAL ACADEMIC PUBLISHING PRACTICES

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Peer-reviewed scientific journals aim to create a forum for exchange of information in a particular field according to the latest developments in science. Over its 30-year history, Biotechnology & Biotechnological Equipment (B&BE) publishes original articles, reviews, short communications and case reports in all fields of modern biotechnology. The stages of development of B&BE as a publishing end-product reflect the advancements in international academic publishing practices in line with the advantages that modern information technologies (IT) have to offer. The present paper focuses on what authors should know about their rights and responsibilities as contributors to academic journals; about the ethics of science publishing and the importance of avoiding any potential conflicts of interest between co-authors, the editor, the peer-reviewers and the publisher. Other topics of discussion include the advantages of copyright protection in science publishing and, particularly, in open-access publishing. Specific emphasis is also put on publishing indices and ways in which authors can make use of modern IT technologies and indexing databases to boost the impact of their research articles. Finally, it is eminent scientists from all over the world who are members of the Editorial Board that are also leading contributors to B&BE. The journal has played and continues to play an important role in the career development of scientists in Bulgaria. The future of B&BE will depend not only on its editorial team but, hopefully and most of all, on these ambitious and energetic young researchers.

V7

PROTECTIVE ACTIVITY OF *CLINOPODIUM VULGARE* L. LEAVES ON *SACCHAROMYCES CEREVISIAE* NUCLEAR DNA

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Object of Research: To evaluate antioxidant, DNA protective, antigenotoxic, antimutagenic and anticarcinogenic potential of *Clinopodium vulgare* leaves extract on a test-system *Saccharomyces cerevisiae*.
Materials and Methods: *Saccharomyces cerevisiae* strains D7ts1 and 551 were used. Three concentrations (10, 100 and 1000 µg/ml) of *Clinopodium vulgare* L. leaves extract are tested. Antioxidant, DNA protective, antimutagenic and anticarcinogenic effects are analyzed based on antiROS, constant field gel electrophoresis (CFGE), Zimmermann and Ty1 transposition tests. The radiomimetic zeocin is used as a DNA damaging agent.
Results: Noprooxidative, DNA damaging, genotoxic, mutagenic and carcinogenic effect is found for concentrations 10 and 100 µg/ml of *Clinopodium vulgare* leaves extract. DNA protective effect of *C. vulgare* L. is found - an acceleration of DSB rejoining, an amelioration of ROS levels - 6-fold and decreased levels of reversion - 4-fold; mitotic crossing-over - 2-fold and transposition – 4-fold is found when 1000 µg/ml *C. vulgare* L. No effect is observed on the mitotic gene conversion.
Conclusions: New information is provided that *Clinopodium vulgare* L. protects nuclear DNA of *Saccharomyces cerevisiae* against the radiomimetic zeocin. Concentration 1000 µg/ml possesses the most pronounced antioxidant, DNA protective, antigenotoxic, antimutagenic and anticarcinogenic properties.

V8

COMPARISON OF STRESS RESPONSE OF TWO CLOSELY RELATED GENOTYPES TO DROUGHT

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Object of Research: To compare stress response of two closely related genotypes to drought stress, induced by polyethyleneglycol (PEG).
Materials and Methods: Cultivar Dobrudjanski 7 (D7) andit's mutant line L8 obtained by Prof. Svetleva were studied. Plants material at the third leaf phase is treated for 24h with 8% and 16% PEG (MW 10000). Stress response of treated samples vs untreated is compared. The levels of malondialdehyde - MDA, total hydrogen peroxides - H_2O_2, proline - Pro and heat shock protein - HSP70B are calculated by Two-way ANOVA (Graph Pad Prism, version 6.04).
Results: No statistically significant differences in the constitutive levels of MDA, $H_2O_2$and Pro for both genotypes are found. About 6-fold higher constitutive content of HSP70B is obtained for D7 comparing with L8. PEG in the range of 8% and 16% could induces light stress increasing about 1.5–2 fold the levels of MDA, H2O2 and HSP70B. No effect of the genotype was confirmed. The other tested marker – Pro accumulation clearly indicate statistically significant difference depending on the concentration (about 4-fold for 8% and 7-8 fold for 16%) and on the genotype (1.2 fold higher levels for L8 compared to D7).
Conclusions: Pro could be considered as the only reliable marker to distinguish stress response to drought stress in genetically closely related genotypes. Here we confirm our previous finding in *Chlorella* genotypes that constitutive levels of HSP70B could be recommended as early warming marker for genotype resistance to oxidative stress.

V9

APPLICATION OF HYPERSPECTRAL REFLECTANCE TECHNIQUE FOR EARLY DETECTION OF DISEASE ON POTATOES

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Potato diseases caused by viruses can lead to potatoes' losses in the form of decreased yields, misshapen tubers or internal discoloration. Potato Virus Y (PVY) is one of the most important viruses infecting potatoes. PVY causes mosaic patterns on leaves and tuber malformations. Symptoms of PVY vary depending upon the strain of the virus and the potatoes' variety. In this study a remote sensing technique, leaf spectral reflectance, was used for detecting the presence of disease in potato plants caused by PVY. For investigations young potato plants, cultivar Armada grown in a greenhouse under control conditions, were used. The plants were divided into two groups. The first group consisted of healthy (control) plants. At growth stage 4-6 expanded leaf, the second group was inoculated with PVY. Hyperspectral reflectance data were collected by means of a portable fibre-optics spectrometer in the visible and near infrared (NIR) spectral ranges. The differences between the spectral data of control and infected plants were assessed by means of statistical (Student's t-test), first derivative analysis and some vegetation indices implemented in green, red, red edge and NIR wavelength intervals. Significant changes in spectral characteristics were observed in all investigated spectral ranges. The strong relationship which was found with the results from serological analysis (DAS-ELISA test) for presence of the PVY infection indicates the importance of hyperspectral reflectance technique for conducting, easily and without damage a preventive screening of potato crop in order to provide a yield of good consumption characteristics.

V10

MIGRATING BIRDS AS CARRIERS OF ZOONOTIC PATHOGENS

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Object of Research: The aim of the presented study was to investigate migratory wild bird species passing the territory of Bulgaria along the Mediterranean/Black Sea Flyway (MBSF) as carriers of important food-borne pathogens. Situated at the crossroad between Europe, Asia and Africa, Bulgaria is an important part of the MBSF used by millions of birds breeding in Eastern Europe, Scandinavia and Russia.
Materials and Methods: 673 wild birds of 46 species were subjected to multi-pathogens assay for the presence of food-borne pathogens from the genera *Campylobacter, Yersinia, Salmonella* and *Listeria* and from the species *E. coli, S. aureus, F. tularensis,* and *Brucella* spp. Bacteriological, serological and molecular biological (DNA isolation, conventional and multiplex PCR) methods were used for identification of the bacterial isolates.
Results: We detected *Campylobacter16S rDNA* gene in 1.34% of birds tested and no occurrence of pathogenic species was found. Thirty four migrants (5.05%) carried nonpathogenic *Yersinia enterocolitica* strains, while pathogenic *Yersinia (ail* gene positive) was detected in one bird only (0.15%). Three birds (0.45%) were identified as carriers of non-pathogenic *Salmonella* strains. *Listeria monocytogenes* was detected in 5 avian samples (0.74%). One out of 33 birds was *Brucella* spp. positive. *E. coli16S rDNA* gene was found in 8.77% of birds and no virulence associated genes were expressed.
Conclusions: These data provide first evidence that the birds migrating via Bulgaria harbor detectable enteric bacteria such as *Campylobacter*, *Yersinia*, *E. coli*, *Salmonella* and *Listeria,* some of them pathogenic. Although the carriage of pathogenic strains of *Y. enterocolitica* and *L. monocytogenes* was low, the migrants could be of risk for other birds, animals and humans as source of important zoonotic pathogens.

Posters

FOOD QUALITY

FQ 2

EVALUATION OF AUTHENTICITY AND QUALITY OF ARGAN OILS SOLD ON THE BULGARIAN MARKET

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Object of Research: Argan oil is one of the most expensive plant oils in the world. Because of its healthy fatty acids and unique sterols composition it is used for both nutritional and cosmetic purposes. Dietary investigations have revealed its hypocholesterolemic, antiatherosclerotic and hepatoprotective effects. In addition to healthful consumption of argan oil, the high oxidative stability allows its using for cooking at high temperatures as well.

Due to its high price the argan oil is quite probable to be illegally diluted with cheaper oils, and estimation of such adulteration becomes an analytical problem. Thus, the purpose of our study was to evaluate the authenticity and quality of argan oils sold on the Bulgarian market, and to compare them with authentic extra virgin argan oil bought directly from producer in Morocco.

Materials and Methods: Six samples of argan oils provided into Bulgarian market by various traders, and one original Moroccan sample, were analyzed by chromatographic (TLC, GC, GC-MS, HPLC), titrimetric and spectroscopic methods about their fatty acids, triacylglycerols, sterols and oxidative stability. Principal component analysis (PCA) was applied for data processing.

Results: All analyzed samples had similar fatty acids composition, with slight deviation in one sample. Triacylglycerols profile confirmed the distinction of this sample, and sterols composition evidenced undoubtedly the presence of other plant oil. This sample had the lowest oxidative stability as well, despite the declared addition of antioxidants.

Conclusions: Five of samples were identical to authentic Moroccan argan oil in their fatty acids, triacylglycerols and sterols. One sample was a blend of argan and other plant oil.

FQ4

STUDIES OF GENOTOXIC EFFECT OF TWO TYPES OF PLANT EXTRACTS ON *HORDEUM VILGARE* AND HUMAN LYMPHOCYTES

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Object of Research: The wide application of plant extracts in human nutrition and pharmacy requires studies on their safety at doses in the pharmacological range.To assess biological effect of extract from *Papaver rhoeas L.* (poppy) and *Salvia officinalis L.* (sage) on the base of endpoints for cytotoxicity and genotoxicity in *Hordeum vulgare L.* and human lymphocytes *in vitro*.

Materials and Methods: Plant extracts of sage and poppy were prepared by extraction with methanol. Cytotoxicity of plant extracts was evaluated based on the mitotic index. Endpoints for genotoxicity were chromosome aberrations and micronuclei. *Hordeum vulgare* L. root tip meristems were treated with 10–100 μg/ml sage extract and with 0.25 - 1 mg/ml poppy extract respectively. Human lymphocytes were treated with 5–100 μg/ml sage extract and with 0.01 - 1 mg/ml of poppy extract. Untreated cells were used as a negative control.

Results: Both extracts have no or weak cytotoxic effect in barley in the concentration range used by us, whereas human lymphocytes are sensitive to the poppy extract. Sage extract shows a low cytotoxic effect on lymphocytes. Well expressed genotoxic activity was obtained after treatment with poppy extract and also with sage extract evaluated as frequencies of chromosome aberrations and micronuclei in both test-systems. The effect has clear concentration dependency.

Conclusions: The cytotoxic and genotoxic effects of plant extracts clearly depend on the test-system used and the concentrations. Human lymphocytes are more susceptible to both of extracts than *H. vulgare*.The results obtained show that plant extracts should be used with caution by medication.

FQ5

ANALYTICAL METHOD FOR DETERMINATION OF 8-ALPHA-HYDROXYMUTILIN IN TISSUES WITH ANIMAL ORIGIN

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Object of Research: To determine the residue of 8-alfa-hydroxymutilin (8α-HM) in porcine tissues after administration of Rodotet premix.
Materials and Methods: The evaluation of the concentration of 8α-HM as marker residue for tiamulin, belong to pleuromutilins group, was done through verification and validation of an analytical method for the quantitative determination of 8-alpha-hydroxymutilin in tissues (porcine muscle and liver). Quantification of 8α-HM was performed with liquid chromatography-tandem mass spectrometry (LC MS/MS).
Results: In order to assess the residues in tissues, an analytical method for 8-alpha-hydroxy-mutilin was verificated and validated in line with accordance of Commission Decision 2002/657/EC, by a set of parameters, which comply with the requirements defined in the Decision and Bioanalytical method validation. Maximum residual levels (MRLs) for porcine tissues have been established in Commission Regulation No 37/2010. This Regulation lay down the presented marker residue for tiamuline as the sum of metabolites that may be hydrolysed to 8-alfa-hydroxy-mutilin: MRLs at 100 µg/kg for porcine muscle, and at 500 µg/kg for porcine liver are determinated.
Conclusions: All analytical experiments were done under the principles of GLP, VICH GL49. Therefore the used verificated method contained a hydrolysis step. To evaluate the matrix effect an internal standard Norfloxacine D5 was added before instrumental analysis. Additionally, to determine behavior of 8-alfa-hydroxy-mutilin (e.g. behavior in matrix and freeze-thaw effect), was also verificated and validated an analytical method without hydrolysis step described before by BVL, Germany.

Key words: 8-alfa-hydroxymutilin, hydrolysis, marker residue, method validation, MRL, Tiamulin

FQ 6

ELECTROCHEMICAL DETERMINATION OF REDOX POTENTIAL IN INFANT FORMULA AND HUMAN BREAST MILK

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Object of Research: Human breast milk is the best dietary choice for newborn baby, and it is considered to be a gold standard.All the manufacturers of infant formula aim to produce these products with composition very similar to that of human breast milk. The objects of research of this study are to investigate the significance of breast milk and infant formula in the prevention of oxidative stress, by electrochemical determination of the total antioxidant potential and commonly used DPPH method, demonstrating the relationship between the antioxidant capacity of milk and postnatal age.

Materials and Methods: Infant formulas supplemented with prebiotics for term and preterm newborn babies, human breast milk and UHT milk were used. Cyclic voltammograms and differential pulse voltammograms were recorded with the glassy carbon electrode as the working electrode, an accessory platinum electrode, and an Ag/AgCl reference electrode. The DPPH assay measures the reducing ability of antioxidants in milk samples towards the DPPH radical using a UV-vis spectrophotometer.

Results: Electrochemical measurements indicates that human breast milk has the highest redox potential (250 mV), while skimmed UHT milk has very low one (100 mV). Infant formulas have also high potential of 180mV. DPPH method confirmed results obtained by electrochemical methods. The free radical scavenging activity is highest for human breast milk (92.51%) and lowest for UHT milk sample (39.94%). Infant formulas have also high free radical scavenging activity (70-91%).

Conclusions: The main advantage of electrochemical methods used to assess the total antioxidant activity of milk was that they directly monitored the electron-donating ability of the compounds and could be used for the quantitative analysis of the total antioxidants of different types of milk.

FQ 7

HYDROXIL RADICAL SCAVENGING ACTIVITY OF PRETERM MOTHERS MILKS IN THE FENTON SYSTEM

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Object of Research: Mother's milk have antioxidative effects against Fenton system *via* HO• radical scavenging. Mother's milk from women after preterm delivery antioxidative properties are not examined in details. We examined hydroxil radical scavenging activity of preterm mother's milks in the Fenton system .

