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*Correspondence to: A. Horna, Institute of Nutrition and Diagnostics, RADANAL, Ltd. Okružní 613, Pardubice, Czech Republic, Tel: +420 466 650 618; Fax: +420 467 027 020; E-mail: info@indc.cz; Homepage: www.indc.cz

The microbiome and man theatre: established concepts and novel prospects in medicine

Rusch V*

Old Herborn University Foundation, Institute for Integrative Biology, D35745Herborn-Dill, Germany, E-mail: volker.rusch@old-herborn-university.de

A rising flood of literature published on the topic “microbiome and man” illustrates the growth of appreciation of a new image of man and microbes as a functional unity and its role of intimate partners in a symbiotic system. Surprising insights into this complex ecological network let familiar paradigms falter and offer new routes in adaptive control of numerous interdependent physiological processes at multiple interfaces leading to an understanding of established concepts in therapeutic and preventive intervention, the promise of better targeted research and new clinical applications. Intensive studies of metazoan and human physiology and its metabolic, immune and other functions reveal that many functions are deeply influenced by symbiotic microbial communities and even brain and behaviour are affected. The gut brain axis links gut and brain activities in a bi-directional communication system utilizing neural, endocrine and immunological signaling. A new comprehension of immune functions emerged and the immune system now appears not only as a defensive system, but also as an institution organising the communication between multicellular organisms and microbes. As a consequence, special attention is focused upon microbes as preventive and therapeutic agents in modulation of immune and other physiological functions, as reflected in rapidly expanding numbers of publications on well designed and controlled studies and mounting evidence on clinical efficacy. Papers mainly deal with lactic acid bacteria and to a lesser extent with other microorganisms in probiotics or microbiotics. The medical community vividly discusses the potential of fecal microbiota transplants and even considers microbes as “psychobiotics”. In Germany, a medical association for microbiological therapy developed a modular system in clinical

application of lactic acid bacteria, *E. coli* and *E. faecalis*. Such concepts win increasing acceptance in clinic and practice. Our group is involved in numerous experimental and clinical studies with microbiotics containing viable or dead *E. coli*, *E. faecalis* and autologous vaccines in sinusitis, rhinosinusitis, bronchitis, tonsillitis, atopy, asthma, the irritable bowel syndrome and other disorders. A mixture of data will be presented to demonstrate significant effects in experimental and clinical situations and how regulatory signals modulate host physiology and immune functions, including most recent findings.

Colorectal cancer and gut microbiota: Probiotics and prebiotics in prevention?

Tlaskalová-Hogenová H*, Klimešová K., Zákostelská Z., Kverka M., Hornová M., Vannucci L., Štěpánková R., Kozáková H., Hudcovic T., and Rossmann P.

Institute of Microbiology, Academy of Sciences of the Czech Republic, Videnska 1083, Prague 4 and Nový Hrádek, Czech Republic, E-mail: tlaskalo@biomed.cas.cz

Colorectal cancer is the most common malignancy of the gastrointestinal tract. More frequent incidence in industrialized countries than in developing once could be explained by differences in dietary habits and lifestyle. The link between diet and pathogenesis of colorectal cancer is likely to be due to an imbalance of gut microbiota. The microbiome encodes huge numbers of catabolic and anabolic pathways in the gut enabling it to use a vast range of substrates. The composition and metabolic profile of the gut microbiota is substantially and rapidly changed after major changes in diet. Balanced interaction between commensal bacteria and the host mucosal immune system is a prerequisite for homeostasis in intestinal tract. Barrier formed by intestinal epithelium separates intestinal microbiota from underlying tissues, preventing bacterial infiltration and subsequent inflammation. Numerous chronic diseases may occur as a result of disturbance of intestinal microbiota composition (“dysbiosis”), impaired mucosal

barrier function and changes in mechanisms regulating mucosal immunity. Impairment of intestinal barrier function was found during development of inflammation leading to colorectal cancer development. Carcinogenesis in the gut could be driven by the presence of potentially harmful microbes or by lack of protective ones, by the production of carcinogens generated by microbes, and by the induction of inflammation and modulation of the immune system. Accumulating data suggest that ingesting of probiotics or/and prebiotics may be able to play a preventive role in the onset of colorectal cancer. Probiotics contribute to development and reactivity of the mucosal immune system by promoting epithelial, dendritic, and T-cell responses. Probiotics may decrease the burden of carcinogens by influencing microbiota composition, moreover, they have stabilizing effects on the gut microbiota, potentially reducing a proinflammatory response. Gnotobiological approach is allowing to analyse the impact of defined microbes on the regulation of mucosal immunity and carcinogenesis. Using gnotobiological methods we have shown that gut microbiota promotes colitis-associated colorectal cancer by increasing the exposure of gut epithelium to carcinogens. Gut microbiota and its metabolic activity could be potential targets for colorectal cancer therapy.

The link between diet, gut microbiota and disease expression in rodent models of human disease

Hansen A. K*

Department of Veterinary Disease Biology, University of Copenhagen, E-mail: akh@sund.ku.dk

An increasing amount of evidence shows that a range of human diseases, such as type 1 and type 2 diabetes, colitis, obesity and cancer are under impact of the diet; an impact, which often works in conjunction with the gut microbiota. In this context it has been essential to perform studies in mice, in which it has been shown that various dietary components within the fractions of fat, carbohydrates, and proteins with a documented impact on the development of disease in mouse models, act through or with the gut microbiota. Sequencing techniques have allowed us a better ability to identify single bacterial species of importance, but it has also shown us that the more overall balance between microbiota members and the time of life we meet these for the first time is of importance. This has generated knowledge for a further understanding of these human diseases, and the way our diet influences their development. However, it will also in a near future change the way we set up mouse models for the studies of human diseases. Examples from the works of our research group will be given.