Materials and Methods: We utilize electron paramagnetic resonance (EPR) spin-trapping spectroscopy to determine and compare activity of premature mother milk, skim milk and whey against Fenton system. The spin-trapping technique is based on the reaction of 'EPR silent' spin-trap with free radical which yields a more persistent EPR active nitroxide spin-adduct. DEPMPO, a sophisticated EPR spin-trap reagent, is applied in order to analyze reactive products of milk with HO• produced in Fenton reaction. Premature mother milk was collected 6 weeks after premature baby delivery, from five exclusively breast-feeding mothers. The milk was then mixed, aliquoted, stored at -80°C. Skim milk was prepared by centrifugation (10000 g, 5 min at 4 °C).Whey was prepared from skim milk by acidification to pH 4.6 with lactic acid, incubation for 30 min at room temperature, centrifugation (as above), and readjustment of pH of the supernatant with NaOH to 6.7. Fenton reaction was performed by combining 1 mM H_2O_2, and 0.2 mM $FeSO_4$.

Results: It can be observed that full breast milk as well as fractions scavenge hydroxyl radical, which results in the production of urate and ascorbyl radicals. The intensities of DEPMPO signals in all milk-containing systems was drastically lower compared to control (Fenton) system indicating that milk samples scavenge HO•.

Conclusions: There was no significant difference between the intensities of signals of urate radical adduct or ascorbyl radical between fractions – full milk, skim milk and whey. Main antioxidants (urate and ascorbate) in mature mother's milk from women after preterm delivery are in whey.

FQ 8

ANTIRADICAL ACTIVITY OF BULGARIAN SOYBEAN GENOTYPES

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Object of Research: Soybean (*Glycine max* (L.) Merr.) is an excellent nutrition source containing essential amino acids, omega-3-polyunsaturated fatty acids, phenolic compounds, vitamins, folic acid and inorganic elements. In Bulgaria series of elite soybean varieties with improved yield and nutrition qualities has been released at the Experimental Station in Soya, Pavlikeni. In the present study seeds from four Bulgarian soybean varieties and two lines were examined and were compared with the American variety "Hodgson" (world standard) for their antiradical potential. Additional antiradical activity of soybean sprouts was assayed.

Materials and Methods: The analysis was performed on Bulgarian soybean varieties: "Richy", "Rosa", "Srebrina", "Daniela", American variety "Hodgson" and two lines. Preparation of extracts: The powdered soybean seeds (2 g) were mixed with 2 ml 0.1 N HCl and 10 ml acetonitrile in a 125-ml flask and stirred for 2 h at room temperature. The solution was filtered and dried under vacuum. Soybean sprouts (1 g) were treated with distilled water into the ultrasonic bath for 30 min. The solution was filtered and dried under vacuum. Free radical scavenging activity: 2,2-diphenyl-1-picryl hydrazyl (DPPH) radicals were used for evaluation of antiradical activity.

Results: Antiradical activity expressed as concentration of extracts needed for 50 % inhibition of radicals (IC_{50}) ranged from 2.73 to 3.84 mg/mL for seed extracts. The extracts of studied samples showed commensurate and in most cases even better values than the world standard "Hodgson". The extract of soybean sprouts had significant higher antiradical activity (1.190 mg/mL) than seed extracts.

Conclusions: Bulgarian soybean cultivars are promising food with antioxidant action. The extracts of soybean sprouts showed higher antioxidant activity than the extracts of seeds that should be taken in consideration when choosing a soybean food product for consumption.

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FQ 9

CAFFEINE CONTENT IN VARIOUS COFFEE SAMPLES DETERMINED BY INFRARED SPECTROSCOPY

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Object of Research: Caffeine and the evaluation of its dietary intake attract much attention in the food and nutrition industry because of its controversial impact on human health. Official methods for caffeine determination in coffee are based on chromatography and spectrophotometric measurement involving time-consuming sample preparation and use of large amounts of organic solvents. Therefore, the application of faster and more environmentally friendly analytical methods such as Furrier-transform infrared (FT-IR) spectroscopy offer a great advantage. This study reports the caffeine content in roasted and instant coffee of various trademarks purchased at the local market determined by a fast and reliable FT-IR procedure.

Materials **and Methods:** The caffeine was extracted from the coffee samples with a small volume of chloroform. All IR spectra were measured on a Brucker Tensor 27 FT spectrometer in a calcium fluoride IR cell at a resolution of 2 cm-1 and 64 scans.

Results: FT-IR spectroscopy offers a reliable way for caffeine determination, based on the absorbance of this compound at 1659 cm–1. This is usually the most intensive band in the spectra of pure caffeine solutions and coffee extracts. A series of standard solutions of caffeine in chloroform as well as the real coffee samples were analyzed and integrated area of the carbonyl band at 1658 cm–1 was determined by a curve-fit procedure. The caffeine content in real coffee samples was determined based on a standard curve for absorbance vs concentration of caffeine. All instant coffee samples showed higher caffeine content than the roasted coffee samples.

Conclusions: The caffeine content in various types of roasted and instant coffee can be conveniently determined by a simple FT-IR procedure after extraction with a small volume of chloroform that reduced drastically both the time required for sample preparation and the use of organic solvents.

FQ 10

BEYOND THE NUTRITIONAL VALUE OF *SAMBUCUS EBULUS*: METABOLOMICS AND ANTIOXIDANT ACTIVITIES

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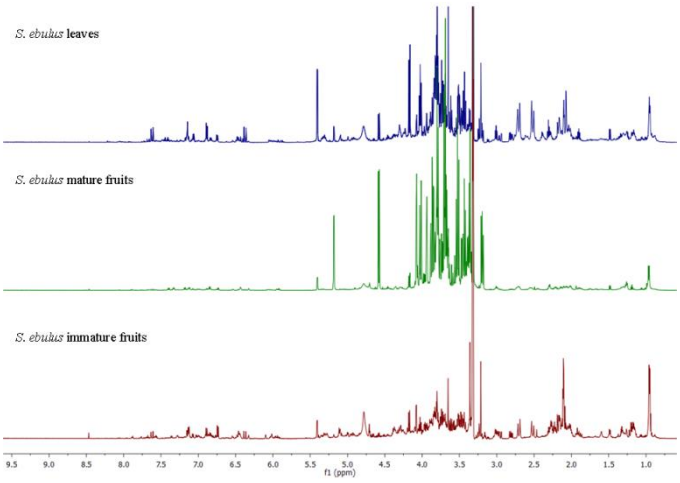
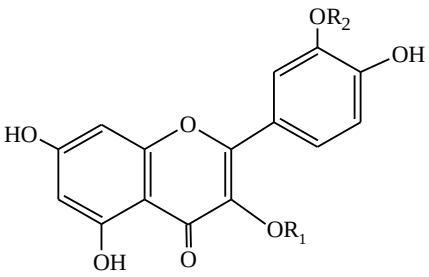
Dwarf elder (*Sambucus ebulus* L., Caprifoliaceae) is a popular medical plant, used for centuries in the folk medicine of Europe and the Balkan Peninsula in particular. *S. ebulus* preparations showed anti-neoplastic, antimicrobial (*incl.* antiviral) and anti-inflammatory properties. Elderberries accumulate sugars and fibers, vitamins and minerals, besides abundant secondary metabolites, as flavonoids, anthocyanins, phytosterols, triterpenes and iridoid glycosides. Here we report the application of ^{1}H NMR metabolic fingerprinting in combination with principal component and hierarchical clustering analyses to reveal the metabolic differences of *Sambucus* mature and immature fruits, and plant leaves. Moreover, we show that immature fruits and leaves of *S. ebulus* have similar metabolome, which apparently undergoes significant changes during the fruit ripening stage. Sambunigrin (cyanogenic glycoside) was not detectable in any sample (1). Further, a scheme for isolation of individual compounds utilizing different chromatographic techniques was developed, resulting in five flavonoid glycosides – quercetin-3-*O*-laminaribioside [**1**], isorhamnetin-3-*O*-laminaribioside [**2**], quercetin-3-*O*-rutinoside [**3**], isorhamnetin-3-*O*-rutinoside [**4**], isorhamnetin-3-*O*-glucoside – isolation [**5**]. The evaluation of antioxidant properties (in $ORAC_{FL}$ and $HORAC_{FL}$) suggests that the dwarf elder fruits might serve as a powerful source of valuable molecules for various purposes (2).





1 R_1 = Laminaribiose R_2 = H

2	R_1 = Laminaribiose	$R_2 = CH_3$
3	R_1 = Rutinose	$R_2 = H$
4	R_1 = Rutinose	$R_2 = CH_3$
5	R_1 = Glucose	$R_2 = CH_3$

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FQ 11

IDENTIFICATION OF COUNTERFEIT OF WHOLE GRAIN BREAD

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Whole grains play an important role in maintaining health and preventing disease. The production of whole grain bread as a healthy alternative to white bread has grown in the last decade. The bread manufacturer sinorder to maximize the irprofits, dark colour ingredients like molasses used for coloring of the white flour. If the colour is not labeled on the packaging or the bread with added colour is declared as "wholegrain" bread, means that the bread was falsified.

The bread samples were evaluated for water and ash content and aw-values. The hardness and porosity of the bread samples were also tested. The color values of bread samples were measured using a reflectance spectrophotometer CIE L* a* b* colorsystem. The samples declared as "wholegrain" bread and bread with colour ingredients were supplied from the local market. The possibility to correlate the determined ash values and estimated browning index (BI) in a qualitative confirmation of the added colour in the bread was investigated in this study.

The average water content and aw values in the analyzed bread samples were 65% and 0.88, respectively. The ash contents in whole grain bread samples (from 1.87 to 2.88%) were two times higher than in bread with added colour (1.26%). The highest BI value of 70.81 was estimated for the bread with colour ingredient. In comparison to the whole grain bread samples, the bread with added colour was characterized with the highest hardness (7 N), small volume and compacted structure without visible pores on the cross section.

FQ 12

INFLUENCE OF PROCESS PARAMETERS ON STIRRED YOGHURT QUALITY

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Yoghurt is one of the most popular dairy fermented product obtained by the action of lactic acid starter bacteria that are responsible for milk acid ification and synthesis of aromatic compounds. The physical and structural quality attributes of yoghurt are influenced by the milk quality, as well as by the major processing factors. Varieties of yogurt have been produced by incorporating health or flavor enchanting components, which influence the yogurt texture. Yogurt texture could be modified by using anexopolysaccharide producing strains of lactic acid bacteria. Apart from sensory and textural attributes, the exopolysaccharides are associated with health benefits.

The objective of this study was to investigate the effects of the starter cultures *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (exopolysaccharide producing and non-producing strains), temperature of cooling after stirring (8, 15 and 20°C) and time of storage at 4°C (1, 4, 8 and 15 days) on the acidity and viscosity of the stirred yoghurt produced from pasteurized cow's milk. The total solids in the milk were adjusted by adding whole milk powder. The acidity was determined by measuring the pH values. The Brookfield viscosymeter (spindle No 3 at 30 rpm) was used in the viscosity measurements.

The pH values of yoghurt samples decreased in the range of 4.48 to 4.24 by increasing the cooling temperature and storage time. The starter cultures slightly affected changes in pH values of the yogurt samples. The influence of the starter strain and cooling temperature on the viscosity was significant. The differences of the viscosity were higher by increasing the cooling temperature and storage time when exopolysaccharide non-producing starter strain was used. The viscosity values varied from 1810−2310 mPas and 2230−2980 mPas for exopolysaccharide producing and non-producing strains, respectively.

FOOD BIOTECHNOLOGY

FB 2

RED MICROALGA *RHODELLA RETICULATA*- POTENTIAL SOURCE OFFOOD ADDITIVES

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Object of Research: Evaluation of the biochemical composition of the red microalga *Rhodella reticulata* was performed. The strain proved to be an important source of functional ingredients andcould be successfully used as food additives. Appropriate conditions for growth and heteropolysaccharide production by the alga were reached by optimization of the medium, thus obtaining greater quantities of the desired algal product.

Materials and methods: The red microalga *Rhodella reticulata* (Rhodophyta), strain UTEX LB2320 was acquired from the Austin University, Texas USA.

Dry weight was measured as algal suspension was centrifuged, the supernatant was removed and the cells were dried at 105 °C for 16 h.

Several semi-continuous cultures were carried out and growth, biochemical parameters, such as lipid, protein, carbohydrate, exopolysaccharide and pigment contents were quantified.

The elemental composition of a new medium was tested. Soil extract was evaluated by atomic emission spectroscopy.

Results: Biochemical composition of *Rhodella reticulata* from dry weight was: Proteins: 35%; lipids - 10%; carbohydrates - 35% and pigments – 8.2%. In the fatty acids profile highest percent (46% from the lipids) was for the polyunsaturated eicosapentaenoic acid, an important feature for this strain.

The optimization of the concentration of the participating nutrients and addition of soil extract in the medium resulted in enhancement of the growth of microalgal cells up to1.6-fold.At the 120^{th}h, the extracellular polysaccharide content using the new medium reached 1.4 - fold.

Conclusions: The results proved that *Rhodella reticulata* ingredients can find application as additives for human and animal feed, as well as natural food colorants. Much greater productivity in a desired ratio of algal biochemical components could be reached using the most suitable type and balance of elements in the cultivation medium.

FB 3

LIPASE-CATALYZED SYNTHESIS OF LOW-CALORIE STRUCLURAL LIPIDS FOR CLINICAL NUTRITION FROM WALNUT OIL

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Object of Research: Lipids are one of the major components of emulsions used in parenteral and enteral nutrition and they provide 15–30% of the total calorie intake to clinically nourished patients. In many feeding formulations, medium-chain triacylglycerols (MCTAGs) are preferable than the long-chain triacylglycerols (LCTAGs) due to their faster hydrolysis and easier absorption from the human intestine. At the same time LCTAGs are source of essential fatty acids and are also needed in diet. Recently, it has been found that tailor-made lipids containing medium-chain acyl residues in the *sn*-1 and *sn*-3 positions of the glycerol backbone, and essential long-chain fatty acyl residues in the *sn*-2 position (MLM-type) combine the desirable characteristics of MCTAGs and LCTAGs. The aim of the research was to establish the potential of walnut oil as a starting material for synthesis of low-calorie structured lipids of MLM-type.

Materials and Methods: Caprylic acid residues were incorporated selectively into *sn* 1,3-positions of triacylglycerols in one step acidolysis using immobilized 1,3-specific lipases from *Rhizomucor miehei* (Lipozyme) and *Rhizopus delemar* (PP-RhDL) as catalysts.

Results: Under the optimal reaction conditions, we achieved an excellent yield of the desired structured lipids (97 mole % with Lipozyme and 96.4 mole % with PP-RhDL). The synthetic activity of PP-RhDL was not influenced by the reaction medium, whereas the Lipozyme was more effective in hexane than in solvent free medium. The same degree of conversion of initial triacylglycerols was observed for the two enzymes in four consecutive reaction cycles.

Conclusions: The amount of MLM-type structured lipids, however, decreased with each following cycle at the expense of mono-substituted with caprylic acid triacylglycerols (MLL).

FB4

LIPASE-CATALYZED SYNTHESIS OF BANANA FLAVOR IN NON-CONVENTIONAL REACTION MEDIA

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Fruity flavor enhancers and fragrance compounds are widely used in food, pharmaceutical and cosmetic industries and their annual market share amounted to around 15 billion euro. The amount of the aroma compounds, obtained from natural sources is insufficient, and their isolation via extraction and distillation is sophisticated and very expensive process. Short-chain esters are responsible for the aroma of many fruits, including apples, pears, bananas, pineapples, strawberriesetc. Conventional synthesis of such esters involves use of hazardous catalysts and high consumption of energy and water. In recent years, application of environmentally friendly technologies incl. biocatalysts, green solvents, etc. have attracted much attention. Lipases and esterases are hydrolytic enzymes which in non-aqueous media catalyze ester-bond formation reaction and there are many excellent results in reported in the literature. Yet, the enzyme-catalyzed synthesis of short-chain esters is a challenge, especially for the industry, due to strong inhibition of the biocatalysts at high concentrations of short-chain alcohols and organic acids.

Here, we report the stabilization of two lipases from *Candida rugosa* (CRL) and *Rhizopus delemar* (RhDL) via physical adsorption on macroporous polypropylene and their application as catalysts for isoamylacetate synthesis (banana flavor production). We assessed also the effect of two ionic liquids on the basis of imidazolium cation and acesulfame anion ([C1Cnim][Ace] on hydrolytic activity and thermal stability of the lipases. The applicability of the seionic liquids as suitable co-solvents was tested in the lipase-catalyzed banana flavor ester. The results obtained by us with CRL-PP and RhDL-PP were comparable and even better than those reported in the literature with other lipases.

FB 5

CRYOPROTECTION OF THREE VALUABLE MEDICINAL SPECIES OF GENUS *HYPERICUM*

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Object of Research: Plant species of genus *Hypericum* are among the most important plants in herbal medicine and their oils have numerous applications in food, pharmaceutical and perfume industries. The aim of the present work is to evaluate the structural and functional changes possibly occurring in three *Hypericum* species regenerated after cryopreservation.
Materials and Methods: The experimental plants, *Hypericum kalmianum, Hypericum tomentosum* and *Hypericum humifusum* were maintained *in vitro* on MS culture medium. Cryopreservation was carried out by the method of vitrification. After fixation in 3% glutaraldehyde, a light microscopy and transmission electron microscopy were performed. Pigments' content was determined according to Arnon (1949). Malone dialdehyde was measured according to Dhindsa et al. (1981). Superoxide dismutase activity was measured after Beauchamp and Fridovich (1971) and catalase activity - according to Aebi (1984).
Results: The three *Hypericum* species showed a good tolerance to cryopreservation. All regenerated plants had typical bifacial leaves. *H. kalmianumand H. humifusum* have the same histological organization while in the regenerated *H. tomentosum* equifacial leaves were structured suggesting abnormalities in leaf histogenesis.
In vitro conditions led to atypical structuring of the chloroplasts and species-specific forms of thylakoids destruction. Chloroplasts of regenerated plants showed well-structured internal membrane system, excepting *H. humifusum* in whom high-grana chloroplasts were formed.
Low-temperature caused a decrease in chl. a and chl. b content in the regenerated plants accompanied with enhanced values of malone dialdehyde, most prominent in *H. humifusum*. The activity of superoxide dismutase was significantly increased in all experimental species. Catalase activity also increased with the exception of *H. kalmianum.*
Conclusions: For all three examined *Hypericum plants* the tolerance to cryopreservation was a species-dependent feature. Possible compensatory subcellular mechanisms were likely to develop to overcome the low-temperature stress.

FB6

HAEMATOCOCCUS PLUVIALIS – NATURAL SOURCE OF BIOLOGICALLY ACTIVE SUBSTANCES FOR HUMAN HEALTH AND NUTRITION

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Object of Research: Astaxanthin is a red pigment (xanthophyll), which exhibits strong antioxidant, anti-lipid peroxidation, anti-diabetic and anticancer activities, as well as cardiovascular disease prevention, and immune-modulation properties. The green microalga *Haematococcus pluvialis* is one of the biggest producers of natural astaxanthin for human consumption. The aim of our work was optimizing the cultivation conditions for growth and carotenoid accumulation in *Haematococcus* cells.

Materials and Methods: *Haematococcus pluvialis* strain BS2 was grown for 30 days in different culture media: BG11 (Rippka et al., 1979); BG11 + 1 g/l NaHCO3; modified BG11 (Boussiba & Vonshak, 1991); modified BG11+ 1 g/l NaHCO3 and medium after Setlik (1967), modified by Georgiev et al. (1978) with ¼ concentration of nutrients (noted as ¼ ChR). The "green" and the "red" stage algal biomass was harvested and analyzed for protein, carbohydrate, pigment, lipid contents and fatty acid composition.

Results: After 30 days of cultivation the maximum cell concentrations were obtained in ¼ ChR media and mod BG11 and the highest carotenoid content was determined in BG11 + NaHCO3 (0.78% of dry weight). Stress induced carotenoid accumulation is associated with a significant decrease in protein synthesis. Spontaneous enhancement of γ-linolenic acid (28.8 % of total fatty acid content) was detected when *Haematococcus pluvialis* BS2 was grown in BG11 nutrient medium.

Conclusions: Growth and biochemical compostion of *Haematococcus* biomass depend largely on the mineral medium used. The presence of NaHCO3 in the medium induce more intensive growth and carotenoid synthesis. Finding the right cultivation conditions for the production and maintenance of high levels of γ-linolenic acid, which has significant role for human health, is worth further efforts.

FB7

IMPROVEMENT OF ANTIOXIDANT PROPERTIES OF PEA

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Object of Research: Pea is a legume species with economical importance because of its nutritive value characteristic with high protein level, vitamins, minerals, and antioxidants. The present work objective is to investigate the effect of plant growth regulators on the pea plants' vitability and related accumulation of phenolics defining the antioxidant activity.

Materials and Methods: Sprouting pea (*Pisum sativum*) seeds were cultivated as a hydroponic culture for 6 days under 16h light /8h dark photoperiod, at 22ºC. Next, plant growth regulators were added for 7 days in different combinations: 1-control, 2-4 µM Abscisic acid /ABA/, 3-3 µM Gibberellic acid /GA/, 4-ABA+GA (in the above concentrations). Pea seedlings have been grown and subjected to analyses, such as: morphometric, cytological, leaf pigment content, as well as phenolics (phenols and flavonoids) quantity and total antioxidant activitiy.

Results: ABA and GA are negative and positive plant growth regulators, respectively. In pea, GA promoted stem elongation and mesophyll cells number, while ABA reduced the number of leaf pairs and the mesophyll cell size. Each treatment caused only mild stress to the plant as indicated by the decreased ratio of chlorophyll a and b, but lack of change in the carotenoid content. Intriguingly, the phenolics content increased only after treatment with GA, which correlated with higher antioxidant activity. The effect of ABA was rather inhibitory.

Conclusions: The phenolics serve the plants as antioxidants that assure protection against abiotic and biotic unfavorable factors but they can also attract pollinators. In human diet, the plants are the main source of antioxidants and improving the antioxidant capacity of crops such as pea is of great interest. Our results suggest that pea plants treated with GA possess higher antioxidant capacity and their growth is stimulated. Therefore, GA could be applied for improving both pea yield and quality.

FB 8

CRYO-CONSERVATION OF NINE *HYPERICUM* SPECIES IN TERMS OF THEIR APPLICATIONS IN MEDICINE AND DIETETICS

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Object of Research: Cryopreservation is an adequate method for long-term storage of living cells and organs and it is widely applied in industrial, medicinal and food technology. The aim of the present work is to examine the influence of cryopreservation protocol on several species of genus *Hypericum* - extensively used herbs in medicine and food industry.

Materials and Methods: Nine different species of the genus *Hypericum: H. perforatum, H. rumeliacum, H. konytchense, H. tetrapterum, H. kalmianum, H. humifusum, H. annulatum, H. tomentosum* and *H. pulchrum* were cultivated in vitro on MS culture medium. Cryopreservation was performed by the vitrification method. Chlorophyll and carotenoid contents were determined according to Arnon (1949). Malone dialdehyde was measured according to Dhindsa et al. (1981). Superoxide dismutase activity was measured after Beauchamp and Fridovich (1971) and catalase activity - according to Aebi (1984). Total antioxidant activity was determined according to Prieto et al. (1999).

Results: Cryopreservation led to a slight reduction in the pigments' content in all regenerated species in comparison with the control plants. The activity of superoxide dismutase in hypericin-producing species was highly increased. The strong increase of catalase activity in cryopreserved *H. rumeliacum* and *H. annulatum* was accompanied by an enhancement of malone dialdehyde content. The greatest increase in total antioxidant activity was observed in the cells of hypericin-producing *H. rumeliacum* and *H. perforatum*.

Conclusions: According to the results, cryopreservation did not cause a significant increase in the levels of reactive oxygen species and further disabilities in the cell membranes in the selected species. The physiological changes observed in the regenerated plants could be an indication of possible adaptive mechanisms after cryopreservation

FB 9

THE ROLE OF LEAF ANATOMICAL CHARACTERISTICS IN ELABORATING THE PROTOCOL FOR MICROPROPAGATION OF *NEPETA NUDA L.*

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Object of Research: The genus *Nepeta comprises* about 250 species distributed in the central and southern parts of Europe, Asia and the Middle East. Several species are used in folk medicine as diuretic, diaphoretic, antitussive, antispasmodic, anti-asthmatic, febrifuge, emmenagogue and sedative agents. *Nepeta* species contain monoterpenes, sesquiterpenes, cyclopentanoid iridoids derivatives and nepetalactones. The activity of nepetalactone and also of its isomers on the olfactory sense of domestic cats was demonstrated and they function also as insect attractants and repellants. Histological analysis of the leaves from in vitro-cultured in test-experimental conditions *Nepeta nuda L.* plants was made in an attempt to determine the suitable growth regulator and its concentration.

Materials and Methods: The Nepeta plants were grown on standard MS medium (control) or on supplemented with indole-3-butyric acid (IBA) or N6-benzyladenine (BA) medium. Each growth regulator was separately added in 10 concentrations from 0.1 to 1 mg/l in 0.1 mg/l increments. IBA and BA affected in varied ways the histogenesis of the leaf lamina.

Results: The IBA addition in concentrations 0.8 mg/l, 0.9 mg/l and 1 mg/l had positive effect on formation of the mesophyll. On the contrary, BA added in the same concentrations had negative effect expressed in hyperhydricity and abnormal leaf structure.

Conclusions: The results suggested that only supplementing IBA in the culture medium was sufficient to accomplish regeneration and *ex vitro* adaptation of *N. nuda plants*.

FB10

MICROBIAL FOOD PRODUCT RICH IN SELENIUM

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Object of Research: Selenium belongs to the group of essential microelements. Their concentrations in tissues and biological fluids are low, but they have a great chemical reactivity. Major route of entry of selenium in the body are food and beverages. It is proven that inorganic selenium in the form of sodium selenite or selenate is less accessible to the organism than the organic selenium existing as selenium amino acids or enriched microbial cells. These considerations motivate the need to examine the possibility of obtaining yeast enriched with selenium. Dosed usage of such yeast will lead to satisfying the needs of the human body for this element.

Materials and Methods: In this work the growth characteristics and maltase activity of bakery yeast in a medium, containing various amounts of selenium ions (Se^{4+}) were investigated. The conditions for maximum biosorption of such ions from the cells were followed. The technological time of their introduction in the culture media was also measured.

Results and Conclusions: The results showed that in the presence of selenium in the culture medium above a critical level (10 $mg.dm^{-3}$), cells change the mechanism of metabolism of the carbon substrate (qs), the growth rate (μ), and the economic coefficient (Ys). Maltase activity decreased at the high concentrations of Se^{4+}. Sorption of ions in the cells increased in proportion to their concentration, as young cells tolerate the selenium shock more easily. The amount of adsorbed selenium from the cells of bakery yeast *Saccharomyces cerevisiae* RD-1, used in the assay, is in the range of 0.15 to 0, 60 mg/g dry cell weight, which is a good indication for practical use.

FB11

IMPROVEMENT OF α-AMYLASE PRODUCTION BY *ASPERGILLUS ORYZAE* PP

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Object of Research: Amylases are among the most important enzymes that have a great importance in present-day biotechnology. Besides their traditional use in food (starch saccharification, baking), detergent, textile, paper and distilling industry, they are also applied in many sectors such as clinical, medicinal and analytical chemistry. To improve the conditions for higher α-amylase production the effect of some basic factors such as carbon source and its concentration in fermentation medium, pH and the method of cultivation were studied.
Materials and Methods: The strain *Aspergillus oryzae* PP was obtained from Microbial Collection of the Department of Biotechnology, Sofia University and was cultivated in modified Czapek-Dox medium at 28°C for 96 h in Erlenmeyer flasks.
Results: Soluble or insoluble starch was used as a sole carbon source in the medium. Soluble starch was found to be the most effective inducer for amylase biosynthesis (812 U/ml). Due to its high price soluble starch was replaced with insoluble starch. pH for the highest amylase activity was 7,5 - 8. The growth of *Aspergillus oryzae* PP was limited by the starch after 48 h of the fermentation. We carried out fed-batch processes by adding 10 ml fresh medium in the first variant and 20ml medium in the second one with dropping of 20ml fermentation medium at 48h of the cultivation. The enzyme production was higher (with 26%) during fed-batch fermentation with dropping.
Conclusions: An improvement of α-amylase production was achieved by using of a proper carbon source, pH optimal for the strain growth and fed-batch cultivation with dropping.

FB12

GLUCOSE EFFECT ON POLYMETHYLGALACTURONASE SYNTHESIS BY IMMOBILIZED CELLS OF *ASPERGILLUS NIGER* 26

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Object of Research: Pectinolytic enzymes have great commercial importance for various industrial applications in juice and wine clarification, cotton pretreatment, paper and pulp manufacturing, waste management, animal feed, textile industry etc. Filamentous fungi from genus *Aspergillus* are considered the best pectinase producers. Cell immobilisation offers numerous advantages over normal suspended cultures, such as cell stability, higher cell densities, enhanced fermentation productivity, and feasibility of continuous processing. The aim of the present study was to study whether the regulatory mechanism catabolite repression takes part in the biosynthesis of polymethylgalacturonase (PMG) by immobilized cells of *A. niger* 26.