Contribution of microbially synthesized vitamers to folate status and impact on inflammatory bowel diseases

Wei BS¹, Engst W², Luna R³, Versalovic R³, Siegmund B⁴, Blaut M⁵, and Saulnier DM^{1*}

¹Microbiome Gastrointestinal and Inflammation Research Group, Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam Rehbruecke, Germany

²Analytics Group, German Institute of Human Nutrition Potsdam Rehbruecke, Germany

³Department of Pathology, Baylor College of Medicine, Houston Tx, USA

⁴Department of Gastroenterology, Charité University Hospital Berlin, Germany

⁵Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam Rehbruecke, Germany, E-mail: delphine.saulnier@dife.de

Folate is one of the key vitamins involved in normal cellular function, growth, and development. Low folate and raised homocysteine levels are risk factors for chronic inflammation. Although evidence is accumulating that microbially derived folates could impact the folate status of the host, their production in the gut has been so far largely ignored. Subclinical but biochemically relevant folate deficiency occurs in 40% of inflammatory bowel diseases (IBD) patients. IBD, whose main manifestations are ulcerative colitis (UC) and Crohn's disease (CD), are the result of an altered immune homeostasis within the intestinal mucosa against the gut microbiota, leading to chronic inflammation in genetically predisposed individuals. Incidence rates of IBD increased dramatically in parallel with social development but etiology of the disease is unclear. Our hypothesis is that serum folate deficiency in ileal CD subjects reflects an altered folate metabolism in the gastrointestinal tract due to a host and/or a gastrointestinal microbiota component. Furthermore, we hypothesize that altered folate metabolism in the gastrointestinal tract contribute to microbial dysbiosis and increased inflammation in ileal CD patients. In the first stage of this project, folate production by selected gut bacterial commensals was assessed in a folate free-medium. Most strains, with the exception of *Faecalibacterium prausnitzii* - a species with anti-inflammatory properties strongly reduced in IBD - were able to produce both intra and extra-cellular folates. In a second stage of this project, we are defining the quantity as well as the different forms of fecal folate vitamers to be obtained from 40 ileal CD patients with different serum folate status by a microbiological assay and mass spectrometry. Furthermore, we are characterizing microbiota composition using next generation sequencing technologies. In the future, we will also investigate the potential of the fecal microbiota of ileal CD patients with high and low serum folate status to synthesize folate vitamers by performing fermentation of fecal samples inoculated with different folate precursors. The results should lead to a greater understanding of the role of microbially synthesized folate. They will facilitate the development of strategies to alter the gut microbiota, improve folate status, and prevent or suppress host inflammation in IBD and other inflammatory disorders.

Clinical application of metabolomics

Friedecký D.^{1,2}, and Adam T.^{1,2,3*}

¹Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University in Olomouc

²Laboratory of Inherited Metabolic Disorders, Faculty of Medicine and Dentistry, Palacký University in Olomouc, University Hospital Olomouc

³Department of Clinical Chemistry, University Hospital Olomouc, E-mail: david@friedecky.cz

Metabolomics has become an important tool in clinical research and diagnosis of human diseases. We applied both targeted and untargeted metabolomic approaches using aqueous normal phase chromatography with triple quadrupole or high resolution mass spectrometers, respectively. The metabolomic workflow consists of several important steps starting from sample preparation through the analysis, mathematical transformations up to presentation of the results and data interpretation. In the last years, we focused on various field covering diagnosis of inherited metabolic disorders in biofluids and identification of new biomarkers, understanding pathobiochemical processes in cells treated by potential anticancer drugs, asphyxia in newborns and metabolic changes in patients suffering from chronic myeloid leukemia, and others.

On-line LC/EC/MS for the prediction of metabolism, toxicity and stability - Usage and future prospect

Horna A.¹, and Portyčhová L.^{1,2*}

¹Institute of Nutrition and Diagnostics, RADANAL Ltd., Okružní 613, Pardubice, Czech Republic

²Department of Analytical Chemistry, Faculty of Science, Palacký University, 17.listopadu 12, Olomouc, Czech Republic, E-mail: horna@radanal.cz

All organisms are in their environment exposed to many artificial substances, xenobiotics (e.g. drugs, toxins, industrial chemicals). These compounds have generally a lipophilic character. They interact with proteins (mainly albumin) in the body and after that they are transported into organs, tissues and subsequently into body cells. Most xenobiotics are not biodegradable, thus they accumulate in fatty tissues and often cause serious diseases. Every living organism has its own defense system by which defends against foreign substances. These compounds are (in phase I) usually oxidized in organisms using catalysis of enzymes, especially cytochromes P450. Gained metabolites of xenobiotics conjugate (in phase II) with glutathione or other endogenous substance. The polarity of the metabolites increases this way and the elimination of these compounds from the body is easier. If glutathione is depleted, reactive metabolites may bind covalently to macromolecules and cause cell damage, mutations, cancer or immunological damage. A prediction of toxicity and metabolism of xenobiotics in a human body is very important for determining of harmfulness of compounds which affect us or substances that we intentionally accept thinking that they aren't toxic. A suitable tool for mimicking the xenobiotics metabolism in a human body is liquid chromatography coupled with mass spectrometry and electrochemistry (LC/EC/MS). Usage of electrochemistry allows simulating of the metabolism catalysed by cytochromes P450. Gained oxidation products

are analysed and identified with mass spectrometry. Moreover it is possible to watch interactions between metabolites and glutathione thanks to injecting of this tripeptide directly to the device. Another usage of EC/MS is a possibility of carrying out a small scale synthesis of standards in a short time. Institute of Nutrition and Diagnostics (the company RADANAL Ltd.) comes under a small amount of workplaces which have and use this instrument.

Neglected and underutilised plants provide opportunities for enhanced nutrition in developing countries

French B.¹, Green B.¹, Mc Phee J.^{1,2}, and Macka M.^{3*}

¹Food Plant Solutions, PO Box 363, Devenport 7310, Tasmania, Australia

²Tasmanian Institute of Agriculture, University of Tasmania, PO Box 3523, Burnie, 7320, Australia

³Australian Centre for Research on Separation Science (ACROSS) and School of Physical Sciences, University of Tasmania, Private Bag 75, Hobart 7001, Australia, E-mail: mirek.macka@utas.edu.au

Food and nutrition systems in many developing countries, especially in the 80 poorest countries, are usually plant based. Over the last 200 years, western influences have introduced many nutritionally inappropriate foods to these countries, while traditional plants have often been neglected. To address the resultant nutritional imbalance and chronic malnutrition, front line agriculturists and nutritionists are beginning to re-focus on what are technically being called "Neglected and Underutilised Species" or NUS. The world's most comprehensive database, covering 26,600 edible plant species, is arguably the Food Plants International database, compiled by Tasmanian agricultural scientist, Bruce French. The aim of this work is to standardise what has become a confusing and scattered set of information about edible plants. The database can identify and highlight useful, but overlooked crops. Agro-ecological information and mapping makes selecting crops for specific biomes achievable. The database also highlights the deficiency of nutritional information that is available for NUS. Improved nutritional data is required for NUS to facilitate selection of appropriate plants to address key nutritional deficiencies in developing countries. These include data on protein, vitamin A, vitamin C, zinc, iron, and other micro-nutrients. NUS therefore represent an important opportunity for nutritional research into novel plants species, with direct relevance and application to reducing the impacts of malnutrition of developing countries. This information then needs to be delivered to the target audience especially for children in rural tropical regions. Food Plant Solutions Rotarian Action Group is using the global footprint of the Rotary International network to help people in developing countries to better understand the nutritional value of traditional food plants and their importance in the diet. This talk will expand on the comparative nutritional merit of some common western and traditional plants, the work being done to promote the use NUS and highlight the deficiencies in the availability of vital nutritional information on food plants.

Immunonutrition: Modulation of oxygen- and nitrogen-dependent microbicidity of phagocytes

Bondarenko I.*

North West Research Centre for Hygiene and Public Health, St.Petersburg, Russia, E-mail: Igor.G.Bondarenko@gmail.com

Reactive oxygen and nitrogen species generated by specific cytoplasmic enzymes of phagocytic cells have been well known as most efficient molecular tools for intra- and extracellular killing of viruses, bacteria, protozoa, fungi, and helminths. Acute and chronic infections and microbial overgrowth (like candidiasis) may, therefore, be counteracted with controlled activation of the first line of the innate immunity, the phagocytes. The relevant enzymic generators of reactive oxygen and nitrogen species that can be stimulated by substrates and allosteric inducers are: myeloperoxidase, NAD(P)H oxidase, inducible NO synthase, and xanthine oxidoreductase. For the practical purpose of immunoregulation in vivo, specific nutritional activators of the aforementioned enzymes can be employed (trace elements, coenzymes, substrates or their precursors, and allosteric modulators). Examples of beneficial clinical effects of the use of coenzymes and an allosteric activator of myeloperoxidase (colloidal silver) are demonstrated and discussed. A special attention is paid to the positive changes in clinical condition of patients with chronic bacterial and parasitic infections, the effects that have been associated with targeted upregulation of xanthine oxidoreductase and resulting production of peroxynitrite as an endogenous anti-infectious agent. Specific nutritional forms of the aforementioned modifiers of phagocytes (ionic minerals, activated vitamins, water-soluble substrate precursors) are discussed, with a particular emphasis on the reasons for their higher bioavailability.

Bifidobacterium longum ssp. Longum protection against dss-induced colitis in mice is strictly strain dependent

Kozakova H.¹, Srutkova D.¹, Hudcovic T.¹, Zakostelska Z.², and Schwarzer M.¹

¹Institute of Microbiology, Academy of Sciences of the Czech Republic, v.v.i., Nový Hrádek, Czech Republic

²Institute of Microbiology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic, E-mail: kozakova@biomed.cas.cz

Chemically induced murine model of intestinal inflammation such as treatment with dextran sulfate sodium (DSS) is the most commonly used system in animal studies due to many histopathological and immunological similarities to human ulcerative colitis. In patients, decreased number of bifidobacteria in the gut was found and supplementation with probiotics seems to be a promising tool of prophylaxis. The aim of this study was to select new probiotic candidates with the potential in prevention of DSS-induced acute colitis. We analyzed ten bifidobacterial isolates from healthy infants and adults to assess their key probiotic properties as resistance to

bile and growth in milieu with low pH. Selected bifidobacterial candidates were identified by molecular-biological methods and their immunomodulatory capacity was evaluated by cytokine determination in co-culture in vitro system of splenocytes and bone marrow dendritic cells isolated from BALB/c mice. For further study we selected two bacteria of *Bifidobacterium* (*B.*) *longum* spp. *longum* strain differing in cytokine production – CCM 7952 (low producer) and CCDM 372 (high producer). These strains were administrated to BALB/c females followed by one week-lasting drinking of DSS solution. We evaluated macroscopical and histological signs of inflammation, IgA production, and level of proinflammatory cytokines. Changes of the permeability of the intestinal barrier were evaluated by expression of tight junction proteins (zonulin-1 and occludin) immunohistochemically and by Western blotting. We observed that *B. longum* ssp. *longum* CCM 7952 protected mice against DSS-induced colitis and that prophylactic effect of bifidobacteria is strictly strain dependent and therefore the exact characterization and determination of their immunomodulatory activity is highly desirable.