Materials and Methods: *A. niger* 26 conidiospores were immobilized in 3% Ca-alginate beads. Cultures were grown in medium containing pectin and enhanced concentration of glucose or glycerol at 28°C for 96 h. In the experiment with immobilized cells the duration of the cultivation cycle was 48 h. PMG activity was measured by the decrease in viscosity of the substrate solution.

Results: Glucose above 0.5% caused concentration-dependent decrease in PMG activity of free and immobilized cells. The decrease was result from a repression of the enzyme synthesis, rather than of inhibition of the enzyme secretion. Our data showed a nonspecific control of PMG synthesis by the carbon sourses. Glucose repression is reversible and operates on the translational level.

Conclusions: PMG biosynthesis by *A. niger* 26 is under high effective control of regulatory mechanism catabolite repression.

FOOD SAFETY

FS 7

THE ANTIVIRAL ACTIVITY OF EXTRACT FROM ST. JOHN'S WORT AGAINST POTATO VIRUS Y (PVY)

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Object of Research: Viral diseases are major problem in stable crop production, especially in vegetative propagated plants such as potato. Economically important viruses on potatoes in Bulgaria are PVY and PLRV. Conventional methods of virus control are limited to use of virus-free seed tubers and chemical control of insect vectors. The object of this study was to test the effect of different extracts from St. John's wort against Potato virus Y in test tobacco plants.
Materials and Methods: Liquid and solid fractions of methanol and n-hexane plant extracts were obtained from St. John's wort by Soxhlet extractor and concentrated by vacuum evaporator. The resultant liquid fractions were dissolved in water in different concentrations and sprayed to the plants. Plants were tested with DAS-ELISA for presence of PVY.
Results: The liquid fractions of 10% of methanol and n-hexane water extracts from *Hypericum perforatum* reduce DAS-ELISA values of PVY inoculated plants under the Cut off straight line. Solid fractions of 3% reduced the virus titer to the DAS-ELISA values of healthy plants.
Conclusions: We found that 10% liquid fraction and 3 % of solid fractions of methanol and n-hexane extracts were effective against plant pathogenic bacteria in vitro and reduce significantly DAS-ELISA values of PVY in virus inoculated tobacco plants.

Key words: PVY, St. John's wort extract

FS 8

VIRAL DISEASES OF POTATOES AS A PRECONDITION FOR DETERIORATION OF FOOD QUALITY

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Object of Research: Potatoes are one of the most common crops. One of the main factors for obtaining high yields and high quality for potato production is the use of healthy planting material, free of viral, bacterial and fungal pathogens. The object of this study was to identify viral diseases on potatoes in different regions in Bulgaria and to determine the viruses with great economic impact on potato food quality and quantity.

Materials **and Methods**: Leaf and tuber samples were taken from potatoes with different symptoms and damages and from symptomless plants too. The samples were tested for virus infection with DAS-ELISA and stain differentiation with Touchdown multiplex RT- PCR.

Results: Around 68 % from the tested plants were virus infected. Most of the virus infections were mixed. Potato leaf samples with the different symptoms expressed were infected with PVY, PLRV, PVX and PVA. Samples from symptomless potato tubers were infected with a lot of viruses – PVY, PLRV, PVM, PVA, PVS, PVX and TRV. Some of the symptomless leaf samples carried PVY and PVM.

Conclusions: Potato viruses with great economic impact on potato production and food quality were Potato virus Y (PVY) and Potato leaf roll virus (PLRV). Mixed infections with these two viruses gave loss of production of potato cv. Agria to almost 90%.

Key words: PVY, PLRV, potato quality

FS 9

THE DISINFECTIONS EFFECT ON SUSPENDED AND BIOFILM FORMATING *LISTERIA MONOCYTOGENES*

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Object of Research: The aim of this study was to investigate the disinfecting efficiency of some commercially available disinfectants, used in the food industry, against *L. monocytogenes.*
Material and Methods: Suspension and surface biofilm test were used in investigation the disinfecting efficacy of 5 disinfectants and 2 cleaners against 3 strains *L. monocytogenes* isolated from surfaces in food processing environment after cleaning and disinfection.
Results: All the disinfectants, were effective against suspended *L. monocytogenes* in clean and soiled conditions. There was no difference between the two cleaning methods. Cleaning with the sanitizer followed by disinfection with QAC 1, QAC 2, and QAC 3 resulted in a complete inactivation of *L. monocytogenes* biofilm. Cleaning with the sanitizer followed by disinfection with sodium hypochlorite (SH) reduced *L. monocytogenes* by 2.9 to 3.8 log cfu/cm^2, compared to controls. Disinfection with peracetic acid (PAA) after cleaning resulted in a 3.9 to 4.8 log reduction of bacterial biofilm.
Conclusions: *L. monocytogenes* formating biofilm on a stainless steel are difficult to remove mechanically from surface and more resistant to disinfectants than free suspended cells. QACs can completely inactivate *L. monocytogenes* biofilm with turbidity approximately log 7.0 cfu/cm^2.

FS10

MONITORING OF LISTERIA SPECIES IN INDUSTRIAL REFRIGERATORS IN BULGARIA

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Object of Research: Samples from inner surfaces of refrigerators in manufactures for ready-to-cook and ready-to-eat foods have been investigated.
Material and Methods: The samples were analyzed according to ISO 11290-1:1996. Each sponge were stomached in 225 ml Fraser Broth (containing 1/2 concentration of inhibitors and incubated 24 h at 30 °C, secondary Fraser Broth enrichment, inoculation on Oxford and Palcam agar and identification of *Listeria* spp. and *L. monocytogenes* morphological, biochemical, cultural and serological tests. The serotyping was done using O-listeria sera.
Results: There were positive for *Listeria monocytogenes* 9 samples (3.8%) from industrial refrigerators for ready-to-cook foods. *L. ivanovii* subsp. *ivanovii* was registered in 1 sample and in 11 samples - *L. innocua*. *Listeria monocytogenes* has been isolated from 6 samples (4.2%) from refrigerators for ready-to-cook meat products. The microorganism has been isolated from only 3 samples (3.1%) from inner surfaces of industrial refrigerators in poultry slaughterhouses. 31 strains *L. monocytogenes* (6.6 %) and 19 strains *L. innocua* (4.1 %) have been isolated from 468 samples from industrial refrigerators for ready-to-eat foods. The highest percent (7.7 %) positive for *L. monocytogenes* samples has been observed in refrigerators for safe keeping of raw materials for producing of salads on milk and mayonnaise basis. *L.monocytogenes* has been determined in 6.9 % and 4.9 % respectively in the samples taken from industrial refrigerators for baked products from minced meat and in raw dried and raw smoked sausages manufactures.
Conclusions: It has been concluded that inner surfaces of refrigerators can be contaminated with *L. monocytogenes* and these are one of the vectors of cross contamination of raw materials and ready production. Especially our attention was provoked by the data referring to ready-to-eat foods, as baked products from minced meat, raw dried and raw smoked sausages and variety kinds of salads.

FS11

REDUCING THE CONTENT OF RADIONUCLIDES IN FOOD PRODUCTS OF PLANT ORIGIN

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Object of Research: At radiation accident or transboundery transfer of radionuclides plants are one of the most affected elements of the environment as they accumulate radionuclides by the air or in the long run through roots when grown on contaminated soils. The radioactivity accumulated in this way reaches man by feeding increasing thus the radiation dose in humans.
Object of research in this work are the means for reduction of the radionuclides accumulated in plants and food products of plant origin to levels which allow their use as food for the population.
Materials and Methods: Conservation (by freezing, drying, canning) allows vegetables to be preserved long enough to collapse the short- lived radionuclides.
In case of surface contamination of fruits and vegetables (in the initial phase after the accident) a significant effect of cleaning is achieved by washing thoroughly with water or other appropriate solutions followed by lavish rinsing.
Results: Many methods for processing of raw materials can provide significant reduction of the radioactive pollution in foods. The plants whose parts are used for food but are protected by the sheet hugger shells are practically clean after removing the husks or adherent soil particles.
Conclusions: We have offered procedures and measures for recovery of plant production in case of radioactive contamination.

FS12

REDUCING THE CONTENT OF RADIONUCLIDES IN FOOD PRODUCTS OF ANIMAL ORIGIN

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Object of Research: The system of measures of precaution in the livestock breeding includes production technologies for food stuff of animal origin. They can be divided into four groups: restrictive organizational, zoo-technical and special. Object of our research are measures concerning the reduction of radioactive contamination of food products of animal origin thus allowing their utilization as food for humans. This can be realized in the personal sector and individual households and businesses in the food industry.

Materials and Methods: Several basic approaches in utilization of radioactively contaminated feed materials can be delineated

- Removal of the most contaminated parts of the raw material – peeling, skinning, boning.
- Depth processing through maceration, marinating and production of durable meet product.

Results: In intermediate phase of the accident the contaminated with J-131 milk may be used as cheese. The traditional technology for processing the milk into cheese has a high effect for removal of caesium. Procedures like cooking, maceration, salting, blanching are rather helpful for removing part of the caesium content in meat. Periodic replacement of the steeping liquor increases the rate of stripping of the meet.

Conclusions: Methods for primary treatment and technological processing of the raw materials are implemented. Ways to prepare food that can provide significant reduction of the radioactive contaminants in food are offered. The solutions used for soaking, blanching or boiling, must be discarded after processing.

FS13

THE INHIBITING EFFECT OF THE ESSENTIAL OIL OF *ORIGANUM HERACLEOTICUM* AGAINST *TOMATO SPOTTED WILT VIRUS*

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Object of Research: *Tomato spotted wilt virus* (TSWV) is wide spread on vegetables, flowers, field crops, essential oil-bearing and medicinal plants. TSWV decreases the yield of vegetables as well as the yield of leaves (herba), roots and seeds of the essential oil-bearing and medicinal plants and deteriorates their market qualities. The control of TSWV and its vector – thrips is important because it will minimize the chemical treatments of vegetable plantations (mainly tomato and pepper) and essential oil-bearing and medicinal crops, especially in view of their use for food and medical drugs.
The study covered the antiphytoviral activity or the inhibiting effect of oregano (*Origanum heracleoticum* L.) essential oil against TSWV and whether it could be used for virus control.
Material and Methods: The essential oil was diluted in different concentrations: 500, 1000, 2000, 4000, 5000 and 10000 ppm. The indicator plants *Petunia hybrida* were inoculated by mixed ingredients – *Tomato spotted wilt virus* (TSWV) infectious sap and different kinds of diluted oils.
Results: According to the systemic reaction on *P. hybrida,* the solution of 5000 ppm (0.5 %) of oregano essential oil had an inhibiting effect against TSWV. No symptoms of TSWV or any phytotoxic effect were observed on the tested plants in the concentration of 5000 ppm. Diluting of the oregano essential oil to 5000 ppm could be recommended for TSWV control.
Conclusions: This is the first report on the inhibiting effect of oregano essential oil against plant viruses in Bulgaria, TSWV in particular.

Key words: *Origanum heracleoticum,* antiphytoviral effect, *Tomato spotted wilt virus.*

FS14

LEAD CONTENT IN CARP AND GRASS CARP HERBIVOROUS DANUBE FISH

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Object of Research: Lead is one of the best recognized toxic water pollutants. It affects most of the human organs and systems as gastro-intestinal tract, muscles, kidneys, peripheral and central nervous system, reproductive, respiratory, urinary, skeletal, cardio-vascuolar, endocrine and hemopoetic systems. Carp and grass carp are among the most often consumed fresh water fishes. The aim the research was to study the herbivorous fish carp (*Cyprinus carpio*) and grass carp (*Ctenopharingodon idella*), collected in January 2015 in Vidin section of the Danube river for lead content.
Materials and Methods: Pb concentrations were evaluated *via* atomic Electrothermal Atomic Absorbtion Spectrophotometry with vapor atomization (ETAAS). Samples were prepared from muscle tissue of the fish and analyzed *via* the standard protocol. Maxumum permission standard values of fish contaminants were discussed according the EC Regulation № 1881/2006.
Results: Our analysis showed concentration 0.0062 mg kg^{-1} of Pb (MPS 0.3) for *Cyprinus carpio* and 0.019 mg kg^{-1} Pb (MPS 0.3) - for *Ctenopharingodon idella*. Both samples demonstrated no lead contamination exceeding values described in the EC 1881 Regulation.
Conclusions: The herbivorous Danube fish carp and grass carp contain Pb concentrations which do not exceed the Maximal residual limits and are safety for consumption by Bulgarian citizens.
Acknowledgements: This work was funded by Grant № 972/2015 of the Central Fund for Strategic Development (CFSR), New Bulgarian University.

Key words: Danube fish, herbivorous, lead toxicity

FS 15

MERCURY IN PREDATORY DANUBE FISH - EUROPEAN CATFISH (*SILURIS GLANIS*) AND PIKE PERCH (*SANDER LUCIOPERKA*)

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Object of Research: 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, proteins and DNA peroxidation. Hg concentration persists in higher concentrations in carnivorous compared to herbivorous fish. Concentrations of total mercury were determined in two muscle tissue samples of predatory Danube fish - European catfish (*Siluris glanis*, Linnaeus 1758) and pikerperch (*Sander lucioperka*, Linnaeus 1758).
Materials and Methods: Catfish and pike perch were collected in November 2014 in Danube River (Vidin section, Bulgaria). Muscle samples were analyzed for Hg content *via* CVAAS (Cold Vapour Atomic Absorption Spectrometry) at the Central Laboratory of Veterinary Sanitary Expertise and Control, Bulgarian Food Safety Agency.
Results: The total mercury concentration of the examined samples did not exceed the hygienic limit for Hg for predatory fish. The results indicate that the Hg concentration in *S. glanis* as an omnivorous benthic/pelagic species was 0.033 mg kg^{-1} (MPL 0.5 mg kg^{-1}). Lower Hg content was registered in the muscles of the carnivorous pelagic species *S. lucioperka* – 0.009 (MPL 0.5 mg kg^{-1}). Results obtained are in concordance with the recommended by the EC values described in the Regulation 1881.
Conclusions: No mercury contamination was registered in the catfish and pike perch Danube fish. However, the Hg is biomagnifying through the food chain no risk for the human consumption is posed in the investigated fish species.