Advanced glycation end-products in health and disease with nutritional considerations

Gamian A.^{1,2,3*}, Bronowicka-Szydelko A.¹, Górska S.², and Pietkiewicz J.¹

¹Department of Medical Biochemistry, Wrocław Medical University, Poland

²Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wrocław, Poland

³Wrocław Research Center EIT+, Poland, E-mail: gamian@iitd.pan.wroc.pl

Modification of basic amino acids residues by reducing sugars or small reactive aldehydes is called glycation, a process occurring in the organism during aging and various diseases associated with diabetes, but also in other processes such as food processing. Proteins and also lipids and nucleic acids undergo glycation, non-enzymatic reactions resulting in the advanced glycation end-products (AGE) formation. Regarding human organism, glycated proteins have various epitopes and their detection in patient serum can be useful in determining the stage of diabetes or other diseases, as well as in monitoring of such products in the observation of the therapy progress. Determination of the level of glycated products in food might be important for its characterization of its quality, especially when the effects of food derived glycation products are not fully understood. So far, only a few compounds formed during glycation in human organism and in food are known, such as: deoxyglucose-imidazolone, pentosidine, N-ε-(carboxymethyl) lysine (CML), N-ε-(carboxyethyl) lysine (CEL), N-ω-(carboxymethyl) arginine (CMA), or pyraline. They are formed as a result of glycation of basic amino acids with monosaccharides or reactive aldehydes (methylglyoxal, glyoxal, hydroxynonenal and other). Elaborated in our laboratory the synthesis in a microwave reactor of completely new model products based on N-acetyllysine, allowed to develop, together with obtained monoclonal antibodies, an

immunochemical assay which is potent to determine the commonly occurring epitope in glycosylated proteins present in patient's sera. The presence of such epitope in sera from patients with renal failure with diabetic complications has been confirmed by properly designed ELISA assay. Presented by our team results of quantitative analysis suggest that with the progress of type II diabetes also increases the amounts of studied glycation products in sera of patients. Determination with this assay of such glycation products in food opens the possibility to study on and production of functional food.

The role of Reg3 proteins in innate defence of the intestinal mucosa

Loonen L.^{1,2*}, and Wells J.¹

¹Host-Microbe Interactomics, Wageningen University, The Netherlands

²Top Institute Food and Nutrition, Wageningen, The Netherlands, E-mail: linda.loonen@wur.nl

The Reg3 protein family of antimicrobial peptides contains conserved C type lectin domains involved in carbohydrate recognition. In the mouse the Reg3 β and Reg3 γ family members are induced in the small intestine during the colonization of germ-free mice or in response to infection. At micromolar concentrations Reg3 γ and the human homologue HIP/PAP were reported to be bactericidal for Gram-positive bacteria in vitro. However, in vivo the bactericidal role of Reg3 γ has not yet been investigated. Here we describe the construction of a novel Reg3 γ -/- mouse, its phenotype and the results of infection with *Salmonella enteritidis* and *Listeria monocytogenes*. These results are compared to similar studies we recently published using a Reg3 β -/- mouse. Minor differences in cytokine production and gene expression were measured in the ileum, mesenteric lymph node cells, spleen and serum of adult Reg3 γ -/- mice compared to wild-type controls. There is an absence of intestinal pathology but differences are observed in the location of the mucus in wild-type and knockout mice and more bacteria are observed in the firm mucus layer of the colon. Infection studies were performed using Gram-negative *S. enteritidis* and Gram-positive *L. monocytogenes*. Fecal counts of *Listeria* and *Salmonella* were not significantly different between wild-type and knockout mice. In contrast to previous infection studies in a Reg3 β -/- mouse model, the Reg3 γ -/- mice were not more susceptible to infection. Additionally we did not see an effect of Reg3 γ on infection with a variant of *L. monocytogenes* which has a mutation in the invasin internalin, conferring higher affinity binding to the mouse E-cadherin receptor. Despite the fact that Reg3 γ has been reported to kill *L. monocytogenes* in vitro we conclude that there is no protective effect of Reg3 γ against this strain in vivo. However, Reg3 γ appears to have effects on the spatial segregation of intestinal bacteria in the small and large intestine for reasons which are currently under investigation.

Lepidium meynii (Maca) influences gene expression level of luteinizing hormone in female rats

Ogata M.^{1*}, Jikyo T.¹, and Uchiyama F.^{1,2}

¹Institute of Preventive and Medicinal Dietetics, Nakamura Gakuen University, Fukuoka 814-0198, Japan ²Graduate School of Nutritional Sciences, Nakamura Gakuen University, Fukuoka 814-0198, Japan, E-mail: ogata-m@nakamura-u.ac.jp

Lepidium meynii (Maca) has been reported many kinds of effects on health including fertility. In our previous study, we demonstrated that Maca pharmaceutically enhances luteinizing hormone (LH) serum level in female rats (Fumiaki, U., Tamaki, J., Misato, O., 2014. J. Ethnopharmacol.). To address the mechanism of LH enhancement on Maca intake, we investigated the differential gene expression related to LH production in the pituitary of female rats on Maca intake. Maca powder was made from the tubers of *Lepidium meynii* Walp collected, dried, and reduced to powder at the plantation in Junin Plateau and was purchased from Yamano del Peru SAC. Two groups of female Sprague-Dawley rats were provided normal feed with or without Maca powder ad libitum 7 weeks. At 1800h of the proestrus stage, pituitary organs were collected. Total RNA was extracted from homogenized the pituitary using RNeasy Plus Universal Kit (Qiagen). The total RNA was labeled and hybridized to oligo DNA microarray using Low Input Quick Amp Labeling Kit (Agilent), One-Color Gene Expression Hybridization Kit (Agilent), and Gene Expression Wash Buffers Pack (Agilent) according to the manufacturer's guidelines. We observed 14,658 gene expression levels per group. In the gene expression profiling, the average of the total gene expression levels was no significant difference between the control and the Maca groups. LH is composed of two subunits, alpha and beta subunit. Each subunit is encoded by chorionic gonadotropin alpha gene (*Cga*) and luteinizing hormone beta gene (*Lhb*). Although the *Cga* expression was the same level in the two groups, the *Lhb* expression level was up-regulated on Maca intake. Moreover, early growth response protein 1 gene (*Egr1*) which is reported to be crucial for the activity of the *Lhb* gene promoter was significantly up-regulated on Maca intake. To confirm the expression levels of *Lhb* and *Egr1*, we measured these expression levels by qRT-PCR. We found that the gene expression of both *Lhb* and *Egr1* on Maca intake was enhanced the level of 1.7-fold (n=3, P <0.01) and 2.6-fold (n=3, P <0.01) increases, respectively. These data indicate that the enhancement of LH serum level on Maca intake may be mediated by the gene expression of *Lhb* through the transcription factor, *Egr1*.

Inhibition of CYP1A and CYP2E1 by quercetin in porcine microsomes

Zamaratskaia G.^{1*}, Woll F.¹, Zlabek V.², Rasmussen M.³, and Ekstrand B.³

¹Department of Food Science, BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden

²South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, Vodnany, Czech Republic

³Department of Food Science, Aarhus University, Denmark, E-mail: galia.zamaratskaia@slu.se

Drug-drug interactions as well as food-drug interactions are known to have large impact on the pharmacodynamic behavior of drugs. Food-drug interactions have not yet been well studied and the factors affecting these interactions, such as gender, age, genetic background et cetera, have not been elucidated. The major emphasis in drug-drug and food-drug interaction research has been on phase I metabolism. The cytochrome P450 enzymes (CYP450) are involved in the Phase I metabolism of various endogenous and xenobiotic compounds. A number of studies determined that flavonoids are potent inhibitors of various CYP450 isoforms and membrane transporters and thus interact with pathways of many commonly prescribed drugs. In this study, we investigated in vitro ability of the flavonoid quercetin to modify activity of the drug-metabolizing isoforms CYP1A and CYP2A in male and female pigs. The porcine microsomes were used because pig shares a number of physiologic characteristics with humans and increasingly being used as an alternative to other species of choice in preclinical toxicologic testing of pharmaceuticals. The activities of CYP1A and CYP2E1 were measured using metabolism of the probe substrates 7-ethoxyresorufin and p-nitrophenol, respectively. Quercetin competitively inhibited CYP2E1 and CYP1A activity in the microsomes from male pigs. No effect of quercetin was observed in the microsomes from female pigs. We concluded that quercetin may interact with substrates for porcine CYP1A and CYP2E1 in a gender-dependent manner. The present study is an important step in evaluation of gender-related differences in food-drug interactions.

Laboratory interpretative nutritional pitfalls

Mabrouk R. R.*

Faculty of Medicine, Ain Shams University, Egypt, E-mail: randareda@gmail.com

Each generation of nutrition professionals takes the profession to higher levels of expertise in medical nutrition therapy. Laboratory assessment of nutritional status is one area that has seen dramatic changes in utilization of data to support the nutrition care process and to demonstrate the effectiveness of intervention strategies. Laboratory test results are tools for the nutrition professional to use. There are potential interpretive pitfalls regarding some nutritional parameters. Interpretation requires the communication between the Clinical Pathologists and the nutritionists. Both groups should focus on the diagnosis working hand in hand with other medical practitioners aiming to reach the best cure results. This short presentation will reveal the relationship between certain laboratory test results, changes in nutritional status, and how to interpret some seemingly pitfalls to reach the final management of the patient.

Beneficial effect of low dose of alcohol in rodents

Kato N. ¹, and Izu H. ²

¹Hiroshima University, Japan

²National Research Institute of Brewing, Japan, E-mail: nkato@hiroshima-u.ac.jp

Accumulating epidemiological evidence suggests beneficial effect of low dose of alcohol on the development of diseases including cardiovascular disease, diabetes, liver cirrhosis, cancers, etc. However, there are very limited animal studies on the effect of low dose of alcohol. This study was aimed to elucidate the effect of low dose of alcohol on the rats fed a high-fat diet and on the SAMP8 (senescence-accelerated prone-8) mice. In experiment 1, male SD rats (5 week old) were fed a high-fat diet (30% beef tallow) for 12 wk. Ethanol was given with drinking water containing 1% and 2% (v/v) ethanol. In experiment 2, male SAMP8 mice (11 wk old) were fed a commercial stock diet for 15 wk. Ethanol was given with drinking water containing 1% or 2% (v/v) ethanol. In experiment 1, the food intake and growth were unaffected by intake of ethanol. Serum levels of glucose and lipids were unaffected. Compared to the control (no ethanol), 1% ethanol intake significantly reduced serum levels of ALT, LDH, and ammonia ($P < 0.05$), whereas 2% ethanol intake did so to a lesser extent. Serum urate was significantly lower in both the 1% and 2% ethanol groups than that in the control group ($P < 0.05$). In experiment 2, to evaluate the degree of senescence, we scored the changes of aging, including behavior and appearance such as skin, hair and eyes. These score data in large part indicated the delay of aging process of the mice by ethanol, especially 1% ethanol intake ($P < 0.05$). Our study provides evidence for the beneficial effect of low dose of alcohol on liver function and aging in rodents in consistent with the J-shaped relationship between alcohol intake and the risks of mortality due to several chronic diseases.