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

Key words: mercury**,** toxicity, Danube fish

FS16

ANALYSIS OF MERCURY POLLUTION IN HERBIVOROUS BLACK SEA FISH - ATERINA, SPRAT AND SARDINA

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Object of Research: Mercury is a pronounced toxic heavy metal which is monitored in all food products. It possess complicated toxicity profile. Exposure of humans to Hg0 vapor as well as to the organic MeHg leads to the Central nervous system injury including neurons death (ALS). Chronic exposure to inorganic mercury cause stomatitis and tremor. The target organ of mercury are kidneys when disposed to mono- and bi-valent mercury salts (Hg^{+} and Hg^{++}).
Materials and Methods: Aterina, sprat and sardina were collected at Black sea near the town of Sozopol, Bulgaria. Muscle samples were analyzed for Hg content *via* CVAAS (Cold Vapour Atomic Absorption Spectrometry). Our data obtained were compared with the Maxumum residual limits of the EC Regulation № 1881/2006.
Results: Our analysis showed Hg concentrations in the investigated fish samples as follows: 0.027 mg kg^{-1} of Hg in aterina, 0,020 mg kg^{-1} – in sprat and 0,029 mg kg^{-1} in sardina. According the Regulation 1881 the MRL of Hg is 0.5 mg kg^{-1}. Regarding the results obtained no Hg pollution was detected in the three investigated fish species.
Conclusions: According the Hg contamination, the herbivorous aterina, sprat and sardina Black sea fish are safety for consumption and meet all EC Food safety standards.

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Key words: Black sea fish, herbivorous, inorganic mercury toxicity

FS17

CADMIUM AND LEAD CONCENTRATIONS IN TWO PREDATORY BLACK SEA FISH SPECIES – BLUEFISH (*POMATOMUS SALTARIX*) AND HORSE MACKEREL (*TRACHURUS MEDITERRANEUS* PONTICUS)

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Object of Research: Cd and Pb damage the respiratory, gastrointestinal, reproductive and nephrous systems. Cadmium leads to bone fractures, calcium demineralization and is pronounced cancer inductor. The bones accumulation of lead and its continuous release in blood could harm the human health for decades. Two Black sea predatory fish species - Bluefish (*Pomatomus saltarix*) – juvenile and mature individuals as well as horse mackerel (*Trachurus mediterraneus ponticus*) were assessed for Cd and Pb content.
Materials and Methods: Bluefish and horse mackerel were collected in October 2014 near the towns Sozopol and Nessebar at Black sea coast and were stored at -20^{o}C prior to analysis. Samples were prepared from muscles tissue. Cd and Pb concentrations were evaluated *via* Electrothermal Atomic Absorbtion Spectrophotometry with vapor atomization (ETAAS). Data obtained were compared with the Maximum residual limits (MRL) of fish contaminants described in the EC Regulation № 1881/2006.
Results: According the Cd concentrations our results demonstrated no contamination in both juvenile and mature bluefish: in the juvenile individual – 0.029 mg kg^{-1} Cd and 0.023 mg kg^{-1} Cd- in the mature one (MRL=0.05 mg kg^{-1}). No excess of this metal over the MRL was registered in the horse mackerel too – 0.005 mg kg^{-1}.
Analysis of lead concentrations showed no excess of the MRL and was in accordance with the Regulation № 1881/2006 as well. In the juvenile and mature Bluefish Pb content was 0.053 and 0.020 mg kg^{-1}, resp. (MRL=0.3 mg kg^{-1}). Our results displayed no lead contamination in the horse mackerel - 0.027 mg kg^{-1}. All results obtained coincide the data of The National Monitoring Program. According to it no Pb and Cd contamination was registered for the period 2000-2015 in the investigated fish species.
Conclusions: Bluefish and horse mackerel Black sea fishes are not polluted by Cd and Pb and meet the food safety standards.

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FS18

HEAVY METALS' UPTAKE BY THE GREEN ALGA *SCENEDESMUS INCRASSATULUS*

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Object of Research: *Scenedesmus incrassatulus* belongs to the group of green algae which wide practical application provides opportunities to the food additives market, cosmetics and pharmaceutical sector. However, it is well known that green algae have the ability to extract and accumulate large amounts of heavy metals. This raises a number of issues about its use and also leaves open the question about the heavy metals' toxic concentrations in algal biomass.
Materials and Methods: *Scenedesmus incrassatulus* R-83was intensively grown in 200 ml vessels on the Setlik's medium (1967) modified by Georgiev et al. (1978), under continuous illumination of 150 μmol phot m-2 s-1. A carbon source was provided by bubbling sterile 2% CO2 in air. Heavy metals' treatment was performed by adding $CdCl_2$, $(CH_3COO)_2Pb$ and $CuSO_4$ at concentrations 50 and 100 μM into the medium. Algal growth was determined on the 10th day by the increase of dry weight. Pigment content was measured after methanol extraction and calculated according to McKinney (1944). Malone dialdehyde was determined after Dhindsa et al. (1981). Heavy metals' uptake was determined by AAS spectrometer Perkin Elmer.
Results: The lowest growth rate of *Sc. incrassatulus* was measured at 100 μM Cd^{2+} in the medium. Strong inhibition of growth was also observed when algae were treated with Pb^{2+}. Chlorophyll a and chlorophyll b contents were decreased in the greatest extent after Cd treatment. Carotenoid content remained slightly affected in all experimental variants. Malone dialdehyde content was significantly increased, especially in Pb-treated cultures. The results demonstrated the exceptional affinity of the strain to uptake Cu^{2+}, Cd^{2+} and Pb^{2+}. All metals were absorbed almost completely from the culture medium following the sequence: Pb>Cd>Cu.
Conclusions: The toxic effects of heavy metals on the growth and pigment biosynthesis of *Sc. incrassatulus* was in the order: Cd, Pb, Cu.

MICROBIOLOGICAL CONTROL OF FOOD

MCF3

MICROBIOLOGICAL CONTROL OF PACKAGING MATERIAL FOR FOOD PRODUCTS

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Object of Research: With the implementation of HACCP systems in industrial facilities, suppliers which are engaged in the production and packaging material of food products must meet appropriate standards. One of the main aims is sterility testing of packaging materials for food products.

Materials and Methods: Three major industrial facilities on territories covering by JZU CJZ Kocani were included. During the three months period the following samples were taken for analysis: 1. Card board packagingNo. 8; 2. Cellophane packing 24 pcs. 3. Laminated packaging miniroll No. 10; 4. Foil packaging No. 8; 5. Plastic bags for packing 20 pcs. Yeast extract agar with chloramphenicol was used for the isolation of yeasts and molds at room temperature of 22-25 ° C for 3-5 days, and isolation of other bacterial contaminants on TSA – Tripticsoy agar. We performed Re-incubation after 3-5 days on YEAC. The results on TSA were read in 24/48 hours.

Results: In cellophane packaging were isolated molds in 10 samples or 41.6 % which did not meet the legislation, while 58.3% correspond to the statut or yregulations.In cardboard packaging, plastic packaging miniroll, foil packaging and plastic bags for packaging were not detected yeasts and molds. All 46 samples corresponding to regulation. Out of the 70 tested samples 10 or 14.28% were with positive findings and isolated molds, while the remaining 60or 85.72% of the samples were negative. Other bacterial contaminants were not detected.

Conclusions: Packaging used in these facilities purchased from a foreign manufacturers provided with the ISO certification and stored in an appropriate way (cardboard, plastic mini roll, foil and plastic bags) satisfy the conditions for production and trade of food products. While cellophane which is homemade with not implemented quality certificate or quality control does not meet the requirements.

MCF4

ANTIBACTERIAL ACTIVITY OF EXTRACTS FROM MEDICINAL AND WEED PLANTS AGAINST PHYTOPATHOGENS OF TOMATO AND PEPPER

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Object of Research: Plant pathogenic bacteria cause serious and devastating diseases every year leading to great crop losses. The management of these diseases is hard, complex and expensive because of the lack of adequate control measures. In the recent years the antimicrobial activity of different plants extracts is intensively being investigated for the purposes of plant protection as an alternative to pesticides. Nevertheless, only a small percent of plant species have been still investigated. The object of this study was to test the effect of different extracts from medicinal and weed plants against phytopathogenic bacteria of tomato and pepper.

Materials **and Methods:** Fresh plant parts (leaves and flowers) were dried to absolute dry weight, ground and stored in air-tight brown bottles. Extractions were prepared in Soxhlet extractor with methanol as solvent and concentrated in vacuum evaporator. The soft and solid fractions were diluted in water using dimethylsulfoxide (DMSO). The *in vitro* test for antibacterial activity was completed by the agar diffusion method.

Results: Good antibacterial activity against *Clavibacter michiganensis* subsp. *michiganensis* revealed the extracts from *Hypericum perforatum* and *Iva xanthifolia*. Extracts from *Melilotus officinalis* and *Datura stramonium* showed weak effect against *Xanthomonas vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis*. The extract from *Chelidonium majus* proved to be effective against the three pathogenic species *C. michiganensis* subsp. *michiganensis*, *X. gardneri* and *X. vesicatoria* and has best potential for their control.

Conclusions: Plant extracts possess good potential for control of plant pathogenic species.

MCF5

STUDY OF PIGS AT SLAUGHTER FOR PATHOGENIC *YERSINIA* STRAINS

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Object of Research: Yersiniosis is the forth reported food-borne zoonosis in the EU, caused by human pathogenic *Y. enterocolitica* (>98%) and *Y. pseudotuberculosis* (EFSA, 2013). Pigs are the main reservoir for human pathogenic *Yersinia*, carrying asymptomatically bacteria, predominantly in their tonsils. In the recent study tonsils of pigs at slaughter are investigated for the presence of pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* bio/serotypes.
Materials and Methods: Samples of pigs tonsils were cut aseptically and homogenized in stomacher. Enrichment was carried out on peptone sorbitol bile salts broth for 48 h at 26 ^{o}C, followed by plating on CIN agar and incubation at the same conditions. Isolated colonies suspected for *Y. enterocolitica* and *Y. pseudotubercolosis* were tested by PCR analysis.
Results: Out of 286 samples examined, 5 pathogenic *Y. enterocolitica* strains were detected within 3 batches coming from Sofia region and 3 pathogenic *Y. enterocolitica* strains in one batch originating from the Shumen region. The estimated prevalence was in total 2.8%. For Sofia region the prevalence was 7% and for the Shumen region - 11.5%. Biotyping, serotyping and phagotyping was performed in *Yersinia* Reference Center at Pasteur Institut in Paris, France and the isolates were confirmed as 4/3/VIII. None of the samples were positive for *Y. pseudotubercolosis.*
Conclusions: Occurrence of pathogenic *Y. enterocolitica* strains in pig tonsils does not exclude the possibility for contamination of pork in some regions of the country being potential risk for the health of costumers.

MCF6

ANTIBACTERIAL ACTIVITY OF CAYENNE PEPPERS

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Object of Research: *Capsicum annuum* fruits are rich sources of vitamin C and vitamin A. Pepper extracts are popular for mycosis and arthritis treatment. They possess a sharp flavor, due to the presence of capsaicinoids, which deter most animals from eating the fruit. The aim of our research was to examine the antibacterial activity of cayenne pepper fruits and seeds against test bacteria *Bacillus subtilis* N°8751 and *Escherichia coli* N°8752.

Materials and Methods: 0.1 mL of test bacteria *E.coli* ($1.7x10^8$ CFU/ml) and *B.subtilis* ($3.2x10^8$ CFU/ml) were spread on Nutrient agar. The peppers were washed with sterile destilled water and cut open with a sterile scalpel. The pepper fruit parts were separated in 3 samples.

Sample 1: The pepper tissue was scraped of skin with a sterile scalpel and the samples were loaded in aseptically perforated Nutrient agar wells.

Sample 2: The pepper fruit was perforated with a sterile perforating tool to obtain 0.5 mm discs, which were placed on the surface of the inoculated agar plates.

Sample 3: The seeds were separated from the fruit and were placed on the surface of the inoculated agar plates.

The samples were cultivated at 37°C for 24 hours.

Results: Our results showed that cayenne pepper fruits and seeds inhibit the growth of both *E. coli* and *B.subtilis*. Sample 1 pepper tissues displayed no antibacterial activity. Sample 2 discs showed antibacterial activity against both *E.coli* (25 mm inhibition zone diameter) and *B.subtilis* (24 mm). The seeds (sample 3) also exhibited inhibition zones of 11 mm against *E.coli* and 7 mm against *B.subtilis*.

Conclusions: Cayenne pepper tissue and seeds possess antibacterial properties against both *Escherichia coli* and *Bacillus subtilis*.

MCF7

METHODS FOR DESTRUCTION OF MICROORGANISMS DURING CLEANING AND DISINFECTION OF THE FERMENTATION TANK FORYOGURT 'KABI' GJILAN PRODUCTION

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Object of Research: The purpose of the study was the safety and quality of yogurt "Kabi" production *via* fermentation.
Materials and Methods: Different media were used for the identification of bacteria**:** for *Enterobacteriaceae* – Violet Red Bile Glucose Agar, at 37˚C for 18-24h, for *Staphylococcus auerus* – Baird Parker agar, at temperature 37˚C for 24-48h, for *Salmonella* - SS–agar, at 41.5˚C for 24h, for *Aspergillus* – CYA agar, at25-30˚C for 3-7 days.
Results: Data according to the evaluation tests are presented in Table 1 and Table 2. We registered mesofile aerobic bacteria, which number before disinfection was ≥10 cfu/cm^2. The mentioned bacterial number was detected, due to the insufficient hygiene.
Conclusions: The pathogens growth was connected with the samples composition and handling. The increased pathogens number was due to hygienic conditions during the processing, as well as factors such as: the pH, the temperature, etc.

Key words: disinfection, microorganisms

MCF8

MICROBIOLOGICAL ANALYSIS OF WATER OF RIVER PËRLEPNICA DURING SPRING SEASON 2010

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Object of Research: Aim of this investigation was to estimate the quality of water of river Përlepnica, Kosovoduring the spring season 2010.
Materials and Methods: In the bacteriological analysis, coliform group of bacteria were differentiated by selective medium. Water samples were collected during the spring 2010. Water samples were collected from three different locations along the river.
Nutrientagar (NA), *Salmonella Shigella*agar, VioletRedBile Glucose Agar, Bileaesculinagar, were used to determine heterotrophic bacteria, *Salmonella* and *Shigella*, total coliform and *Streptococcus faecalis*, respectively. All plates were incubated at 350°C for 24h.
Results: The paper discusses results of bacteriological analysis done on various water samples taken from different (3) locations in the river. The total viable count of bacteria for all water samples were quite highranging from 6.3 x 10^2cfu/ml to 2.01 x 10^2cfu/ml.
The isolates were characterized and identified as total coliforms, *Streptococcus*, *Heterotroph* , *Salmonella*, *Shigella* and fungi.
Conclusions: The present study indicates the water in the river Përlepnica was polluted with different bacterial species,which will have serious effects.

Keywords: bacteria, river Përlepnica, spring.

MCF9

MICROBIOLOGICAL ANALYSIS OF THE WATER OF RIVER PËRLEPNICA

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Object of Research: Waterborne bacterial pathogens were isolated from waters in river Përlepnica, Kosovo.
Materials and Methods: In the bacteriological analysis, coliform group of bacteria are differentiated by selective medium. Water samples were collected during the spring season 2011 from three different locations along the river.
Results: The paper discusses results of bacteriological analysis done on various water samples taken from different (4) locations in the river. The isolates were characterized and identified as Total coliform, *Streptococcus, Heterotroph, Salmonella, Shigella* and fungi.
Conclusions: The study therefore, stresses on the need to control the fecal pollution in the water.