Resveratrol is not making wine healthy

Šamánek M. ¹, and Urbanová Z. ²

¹Kardiocentrum, University Hospital Motol, Prague, Czech Republic

²Dept. of Pediatrics, First Faculty of Medicine, Charles University, Prague, Czech Republic, E-mail: milan.samaneck31@seznam.cz

Alcohol has been used medicinally throughout recorded history for prevention of intestinal infections (mainly dysentery), for reduction of digestive ailments, treating wounds, as anesthetics, for reduction of anxiety and increase of self-confidence. Alcohol drinking in moderation causes reduction of heart attacks, heart attacks in hypertension, stroke, the incidence and mortality of diabetes and decreases the all-cause mortality and causes the prolongation of life in better physical and mental conditions. In 1992 presence of resveratrol in wine was suggested as the explanation for positive effects of wine. Resveratrol (3, 5, 4'-trihydroxy-trans-stilbene) is produced in grape skin (50-100 µg/g) against growth of fungal pathogens. Anti-cancer, anti-inflammatory, anti-aging activities or cardioprotective effects were proved only in test tube studies (animal and human cells, baker's yeast) and animal studies (mice, rats, short-lived fish, worms, fruit flies). No results of any human clinical trial have been reported. In human, less than 5% of oral dose is observed in blood. Resveratrol is very rapidly metabolized in liver and eliminated. Therefore, health benefits are unrealistic and greatly exaggerated. Moreover, reasonable therapeutic human dose based on animal studies is 500 – 4 000 mg/day which means 120 – 1000

l of wine per day. Why should we not replace wine with pills? Little was known about the safety of high doses of resveratrol. Pharmaceutical Co. GlaxoSmithKline terminated the study on resveratrol pills in December 2010 due to safety concerns. In 2012, the media called red wine studies into question because 145 counts of falsification were found in the beneficial properties of resveratrol declared by the Director of the Cardiovascular research center in Connecticut professor Dipak K. Das. Recently, the results of Chianti study (1998–2009) found that the urinary resveratrol metabolite concentration was not associated with inflammatory markers, cardiovascular disease or cancer prevalences and all-cause mortality of 783 men and women aged ≥ 65 followed from 1998 till 2009. In conclusion, resveratrol in humans is not associated with positive effects of everyday wine drinking in moderation.

Energy value of beer

Olšovská J., and Čejka P.*

Research Institute of Brewing and Malting, PLC, Czech Republic, E-mail: olsovska@beerresearch.cz, cejka@beerresearch.cz

Beer is an important source of crucial nutritional compounds such as carbohydrates and proteins. Therefore, beer has become an indispensable part of the diet in many cultures. Apart from carbohydrates and proteins, also alcohol and glycerol contribute to the total energy value of beer. The rules for food labelling and calculation of the energy value are applicable to all food and beverages including beer. These rules are defined in Regulation (EU) No. 1169/2011 of the European parliament and of the Council. The presentation provides a brief overview of energy value of various types of beer and compares it with other beverages. Further, the assessment of individual components contributing to the total energy value of beer is performed. Finally, the newly developed method for determination of energy value of beer is introduced; the method is more effective and accurate comparing with two current methods.

Biotransformations of prenylated hop flavonoids

Dostálek P.*, Hudcová T., Jelínek L., and Karabín M.

Department of Biotechnology, Institute of Chemical Technology, Technická 5, Praha 6, CZ 16628, Czech Republic, E-mail: Pavel.Dostalek@vscht.cz

Many studies have confirmed that some hop prenylflavonoids effectively inhibit the proliferation of tumor cells and prevent cancer growth and metastasis. Another important effect of some hop prenylflavonoids is their estrogenic activities that mimic human steroid hormones - estrogens, and thus suppress critical symptoms or reduce the risks of hormone-associated cancers. Prenylflavonoid 8-prenylnaringenin has been identified as the most potent phytoestrogen known to date. The level of xanthohumol, the major prenylflavonoid of hop cones in beer is usually about 0.1 mg/l, while in fresh hops, this value is an order of magnitude higher. This is due to the isomerization of xanthohumol into isoxanthohumol at high temperatures during wort boiling. Therefore the predominant prenylflavonoid in beer is isoxanthohumol, whose concentration can be up to 2 mg/l. Levels

of other prenylflavonoids in beer, such as 8-prenylnaringenin, is unfortunately almost negligible. Therefore moderate beer consumption has very few prenylflavonoid-related positive effects on health. However, an interesting fact is that prenylflavonoids ingested via beer consumption interact with the human gut microflora, particular the anaerobic bacterium *Eubacterium limosum*, which is capable of O-demethylation of isoxanthohumol into 8-prenylnaringenin, with up to 80% efficiency. The amount of 8-prenylnaringenin in the blood thus is increased up to 10 times compared with the amount obtained directly via beer consumption. Unfortunately, this biotransformation occurs in only one-third of the human population due to inter-individual differences in intestinal microbiota. Moreover, the rate of conversion is variable between individuals, depending on factors such as age, genetic makeup, and the medical condition of the individual. This biotransformation reaction is interesting due to the estrogenic properties of 8-prenylnaringenin and the administration of probiotic preparations may be one way to increase uptake of 8-prenylnaringenin into the human body.

Food supplements and functional foods enriched with natural polyphenols for treatment of diabetes

Dragan S.*

University of Medicine and Pharmacy “Victor Babes” Timisoara, Romania and Center for Interdisciplinary Research, University of Medicine and Pharmacy “Victor Babes” Timisoara, Romania, E-mail: simona.dragan@umft.ro

In recent years, there is growing evidence that polyphenols-rich natural products, like nutraceuticals and food supplements, may offer unique treatment modalities for various aspects of type 2 diabetes mellitus, due to their biological properties. Natural products modulate the carbohydrate metabolism by various mechanisms, restore integrity and functioning of β -cells, improve insulin releasing activity, glucose uptake and utilization. Sea buckthorn berries, red grapes, bilberries, chokeberries and popular drinks like cocoa, coffee and green tea are all rich in polyphenols and may blunt the insulin response, offering a natural alternative of treatment in diabetes. Therefore, researches are now focused on potential efficacies of different types of polyphenols, including phenolic acids, flavonoids, stilbenes, lignans and anthocyanins. Animal and human studies showed that polyphenols modulated carbohydrate and lipid metabolisms, decreased glycemia, atherogenic dyslipidemia and insulin resistance, improved adipose tissue metabolism, and alleviated oxidative stress and inflammatory processes. It is important to understand the proper dose and duration of supplementation with polyphenols-rich extracts in order to guide effective therapeutic interventions in diabetic patients.

Anthocyanins as a potential therapy for diabetic retinopathy

Daglia M.¹, Nabavi SF.^{2,3}, Habtemariam S.⁴, Shafiqhi N.³, Barber AJ.⁵, and Nabavi S. M.²

¹Department of Drug Sciences, Medicinal Chemistry and

Pharmaceutical Technology Section, University of Pavia, via Taramelli 12, 27100 Pavia, Italy

²Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

³Deputy of Research, Baqiyatallah University of Medical Sciences, Tehran, Iran

⁴Pharmacognosy Research Laboratories, Medway School of Science, University of Greenwich, Chatham-Maritime, Kent ME4 4TB, UK

⁵Department of Ophthalmology, Penn State Hershey Eye Center, Penn State Hershey College of Medicine, Hershey, Pennsylvania, USA, E-mail: maria.daglia@unipv.it

Diabetic retinopathy is one of the most common complications of diabetes. A plethora of literature indicates that oxidative stress may play a central role in the pathogenesis of diabetic retinopathy. Therefore, we could hypothesise that antioxidant therapies may be protective for diabetic retinopathy. Anthocyanins are important natural bioactive pigments responsible for red-blue colour of fruits, leaves, seeds, stems and flowers in a variety of plant species. Apart from their colours, anthocyanins are known to be health-promoting phytochemicals with potential beneficial properties useful to protect against oxidative stress in some degenerative diseases. They also have a variety of biological properties including anti-inflammatory, antibacterial, anticancer, and cardio-protective properties. Some reports further suggest a therapeutic role of anthocyanins to prevent and/or protect against ocular diseases, but more studies are needed to examine their potential as alternative therapy to diabetic retinopathy. This presentation reviews the available literature concerning the beneficial role of anthocyanins in diabetic retinopathy.

Diet rich in polyphenols decreases metabolic parameters and oxidized LDL in diabetic patients

Serban C.^{1,2*}, Andrica F.^{1,2}, Petcu F.^{1,2}, Ardelean F.^{1,2}, Tudor A.¹, and Dragan S.^{1,2}

¹University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania

²Center for Interdisciplinary Research, University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania, E-mail: dr.corinaserban@yahoo.com

Background: The benefits of grape seed (*Vitis vinifera*) and blackcurrant (*Ribes nigrum*) supplements have increased the popularity of polyphenols use in management of diabetes mellitus. Material and method: In this study, we randomized 40 patients with type 2 diabetes mellitus on a basic diet (35 kcal/day) to one of four food supplements: 10 patients in group 1=apple balsamic vinegar enriched with blackcurrant: 108 mg total polyphenols (TP)/day, 10 patients in group 2= apple balsamic vinegar enriched with buckthorn berries (72 mg TP/day), 10 patients in group 3=the VITIS product (22.5 mg TP/day) and 10 patients in CONTROL group=apple balsamic vinegar (5.5 mg TP/day). TP were determined by the Folin-Ciocalteu method. In

all patients, metabolic parameters such as weight, body mass index, fasting blood sugar values, fasting insulin, HOMA-IR and visceral fat area were measured. Oxidized LDL (ox-LDL) and high sensitivity C reactive protein (hsCRP) were determined by ELISA. Visceral fat area was determined with Inbody520 body composition analyzer. Results: Fasting glycemia decreased in group 1 from 107.3 ± 24.78 mg/dl to 96.8 ± 18.56 mg/dl (p=0.007), from 98.8 ± 10.39 mg/dl to 89.4 ± 10.39 mg/dl in group 3 (p<0.001) and from 113.7 ± 25.75 mg/dl to 103.1 ± 18.13 mg/dl in CONTROL group (p=0.008). Visceral fat area decrease in group 1 from 160.18 ± 39.60 cm² to 152.22 ± 34.07 cm² (p=0.005), in group 2 from 181.64 ± 45.80 cm² to 166.7 ± 38.6 cm² (p=0.011), in group 3 from 173.85 ± 34.72 cm² to 166.53 ± 32.28 cm² (p=0.002) and in CONTROL group from 197.6 ± 64.33 cm² to 186.58 ± 59.96 cm² (p=0.013). From all products tested, only the Vitis product significantly lowered ox-LDL values, from 108.78 ± 24.52 mg/dl to 91.02 ± 23.97 mg/dl in group 3 (p=0.036). hsCRP values decreased from 2.9 ± 1.37 to 2.2 ± 0.92 mg/L in group 1 (p=0.034), and from 2.8 ± 2.1 to 2.2 ± 1.75 mg/L in group 3 (p=0.024). Data analysis revealed a significant Pearson correlation between ox-LDL values and hsCRP in all study groups (r=0.245, p=0.025). Conclusion: Food supplements rich in polyphenols decrease glycemia, visceral fat area, ox-LDL and hsCRP in diabetic patients. These supplements could contribute to a better metabolic control in type 2 diabetic patients.