Keywords: *E.coli, Salmonella, Shigella*, water, river, Përlepnica.

MCF10

MICROBIOLOGICAL ANALYSIS OF WATER FROM THE RIVER ERENIK (KOSOVO) DURING SPRING SEASON 2010

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Object of Researh: The objective of this study is to assess the quality of water, of the river Ereniku during the spring season 2010 year, through the microbiological analysis.
Materials and Methods: Microbiological explorations of the microorganisms in river Ereniku reservoir were carried out during the spring season 2010. Water samples were collected monthly from three different locations along the river. Nutrient agar (NA), *Salmonella Shigella* agar, Violet Red Bile Glucose Agar, Bile aesculin agar, were used to determine heterotrophic bacteria, *Salmonella* and *Shigella*, total coliform and *Streptococcus faecalis*, respectively. All plates were incubated at 35^0C for 24 h.
Results: The results obtained were compared with reports of WHO and EPA standards for Drinking and Recreational Water. All groups of the bacteria exceed the maximum permission standard values of bacterial number.
Conclusions: Based on the obtained results we could conclude:
The waters of river "Ereniku" is highly polluted by bacteria at all locations.
A high number of all microorganisms, at all localities was registered.
On the base of coliform bacteria number according to Tumpling system, the water of "Ereniku" river belongs to fourth class of pollution.

Key words: irrigation, water, bacteria, analysis, Ereniku, Kosovo

MCF11

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF *GEUM URBANUM* EXTRACTS

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Object of Research: Pathogenic bacteria have always been considered as a major cause of human and animal infections. Even though pharmaceutical companies have produced new antibacterials in the last years, resistance to these drugs has increased and has now became a global concern. Many plants used in traditional medicine are valuable source of new substances with therapeutic potential. In the recent study the chemical composition of different extracts of the herb *Geum urbanum* and its antibacterial activity were investigated.

Materials and Methods: Dry mass of aerial and underground parts of the herb was extracted with methanol and re-extracted with petroleum ether, ethyl acetate and butanol MIC and MBC were determined by using liquid broth microdillution method.

Results: All extracts displayed varying antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Listeria monocytogenes*). No activity was found against Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) and *Candida albicans* as well. The most sensitive bacteria are *Staphylococcus aureus* and *Staphylococcus epidermidis*. The ethyl acetate extracts of aerial parts and roots were the most active. We isolated five chemical substances using different chromatographic techniques. The compounds were identified by NMR – catechine, gein, ellagic acid glucoside, wich is new for the species and two ellagic acid acetylrhamnosides, which are new for the genus *Geum*.

Conclusions: *Geum urbanum* possesses antibacterial activity against Gram-positive bacteria. The activity of individual substances will further be studied which will contribute to the sustainable use of Bulgarian natural resources and treatment of various skin sequelae, food infections, etc., which are related with the corresponding bacterial pathogens.

MCF12

EVALUATION ON INFLUENCE OF FEED ADDITIVE SPIRULINA PLATENSIS ON LIVESTOCK REPRODUCTION

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Spirulina platensis is microalgae and potential food for many agriculture animals. His influence on the development of animals comes from its nutritional composition rich proteins, minerals, micro-and macronutrients. The purpose of this article is to review the findings to date on the use of spirulina as a supplement feed and its effect on animal health, performance and reproduction. Research results with algae showed its positive effect on productive and reproductive performance of animals, including health status, which are valid also for human, because *Spirulina* is modern feed additive in last year's. However, the present knowledge of the animal's response to nutrient *Spirulina platensis* is relatively scarce. Research on biological mechanism of action of *Spirulina platensis* will improve their knowledge and future applications as healthy additive.

Key words: *Spirulina platensis,* reproduction

PREBIOTICS AND PROBIOTICS

PP 4

ISOLATION AND INITIAL CHARACTERIZATION OF LACTIC ACID BACTERIA FROM TRADITIONAL DAIRY PRODUCTS

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Object of Research: Traditional dairy products are widely accepted as functional foods with positive effects on human health. Therefore, it is important to study their autochthonous microflora in order to preserve traditions in dairy production and design new functional foods. With this aim, new strains of lactic acid bacteria (LAB) were isolated from different samples of home-made yoghurt and white brined cheese.

Materials and Methods: Isolation procedure for LAB was adapted in accordance with the different milk products. The pure cultures were isolated on MRS and Rogosa agar plates after 48 h cultivation at 37ºC (for isolates from white cheese) and 42ºC (from yoghurt). In addition, antimicrobial and caseinolytic activity were examined by classical agar-diffusion methods.

Results: A new advanced protocol, including a step of overnight cultural enrichment and a modification of the MRS medium with yeast extract/peptone (0.1% v/v), was used. A high viability of LAB microflora was proved. Ten strains (from buffalo milk yoghurt), 14 strains (different types of white cheese) and 1 (yellow cheese) were collected and initially characterized as *Lactobacillus* sp. They showed low caseinolytic activity. The exponential LAB cultures in MRS broth and in skimmed milk (10% w/v) showed a better activity against Gram-positive (*Bacillus subtilis* strain ATCC 6633) than Gram-negative (*Escherichia coli* K12) bacteria.

Conclusions: Obtained results are only an initial step in characterization of the newly isolated lactobacilli. Further technological and functional evaluation is needed and is still in progress.

Keywords: *Lactobacillus,* antibacterial and caseinolytic activity

PP5

ANTIFUNGAL ACTIVITY AND ENZYME PROFILE OF *LACTOBACILLUS BULGARICUS* GLB44 (PROVIOTIC®)

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Object of Research: Moulds and yeasts are the main spoilage microorganisms, responsible for significant economic losses and several healthy risks in human food chain. The antimicrobial activity is an important criterion for the selection of bio-protective lactic acid bacteria (LAB). A limited data exists on the antifungal activity of Bulgarian LAB and their enzyme profile. With this aim, the activity of the commercially available probiotic Proviotic®, containing the strain *Lactobacillus bulgaricus* GLB44, against yeasts and deteriorative and toxigenic moulds, and the presence of key enzymes, were studied.

Materials and Methods: *Lactobacillus bulgaricus* GLB44 (property of Genesis Laboratories LTD) was screened for antifungal activity against five mould species - *Aspergillus flavus*, *Aspergillus niger*, *Fusarium graminearum*, *Trichoderma viride* and *Penicillium claviforme* and three yeast species - *Saccharomyces cerevisiae*, *Kluyveromyces marxianus* and *Rhodotorulla* sp., using agar diffusion method. The enzyme profile of the *L. bulgaricus* GLB44 was determined using API ZYM miniaturized test (BioMerieux, France), following the manufacturer's instructions.

Results: *Lactobacillus bulgaricus* GLB44 possess a high amino-peptidase, acid-phosphatase and β-galactosidase enzymatic activity and a complete lack of the associated with the colon carcinogenesis β-glucuronidase activity.

The *in vitro* tests revealed a broad spectrum of antifungal activity. *L. bulgaricus* GLB44 completely (100%) suppress the growth of *Aspergillus flavus*, *Fusarium graminearum*, *Trichoderma viride*, *Penicillium claviforme*. With regard to *Aspergillus niger*, a lower inhibitory activity (66.6%), was observed. Proviotic® demonstrated a stronger inhibition against *Saccharomyces cerevisiae*, compared to the used as a control commercial antibiotic Fungostatin.

Conclusions: The demonstrated strong proteolytic activity of Proviotic® makes the *Lactobacillus bulgaricus* GLB44 interesting for use in the production of antihypertensive and immuno-modulatory products and also in the manufacture of different dairy products. The antifungal activity of Proviotic® is a promising advantage, suggesting its potential applications in different food technologies as a bio-preservative agent and a health promoting products against fungi.

PP6

LACTIC ACID PRODUCTION FROM INULIN-TYPE FRUCTANS BY BULGARIAN LACTIC ACID BACTERIA

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Object of Research: Inulin and its partially hydrolyzed derivative oligofructose are made up of linear β-(2-1) glycosidic bonds of D-fructose, often with a terminal glucose moiety that is linked by an α-(1-2) glycosidic bond, as in sucrose. Despite the increasing evidence of the fermentation of inulin-type fructans by certain lactobacilli, no information on the kinetics of their conversion is available. The aim of this work was to investigate the ability of Bulgarian LAB isolates to ferment inulin-type fructans and to study their kinetics of growth and fructan degradation.
Materials and Methods: Three strains, belonging to genera *Lactobacillus* and *Pediococcus* were used. Batch fermentations in MRS, containing 20 g/l inulin, instead the glucose, were carried out without pH control at 37ºC. The quantification of carbohydrates and lactic acid concentrations was done by HPLC system equipped with RID and HPLC column Aminex HPX-87H at 65°C.
Results: All strains were able to degrade inulin-type fructans. Lactic acid was the main metabolic end product, as its final concentration varied between 8.2 and 16.9 g/l in 48 h. One of the strains was hetero-fermentative and produced also 3.52 g/l ethanol and 1.08 g/l succinic acid.
Conclusions: The capability of Bulgarian lactic acid bacteria to ferment prebiotics made them useful probiotic supplement in food and beverages.
Acknowledgements: This work was supported by the Grant NSF-B02/27 of Scientific Research Fund of Republic of Bulgaria.

PP7

MOLECULAR IDENTIFICATION OF LACTIC ACID BACTERIA CONVERTING FRUCTO-OLYGOSACCHARIDES

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Object of Research: Lactic Acid Bacteria (LAB) play important roles in the food production and are increasingly used as a health-promoting probiotics. Fructo-oligosaccharides (FOS) are often used as alternative sweeteners by the food industry. FOS can be produced by degradation of inulin and comprise poly-fructose - a polymer of D-fructose residues, linked by β(2→1) bonds with a terminal α(1→2) linked D-glucose.*LAB* strains that convert inulin are rare and desirable as a probiotic additive to prebiotic food supplements.
Materials and Methods: Three LAB strains were isolated from traditional Bulgarian food. Their capability to ferment inulin was proved in MRS, containing 20 g/l inulin, instead the glucose. The genomic DNA was isolated from 24 h cultures using GeneJET Genomic DNA Purification Kit. PCR amplification was performed with 16S universal primers in QB-96 Satellite Gradient thermal cycler, under the following temperature profile: 3.5 min, 94 °C; 35 cycles: 1 min, 94°C, 30 s, 56 °C, 30 s, 2 min, 72 °C; final elongation 7 min at 72 °C.
Results: The ability of LAB strains to convert inulin-type FOS was proved. Molecular identification of the strains was done by the methods ARDRA (with *Sau*3A restriction endonuclease), RAPD (using primer M13), and 16S rDNA sequencing. Strains' identification, based on genetic criteria showed that two of the strains belong to the species *L. paracasei*, and one strain - to *Pediococcus acidilactici*. RAPD by the use of M13 primer was not indicative for the species affiliation.
Conclusions: Isolation and species identification of LAB strains, converting FOS attract increased academic and industrial interest, due to their application as potential probiotics.
Acknowledgements: This work was supported by the Grant DFNI B02/27 of Scientific Research Fund of Republic of Bulgaria.

PP8

CHARACTERIZATION OF POLYSACCHARIDES OBTAINED FROM ESSENTIAL OIL PLANTS WASTES

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Object of Research: The wastes generated by essential oils industry are promising source of polysaccharides and to our knowledge this problem is unexplored. The aim of the present work was to investigate the potential of wastes of three essential oils plants – rose, marigold and chamomile, for obtaining of polysaccharides and characterization of their physico-chemical properties.
Materials and Methods: The rosepetals waste (*Rose Damascene* Mill) was obtained from Zlatna roza distillery, (Zlatosel, Plovdiv, Bulgaria – 2012 crop). The chamomile (*Matricaria Chamomila*) and marigold (*Calendula officinalis*) wastes were obtained from Ecomaat Ltd. (Sofia, Bulgaria – 2012 crop). The material was kept frozen before further treated.
Results: Water soluble polysaccharides were obtained from the wastes of rose petals, chamomile and marigold flowers. The higher yield was observed for waste rose petals pectin – 11%. Their chemical composition was investigated and was found that all of them are medium-metoxyl pectins (DM around 50%) with galacturonic acid content more than 50%. The lowest surface tension value of 0.6 % solutions showed chamomile pectin and it is comparable with beet pectins and locust bean gums. DTA analysis showed that the pectins were thermally stable until 220°C.
Conclusions: This investigation showed that the chamomile, marigold and rose wastes are promising source of pectic substances. They could be used also as flour for incorporation in thermally treated foodstuffs for enrichment of their dietary fiber contents. To our knowledge this is the first report for physico-chemical characterization of pectic substances isolated from certain essential oil plants wastes.

PP9

SELECTION CRITERIA FOR LACTIC ACID BACTERIA TO BE USED AS STARTER AND PROBIOTIC CULTURES

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Object of Research: Production of starter and probiotic cultures of lactic acid bacteria (LAB) occupies a strategic place in modern biotechnology determined by the health potential of these microorganisms. The global dairy industry is constantly exploring new ways to improve products by looking for strains with different properties that can guarantee stability and high quality production.

Materials and Methods: With the aim of developing new starter and probiotic culture each newly isolated strain goes through three scales of research. (i) Laboratory scale - the initial characterization of the isolates by a set of phenotypic and genetic methods as well as evaluation of some technical parameters and/or the *in vitro* health effect depending on the strain's application. (ii) Production scale - selection of suitable growth media, optimization of the processes of fermentation and freeze-drying for the production of active cultures. (iii) Industrial scale- assessment the technological effectiveness in a real production process and conducting clinical trials corresponding to the target of the probiotic product.

Results: As a result of the company research a large number of unique LAB strains have been selected.

Conclusions: On the base of their functional and technological characteristics reliable and competitive starter cultures for the dairy, meat and bakery industries are developed as well as effective probiotic products that are available on the market for wealth and functional foods.

REPORTS ON CORRESPONDING TOPICS (VARIA)

V11

GLOBAL NUTRITION REPORT 2014 ACTIONS AND ACCOUNTABILITY TO ACCELERATE THE WORLD'S PROGRESS ON NUTRITION

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The good nutrition is the center of human prosperity and well-being and sustainable development agenda. The First Global Nutrition Repot was launched in November 2014. It offers a number of findings regarding the progress in improving nutritional status all over the world. The objective of research is to address the global nutritional challenges and to present and analyze the data for Bulgaria. For achieving this objective a documental method was used, covering review of data resources from 193 United Nations members. Data for Regional and Country progress on World Health Assembly Indicators were presented, as well as data for economics and demography, child anthropometry, adolescent and adult nutrition status, etc. The research shows nearly every country in the world is affected by malnutrition. For instance, the rate of Vitamin A deficiency in preschooler age is <1 % only in two countries, reaching 84.4 % in Kenia. Data for Bulgaria shows that the major national problem reported is the adult overweight with a prevalence rate 54.3%, compared to a global average level of 33 %. The rate of anemia in women in reproducible rate age is slightly higher than the global average. In the country, the availability of fruits and vegetables has decreased almost twofold during the last 15 years. As a conclusion, it can be summarized that the complex, overlapping forms of malnutrition (wasting and obesity) is the "new normal", challenging the nutritional policy-makers to take focused action and to send right communications. Evidence show that improvement in nutrition status will make large contributions to eradicating poverty and improving food, health, education, gender and employment status of the population.

V12

COMPARATIVE ANALYSIS OF CATCH PER UNIT EFFORT OF TWO COMMERCIAL FISHING VESSELS TRAWLING FOR SPRAT (*SPATTUS SPRATTUS*) IN THE SOUTHWESTERN SECTOR OF THE BLACK SEA

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Fish are an important source of wild protein for the mankind worldwide. In fisheries and conservation biology catch per unit effort (CPUE) is an indirect measure of fish stock abundance and density.

In this study we compare the fishing capacity of two commercial fishing vessels, indicated by their CPUEs, over a large period of time. The vessels (RK 26 and RK 28) have the same technical specifications, and during the studied period operated in the same time and space (Burgas Bay), and targeted the same species of interest (*Sprattus sprattus*).

The aim was to compare the vessels' CPUE values, and if they were found to be similar or equal, to collect data for fish stock assessments from only one ship of the local fleet.

The analyzed dataset covers 2006 – 2013 period, and contains information about the total sprat catches, the number of fishing operations, and the locations of their trawling areas. The CPUE indices of the vessels were compared by means of the statistical t-test.

Throughout most of the studied period, RK 26 was characterized by higher CPUE values than RK 28.The inverse trend, with higher CPUE values for RK 28, was recorded for the last year (2013) of the investigation, when the captain of this vessel was substituted.

When using CPUEs for identification of fishing capacity of commercial fleets and for evaluation of fish stock abundance, all catch data from the different vessels should be included in order to reduce the errors in fish stock assessment.

Key words: CPUE, sprat, commercial vessels

V13

FIRST RECORD ABOUT NATURALLY OCCURRED ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA* ON *TUTA ABSOLUTA* (LEPIDOTERA: GELECHIIDAE) IN BULGARIA

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Object of Research: *Tuta absoluta* (Lepidoptera: Gelechiidae) is an economically important pest of tomato crops in greenhouses and in open field. The tomato borer is an invasive species for Europe, North Africa and Middle East with an origin South America. Searching for its natural enemies in new territories is needed in order to record native beneficial organisms (pathogens, parasitoids and predators) which could be included in IPM strategies for pest control. The aim of the current study was to identify the pathogen on a larva of the tomato borer found dead in the field in the region in Plovdiv, Bulgaria.

Materials and Methods: Microbiological analyses included isolation of the pathogen in pure culture and its identification according to morphological characteristics. Dead larva of *Tuta absoluta* after surface sterilization was placed in a humid chamber and conidia from the sporulated fungus were transfered to Sabouraud dextrose agar with yeast extract plate in a Petri dish. Morphological characteristics of conidia and conidiogenous cells from the obtained pure culture of the isolate and from the insect cadaver were studied using a transmission interference microscope.

Results: Microbiological analyzes showed that the death of *Tuta absoluta* larva was caused by a fungal pathogen which was isolated in pure culture and identified as *Beauveria bassiana*. Morphological characteristics of the isolate of the entomopathogenic fungus were examined and presented.

Conclusions: This is the first report in Bulgaria about *Tuta absoluta* as a new host of *Beauveria bassiana*.

Keywords: entomopathogenic fungus, *Beauveria bassiana*, *Tuta absoluta* (Lepidoptera: Gelechiidae)

V14

ROLE OF ANTIOXIDANT SUPPLEMENTATION DURING EXERCISE TRAINING

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During intensive exercises reactive oxygen species (ROS) are produced in skeletal muscle. They are associated with muscle damage and impaired muscle function. Thus antioxidant supplements have been proposed to prevent or reduce oxidative stress, decrease muscle damage and improve exercise performance. However, the results of scientific researches on the use of antioxidants in sports practice are very contradictory. The aim of this survey was to summarize the available data. Most observations have shown that antioxidants help to overcome the negative effects of exercise-induced oxidative stress. But there are data reporting no effects of antioxidant supplementation on athlete's performance. Moreover, a growing body of evidence indicates detrimental effects of antioxidant supplementation on the performance benefits of exercise training, likely because ROS mediate important physiological processes and play an essential role in adaptive processes. Evidence-based guidelines regarding the use of antioxidant supplementation during exercise training are needed in order to achieve an optimal pro/antioxidant status in exercising individuals with consequent improvement of athletic performance.

V15

ASSESSMENT OF REGENERATION POTENTIAL OF IN VITRO AND EX VITRO LEONURUS CARDIACA L. PLANTS BY ANATOMICAL MARKERS

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Object of Research: The object of the present anatomical study is to determine the structural organization of the newly formed leaves and roots of *in vitro* micropropagated and *ex vitro* adapted *Leonurus cardiaca* L. plants and to estimate the regeneration potential of this valuable medicinal plant by indicating the major morphological differences in comparison with the leaves of the *in vivo* plants.

Materials and Methods: The *in vitro* shoot culture were induced from sterilized mono-nodal stem segments of *in vivo* growing plants and the explants were inoculated under aseptic conditions on basal MS medium. For *ex vitro* acclimation the *in vitro* grown plantlets were transferred first in a growth chamber and afterwards in a greenhouse for further adaptation. Anatomical studies of the leaves and roots of *in vivo*, *in vitro* and *ex vitro* plants were made by applying the light microscopy technique.

Results: Generally, the leaf structural organization of *in vitro* cultured and *ex vitro* adapted *L. cardiaca* plants resembled the control *in vivo* plants. Slight differences in the shape and arrangement of the mesophyll cells were observed. Concerning the root histology there were differences in the vascular tissue organization between *in vitro* cultured and *ex vitro* grown plants. The research indicated that leave's and root's histological features of *in vitro* and *ex vitro* plants are structural markers of a great importance for assessment of the regeneration potential of the cultivated species.

Conclusions: The results of the anatomical study gave reasons for accepting that *L. cardiaca* plants, cultivated on MS medium, possessed the regeneration potential required for a successful *ex vitro* acclimation.

V16

HISTOLOGIC FEATURES OF SALIVARY GLANDS CAUSED BY INFLUENZA VIRUS INFECTION IN MICE

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Object of Research: Influenza is a seasonal viral infection a significant threat to human health associated with work disability and significant morbidity and discomfort. The clinical symptoms of flu include robust pulmonary inflammatory response, as well as often extra pulmonary pathology by causing serious health complications.

The purpose of this work was to examine pathologic changes in the salivary glands of mice in conditions of experimental model of influenza virus infection.

Materials and Methods: For the purpose of the investigation male mice were used, line ICR (14-16 g). The animals were divided into two groups:

Group I - controls (healthy non-infected animals);

Group II - mice infected with influenza A/Aichi/2/68 H3N2 (2.5 to LD50) by intranasal inoculation.

The animals were housed in cages with free access to water and food, at 22-24 °C with a 12 hour light/dark cycle. On the 5^{th} day after virus inoculation, animals were anesthetized and decapitated. Salivary glands were dissected and fixed with 10% formalin solution and prepared for light microscopy by standard protocol of the Medical University of Sofia. In this study were used routine methods of staining Nissl.

Results: The results obtained indicated that influenza infection produces changes in the salivary glands not only on tissue, but also on a cellular level. There were a pronounced inflammatory edema, hyperemia, and partial hypertrophy of some cells. The volume of the container and the pressure were elevated, the content of plasma proteins were reduced. The functional state of endothelial cells has been changed. Crossing the water outside the vessel wall was observed.

Conclusions: In conclusion, current research shows a part of the extra pulmonary expression of influenza viral infection. It could be a basis for further investigations.

V17

HISTOLOGICAL CHANGES OF SMALL INTESTINE CAUSED BY EXPERIMENTAL MODEL OF INFLUENZA INFECTION IN MICE

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Influenza viruses are RNA viruses belonging to Orthomyxoviridae family. They cause epidemics every year all over the world. Symptoms of flu are manifested through acute respiratory inflammation accompanied by high fever, body aches, and loss of appetite in humans as well in experimental mice models. At first, the influenza virus is introduced into the respiratory tract and starts to replicates in epithelial cells. Later the infection affects other organs in the whole body - have an influence upon stomach, brain as well hepatic metabolism. The purpose of this investigation was to find pathologic changes, caused by influenza virus in the small intestine of mice in conditions of experimental flu model.

For the purpose of the investigation male mice were used, line ICR (14-16 g). The animals were divided into two groups:

Group I - controls (healthy non-infected animals);

Group II - mice infected with influenza A/Aichi/2/68 H3N2 (2.5 to LD50) by intranasal inoculation.

The animals were housed in cages with free access to water and food, at 22-24 °C with a 12 hour light/dark cycle. Experiments were conducted by the standard protocol approved by the Bulgarian Academy of Sciences.

The animals were anesthetized and decapitated on the 5th day after virus inoculation. Tissues were fixed with 10% formalin solution and prepared for light microscopy by standard protocol of the Medical University in Sofia. In this study were used routine methods of staining of Nissl.

In the infected mice influenza virus infection causes changes in all layers of structure of the small intestine. Light microscopic observations showed:

- Damage of the enterocytes structure;
- Increased number of mucus cells in Lieberkuhn's glands;
- Dilation of the vessels in the lamina propria of the mucosa;
- Perivascular effusion;
- Petechial hemorrhages in the serous coat of the intestine;
- Changes in the structure of the lymph follicles constituting the Peyer's patches

The morphological studies on small intestine of mouse 5 days after inoculation with influenza virus A/Aichi/2/68 (H3N2) have suggested that this model of flu causes progressive damage of intestinal tract, probably triggered by inflammation of lung. The mechanism of these disorders probably is associated with enhanced immune response.

In conclusions, we suppose current histological investigation could be used for more clarifying of the mechanism of influenza pathogenesis as informative and adequate markers of viral pathogenesis, and as well they could serve for evaluation of the preventive effects of some drugs in course of medication of influenza virus infection.

V18

TROPHIC RELATIONSHIPS BETWEEN RIVERINE MACROINVERTEBRATES

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Object of Research: Food relationships in river systems are essential for the transport of organic matter in different trophic levels and the flow of energy through the ecosystem. The object of this research is benthic macroinvertebrates community as representative of the trophic levels as consumers ,in which organic matter is transformed.

Materials **and Methods:** This study examines the macroinvertebrates of rivers Mesta, Tundzha, Vit and Veleka, by two-year study period (2011-2012). In total 66 benthic samples were collected by multihabitat sampling method of Cheshmedjiev et al. (2011). Depending on the diet and the morphology of the oral structures, the identified taxa were referenced in functional feeding groups or FFGs (shredders, scrapers, collectors, filterers, deposit feeders and predators) following mainly Cummins (1973), Wetzel (2001) and Cheshmedjiev and Varadinova (2013).

Results: Properly utilization of food resources in river ecosystems is indicative of its stability and sustainability. The presence of all FFGs of benthic fauna in a river stretch, is indicative of the optimal utilization of food resources along the river. In the upper reaches of rivers, where in the watershed had significant amounts of fallen leaves, predominanting group are shredders. In river parts with presence of periphyton, the group of scrapers prevails. Deposit feeders is massively in sectors with organic pollution and deposited organic matter, which they used to consume. Filtering are dominant by river areas rich of fine particulate organic matter. The collectors are the most under represented groups, the predators are relatively equally distributed along the river continuum.

Conclusions: So available, affordable and sufficient quantity and quality of food base, fully determines the structuring and dynamics of trophic types of benthic communities in rivers.

V19

LINKING OF DEPOSIT FEEDING AQUATIC OLIGOCHAETA AND QUANTITY OF THEIR TROPHIC RESOURCES

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Object of Research: The object of this study is class *Oligochaeta* as a component of benthic macroinvertebrates communities, and that part of aquatic worms, which feeding is associated with particulated organic matter, deposited on the bottom of riverine water bodies.

Materials and Methods: This study included the macroinvertebrates of the rivers Mesta, Tundzha, Vit and Veleka, by two-year study period (2011-2012). The benthic samples were collected by multi-habitat sampling method (Cheshmedjiev et al., 2011). The identified oligochet taxa were referenced in three functional feeding groups (shredders, scrapers and deposit feeders) mainly by Cummins (1973), Wetzel (2001), Cheshmedjiev and Varadinova (2013). Also, the amount of organic carbon in river sediments was analyzed by the method of burning (Olsen and Townsend, 2005; Kenderov, 2007).

Results: For the whole study period and based on the four model river ecosystems, determined by total number of taxa, the most *Oligochaeta* species were found in the trophic group of deposit feeders, followed by those belonging to shredders. The least species/taxa were feed by scraping. The amount of *Oligochaeta* along the rivers, showed a tend to gradual increase, as the amount of deposit feeders was highest by river stations with organic load sand significant levels of organic matter deposited on the bottom layer. At the same time, the analysis of the quantity of organic carbon in river sediments, showed similar results - namely, an increase along the river continuum, and stations with level of organic pollution.

Conclusions: Thus, availability and sufficient trophic resources was assessed as key factors for the structuring of community trophic structure along the river continuum.

V20

ALPHA-LIPOIC ACID AS DIETARY SUPPLEMENT

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Alpha-lipoicacid (LA) is a naturally occurring dithiol compound which is synthesized in the mitochondria from octanoic acid. LA is a necessary cofactor for mitochondrial α-ketoacid dehydrogenases which are important for mitochondrial energy metabolism. Apart from its role as a cofactor, free LA has many other important biological actions. There are evidences that orally administered through dietary sources or supplements LA is not used as an enzyme cofactor. Indeed, free LA and its reduced form dihydrolipoic acid are proven universal antioxidants. They reduce the content of free radicals by direct action or by regeneration of the cell antioxidants, such as glutathione, vitamin C and E or indirectly affects gene expression of antioxidant enzymes, possess a chelating effect for transient metal ions down-regulating the formation of hydroxyl radicals and in this way reducing their detrimental effects on cell membrane lipids. LA increases the insulin stimulation of glucose uptake from cells and reduces insulin resistance. There are data that it improves memory and has a beneficial action on neurodegenerative diseases. LA possesses a hypotensive effect and has positive influence on the cardiovascular system. Its anti-inflammatory action is beneficial in patients with rheumatoid arthritis, atherosclerosis and asthma diseases. There is not an upper limit for LA intake for humans. Rapidly absorbed from the gastrointestinal tract by various types of protein transporters LA passes in the plasma and is distributed in various organs and tissues. Some reports suggest that LA crosses the blood-brain barrier. The transport into tissues, glomerular filtration and renal excretion of LA lead to its clearance within 24 hours. Therefore, the LA as a supplement could have beneficial pharmacotherapeutic effect against many pathophysiological conditions.

V21

SIDERITIS SCARDICA GRISEB. - ONE OF THE VALUABLE MEDICINAL PLANTS IN BULGARIA WIDELY USED AS A FOOD SUPPLEMENT

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Object of Research: *Sideritis scardica* Griseb. is a Balkan endemic species, a member of Lamiaceae family. In Bulgaria it is popularly known as Mursalitza Tea, Pirinski Tea, Alibotushki Tea or Mountain Tea, which is due to its distribution limited to a few localities in Pirin Mts, Rhodope Mts and Slavyanka (Alibotush) Mts. In addition to the use as a tea, the medicinal plant could be used in different forms varying from powders to extracts. The aim of this study was to make a survey on all these products.

Materials and Methods: Methods include collection, processing, analysis and summarization of information published in scientific journals.

Results: The chemical components found in *Sideritis scardica* Griseb. include terpenes, flavonoids, iridoids, essential oils and a wide range of micro- and macronutrients responsible for the observed pharmacological activities. It is used as antioxidant, anti-inflammatory, antimicrobial, vulnerary, analgesic, carminative and antiulcerative agents. Therefore, more than 20 companies produce bio products based on the healing properties of *Sideritis scardica*. The vast majority of them packaged tea for daily intake. Eight of the companies produce syrups based on water or alcoholic extraction. One of the companies produces tablets for increasing of body power and endurance. Two types of cold refreshing beverage made from *Sideritis scardica* can be found in the stores. A beer with an extract of the Mursalitza Tea is manufactured too.

Conclusions: All listed products are produced by cultivated *Sideritis scardica* and this is of great importance for conservation of this valuable medicinal plant on the one hand and for control the process of obtaining quality herbs on the other hand.

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V22

MICROBIOLOGICAL PREPARATION OF BUTYRIC ACID. SELECTION OF STRAIN AND OPTIMIZATION OF CULTURE CONDITIONS

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Object of Research: Butyric acid, has many applications in different industries. Pure butyric acid is used in food flavors, and its esters are widely used as additives in the perfume industry. Butyric acid might also find application as an intermediate in the production of one of the next generation of biofuels, biobutanol. Experiments were carried out with the purpose to select a strain with high production capacity for butyric acid and optimize medium composition in view to increase the amount of the product by anaerobic fermentation with the selected strain.

Materials and Methods: Fourteen strains of genera *Clostridium*, were used. Strains were cultivated on various media: meat-peptone broth (MPB) with different carbon source (glycerol, glucose, arabinose and xylose), triptone-thiogluconate medium (TTM), Clostridia Medium (CM) and chickpeagrains medium (CHM). High-performance liquid chromatography (HPLC) was used to analyze all the fermentation compounds. The HPLC system equipped with Rid and HPLC column Aminex HPX-87H at 65°C. Cell concentration was measured by optical densityat 600 nm, using a spectrophotometer SPEKOL 11.

Results and Conclusions: The results showed that the strain 4A1 grown on TTM with 5% glucose produced the higher quantity of butyric acid. The medium optimization showed the best results in TTM. At a concentration of glucose in the medium 10, 15, 20 g/L, the best results are achieved in TTM - 10 and 15 g/L of substrate, at 20 g/L is possible to have a substrate inhibition.

V23

TECHNOLOGY FOR OBTAINING OF BREAD FROM EINKORN

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Einkorn is an ancient grain which is a predecessor of the common wheat. The cultivated einkorn (*Triticum monococcum* L.) refers to the cover wheat's - fine grains with hard covering, which is difficultly separated. It is one of the most early cultivated wheat forms. The objective of the investigations to establish a technology for obtaining of bread from einkorn. Bread from einkorn with very good qualitative characteristics can be obtained by applying single- phase preparation of the dough at fermentation temperature 30^{o}C and duration 50 minutes. For the kneading of bread from einkorn without additives, the water quantity is of substantial importance for the form and the quality of the bread. This bread is kneaded with less water quantity. From the carried out investigations it has been established that the qualitative characteristics are higher for the improved bread from einkorn, followed by the bread from einkorn flour without any additives and on the last place with xanthan rubber additive. The sensory assessment of the three bread types is very good as the porosity of the natural bread is finer and the crumb is more dry and loose. The adding of xanthan rubber makes the bread moister and less crumbly. This bread gets dry more slowly.

Keywords: einkorn, bread, xanthan rubber, qualitative and sensory characteristics

V24

STUDYING THE CHANGE IN BIOCHEMICAL COMPOSITION OF FRESH AND DRIED *CHAENOMELES* FRUITS

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The object of research were three perspective forms of *Chaenomeles*, distinguished by a number of significant vegetative and reproductive indicators. The study was conducted in 2014. The chemical composition and degree of preserving of different components in the dried fruits were studied. The following indicators were studied: dried matter according to Re%, total sugars, inverted sugar, sucrose, organic acids, ascorbic acid, anthocyanins, tanning substance and pectin. The three forms of *Chaenomeles* were grown under nonirrigated conditions, with the application of the necessary agrotechnical events. *Chaenomeles* fruits are distinguished by their high content of ascorbic acid, which is in the range of 79.20 mg%-109.12 mg%. The decrease of the same indicator in dried fruits was significant, which varied in the different forms from 2.9 times to 5.3 times, in comparison with fresh fruits. Significant differences were also observed in the values of other examined components. The aim of the study is to compare the changes in the chemical composition between fresh and dried fruits.

Key words: ascorbic acid, chaenomeles, chemical composition, dried fruits;

V25

NANOTECHNOLOGY IN THE FOOD CHAIN: IS IT SAFE?

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Nanosciences and nanotechnologies are highly promising and rapidly progressing disciplines in research and industrial innovation. The term "nano" refers to the measurement of size; a nanometre (nm) is a millionth of a millimeter. By way of illustration, a nanometer is about 1/50,000th the width of a human hair, and a sheet of normal office paper is about 100,000 nm thick. A nanoparticle (NP) is usually considered to be a structure between 0.1 and 100nm.

The potential benefits of nanotechnology have been recognized by many industrial sectors, and products based on nanotechnology or products containing NPs are already manufactured such as in the field of microelectronics, consumer products and the pharmaceutical industry. Also with respect to food and agriculture, a number of promising applications are emerging, such as smart packaging, nanosensors for pathogen detection or registration of storage conditions, nanoformulations of agrochemicals, nano-encapsulation/nanodelivery of food ingredients, etc.

Although nanotechnology or NPs have the potential to bring significant benefits to both the industry and consumers, they may also introduce potential risks for human health and the environment.

V26

TENEBRIO MOLITOR AND ZOPHOBAS MORIO A PROMISING ALTERNATIVE AS PROTEIN SOURCE IN OUR DIET

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On a constantly growing population on our planet, soon we are about to face a huge problem how to feed the Earth's population. The available soil for farming is constantly declining due to many different reasons. Raising cattle is a resource consuming and the weather anomalies are not helping. If we soon don't find an alternative food source our wellness as a civilization might come to an end. FAO of the United Nations estimates that world food production needs to be increased by 70% because of world population increasing (9 billion by the year 2050).The most abundant representatives of the animal kingdom on our planet are insects. Insects as a source of energy have been commonly used in different parts of the world as a part of some national cuisines [1]. Although, it is not a common case in the west cultures of the world. The main reason according to specialists in the field is psychological. Different investigations reveal that insects contain two to three times more proteins than traditional meats like beef, pork and chicken and it is quite a big waste of resources not using their good nutritional values [2].
Our current goal is to raise the larvae of 2 species of insects *Tenebrio molitor* and *Zophobas morio*, measure the nutritional values, separate their fats from the proteins in dry matter and further to propose the residue in appropriate outward for eating without causing any psychological and other barriers for the average person.

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V27

AUTHORITIES OF THE MINISTER OF HEALTH IN THE FIELD OF FOOD SAFETY

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According to the Food Law in Bulgaria, the state policy in the food safety regulations is executed by the Minister of Agriculture and Food and the Minister of Health.
The Minister of Health in coordination with the respective ministers issues bylaws regulating public relation sin the field of food safety. According to the Food Law, the Minister of Health is responsible for the the competent authority for permit and registration regimes, as well as imposition of compulsory administrative measures.
The Ministry of Health implements a number of administrative functions related to the receipt of applications, maintenance of records, etc.

V28

ANALYTICAL REVIEW OF THE PRICE FLUCTUATIONS OF BASIC FOOD PRODUCTS (RICE, SUGAR, OIL AND MEAL) FOR THE PERIOD 2007-2014 YEAR IN BULGARIA

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Object of Research: The object of research were the price fluctuations of basic food products - rice, sugar, oil and flour. Price levels of average monthly basis for the period 2007-2014 were analyzed.
Materials and Methods: The comparative price analysis for the period 2007-2014 year of average monthly price of basic foodstuffs - rice, sugar, oil and flour was conducted on data by the State price commission.
Results and Conclusions: Key factors which influenced the price fluctuations of the selected food commodities (rice, sugar, oil and flour) for the period 2007-2014 year in Bulgaria were displayed and discussed. The four basic products price fluctuations were analyzed in connection with the recent worldwide financial crisis.

V29

MICROBIOLOGICAL ANALYSIS OF RIVER VALBONA (ALBANIA), DURING SPRING SEASON 2013

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Object of Research: The objective of this study was to assess the quality of the water of river Valbona during the spring season 2013 *via* microbiological analysis. River Valbona is located in the North-East part of Albania and pass near the city Bajram Curri.
Materials and Methods: Water samples for the microbiological analyses were collected at three locations along the river. Water samples were collected monthly at three different locations along the river. Nutrient agar (NA), *Salmonella Shigella* agar, Violet Red Bile Glucose Agar, Bile aesculin agar, dextrose agar were used to determine heterotrophic bacteria, *Salmonella* and *Shigella*, total coliform, *Streptococcus faecalis* and fungi. All plates were incubated at 35^0C for 24 h.
Results: All groups of bacteria exceed the allowed values of bacterial number. The total coliform bacteria ranged from 21 cfu/mL to 57 cfu/mL. The *Streptococcus* ranged from 24 cfu/mL to 63 cfu/mL. *Salmonella* and *Shigella* were enumerated from 14 cfu/mL to 45 cfu/mL. Heterotrophic bacteria count was from 78 cfu/mL to 350 cfu/ml. Fungi number was from 3 cfu/mL to 25 cfu/mL.
Conclusions: The waters of river Valbona is not highly polluted by bacteria at all locations. According to the Tumpling system the water of river Valbona belongs to the Second class of polluted rivers.

Keywords: microbiological, analysis, water, river Valbona

V30

ELBY SYNBIOTIC FERMENTED MILK HEALTHY AND DIETARY PRODUCT

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In recent years, many results by a number of clinical observations on the influence of fermented milks, calcium and probiotics in the fight against obesity have been published. In the prevention of obesity a variety of effective food additives and/or biologically active agents, probiotics, and prebiotics are used. In recent years the interest in the food enriched with calcium to prevent and reduce the risk of osteoporosis, hypertension, and the regulation of lipid metabolism has increased.
In a number of our studies, the technological, biochemical and microbiological parameters of fermented milk fortified with calcium, prebiotics or probiotics were reported. Those functionlal fermented milks are protected by trademarks "Tonus", "Stimko", "Bifidovital".
The purpose of this study was to establish a functional "synbiotic" fermented milk fortified with calcium. Calcium gluconate was used for fortification of milk. Galactooligosaccharide was used as a prebiotic supplement.
As probiotics the selected starter cultures *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for Bulgarian yogurt and established with *in vitro* immunomodulatory effect hypolipemiant effect and *Bifidobacterium longum* strains with immunomodulatory and hipolipidemical were used. The study of lactic acid microorganisms on the final product during storage (21 days at 50°C) indicates the presence of a large number of viable cells - *L. bulgaricus, S. thermophilus* $\geq 1{,}0 \times 10^8$ cfu/g, *Bifidobacterium longum* $\geq 1{,}0 \times 10^6$ cfu/g.

V31

FOOD IN THE BIBLE TEXT

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In this study were presented and discussed the main food groups described in the BIble texts as grains, fruits, vegetables, meat, fish, dairy products, herbs, spices, nuts and miscellaneous. Exact citation is describes as follows: Dairy: Cheese (2 Samuel 17:29; Job 10:10); Butter (Proverbs 30:33); Milk (Exodus 33:3; Job 10:10; Judges 5:25); Animal Meats: Calf (Proverbs 15:17; Luke 15:23); Lamb (2 Samuel 12:14); Oxen (1 Kings 19:21); Sheep (Deuteronomy 14:4); Vegetables and Legumes: Beans (2 Samuel 17:28; Ezekiel 4:9); Cucumbers (Numbers 11:5); Onions (Numbers 11:5); Grains: Wheat (Ezra 6:9; Deuteronomy 8:8); Bread (Genesis 25:34; 2 Samuel 6:19; 16:1; Mark 8:14; Barley (Deuteronomy 8:8; Ezekiel 4:9); Flour (2 Samuel 17:28; 1 Kings 17:12); Fish (Matthew 15:36; John 21:11-13); Fruits and Nuts: Apples (Song of Solomon 2:5); Pomegranates (Numbers 20:5; Deuteronomy 8:8) Grapes (Leviticus 19:10; Deuteronomy 23:24); Pistachio Nuts (Genesis 42:11); Olives (Isaiah 17:6; Micah 6:15); Melon (Numbers 11:5; Isaiah 1:8); Almond (Genesis 43:11; Numbers 17:8); Raisins (numbers 6:3; 2 Samuel 6:19); Spices and Herbs: Mint (Matthew 23:23; Luke 11:42); Mustard (Matthew 13:31); Salt (Ezra 6:9; Job 6:6); Cumin (Isaiah 28:25; Matthew 23:23); Coriander (Exodus 16:31; Numbers 11:7); Cinnamon (Exodus 30:23; Revelation 18:13); Miscellaneous: Honey (Exodus 33:3; Deuteronomy 8:8; Judges 14:8-9); Grape Juice (Numbers 6:3); Eggs (Job 6:6; Luke 11:12); Olive Oil (Ezra 6:9; Deuteronomy 8:8); Vinegar (Ruth 2:14; John 19:29); Wine (Ezra 6:9; John 2:1-10); Fowl: Pigeon (Genesis 15:9; Leviticus 12:8).

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NOTES

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MICROOPTICA

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MEDICAL BULGARIA