Dietary flavonoids and type 2 diabetes: Current insights and future perspective

Xiao J.^{1,2*}, and Högger P.¹

¹Institut für Pharmazie und Lebensmittelchemie, Universität Würzburg, Am Hubland, 97074 Würzburg, Germany

²Department of Biology, Shanghai Normal University, 100 Guilin Rd, 200234 Shanghai, China, E-mail: jianboxiao@yahoo.com

Diabetes mellitus is one of the most significant public health problems in the world. WHO has reported that there are about 347 million people worldwide have diabetes in 2013. The majority is seen in developing countries (South America, China, and India) undergoing westernization. For example, more than 100 million Chinese developed to type 2 diabetes in 2012. It accounts for about 9.3% of total population of China. Significant evidence has shown that the polyphenol-rich diets have the ability to protect against diabetes. Since the last several reviews focused on the nutrition and health effects including type 2 diabetes of polyphenols in 2007-2008, a number of related original publications have appeared in this area. Here we summarized important advances related to influence of dietary polyphenols and polyphenol-rich diets on preventing and managing type 2 diabetes, as well as diabetes-mediated changes in pharmacokinetics and bioactivities of dietary polyphenols. It looks like that anthocyanins or anthocyanin-rich foods intakes are related to the risk of type 2 diabetes, but there is no association for other polyphenol subclasses. It illustrated that procyanidins are more active when administered individually than when mixed with food. The benefits of dietary polyphenols for type 2 diabetes can be summarized as: protection of pancreatic β-cells against glucose toxicity, anti-

inflammatory, antioxidant, inhibition of starch digestion, inhibition of α -amylases or α -glucosidases, and inhibition of advanced glycation end products formation. Moreover, type 2 diabetes also significantly influence the benefits of dietary polyphenols, although there are very limited studies have been conducted so far. Although there is very limited information available so far, it is proposed that type 2 diabetes influences the pharmacokinetic behavior of dietary polyphenols including: i) competition of glucose with polyphenols regarding binding to plasma proteins; ii) weakened non-covalent interaction affinities of plasma proteins for natural polyphenols due to protein glycation in type II diabetes; iii) the enhanced clearance of polyphenols in type 2 diabetes. However, how type 2 diabetes impact the pharmacology of dietary polyphenols are not well understood. An understanding of type 2 diabetes-mediated changes in pharmacokinetics and bioactivity of dietary polyphenols will lead to improve the benefits of these phytochemicals and clinical outcomes for type 2 diabetics.

Are multivitamin and mineral supplements good for healthy aging

Jansen E.*

National Institute for Public Health and the Environment, The Netherlands, E-mail: eugene.jansen@rivm.nl

Introduction: The supplementation of antioxidants and vitamins to our nutrition is considered as being beneficial. Aging, however, is not always considered as an important issue. Our specific aim for this project is to test the hypothesis that supplementation of antioxidant vitamins in humans have positive health effects only in later stages of adult life by counteracting the detrimental effects associated with ageing. This biomarker driven project consists of two parts, a short-term human intervention study and a lifetime mice study. In this presentation a survey will be given of the available supplements in the Netherlands. In addition, an overview will be given of the result of both studies. **Methods:** The mice study was a lifetime study with in total 600 mice. The human intervention study was a short randomized intervention trial of 8 weeks with 80 adult subjects, stratified by sex, age and smoking status. Biomarkers of vitamins, oxidative stress, antioxidant and redox status have been measured in serum and erythrocytes (human study) and in serum, erythrocytes and tissue homogenates (mice study). **Results:** From both human and mice study it appeared that biomarkers of the vitamins increased significantly. As a result, the effect biomarker homocystein decreased substantially. Serum biomarkers of oxidative stress (dROM), total antioxidant (BAP), however, did not change during the course of the study. Only the redox biomarkers showed changes both in serum, erythrocytes (human study) and tissue homogenates (mice study). The antioxidant enzymes of the glutathione pathway increased significantly whereas glutathione did not change. **Conclusions:** Supplementation of multivitamins/minerals resulted in an improvement of the vitamin status. Oxidative stress biomarkers did not change in serum, but only in the erythrocytes. Changes in the redox status in both studies were observed as a result of a low-level supplementation of multi-vitamins and minerals.

Antioxidants protect tumor cells from reactive oxygen species

Bergo M.¹, Lindahl P.², Sayin V.¹, Ibrahim M.¹, Le Gal Beneroso K.¹, and Larsson E.³

¹Sahlgrenska Cancer Center, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden

²Wallenberg Laboratory, Institute of Biomedicine, Sahlgrenska University Hospital, University of Gothenburg, Sweden

³Institute of Biomedicine, University of Gothenburg, Sweden, E-mail: martin.bergo@gu.se

Antioxidants neutralize reactive oxygen species (ROS) in cells and thereby prevent oxidative DNA damage that may otherwise lead to tumorigenesis. Therefore, antioxidant-rich food and supplements are widely used to protect against cancer. However, large randomized clinical trials have demonstrated that antioxidant supplementation either has no impact or actually increases cancer incidence, at least in the lung and prostate. Furthermore, molecular analyses of human lung cancer and experimental studies in mice suggest that tumor cells proliferate faster when levels of ROS and DNA damage are kept low. Tumor cells may keep ROS levels low by activating the expression of endogenous antioxidant genes or by benefiting from exogenous antioxidant in the diet. We have found that supplementing the diet with the antioxidants vitamin E or N-acetylcysteine markedly increases tumor growth and reduces survival in mice with K-RAS- and B-RAF-induced lung cancer. The antioxidants increase tumor cell proliferation by reducing ROS, DNA damage, and p53 expression in mouse and human lung tumor cells. The results suggest that antioxidants may be unsafe in people with increased risk of lung cancer such as smokers and patients with chronic obstructive pulmonary disease. p53 is often inactivated late in tumor progression; therefore, antioxidants may primarily affect precancerous lesions and early tumors. Although antioxidants may protect a healthy cell—or tumor-free person—from future tumorigenesis, we propose that antioxidants also protect tumor cells from ROS and thereby accelerate tumor progression. This could potentially explain the outcome of the clinical trials which likely included both tumor-free people and people with precancerous lesions or undiagnosed tumors.

Antioxidant activities of pomegranate (*Punica granatum* L.) fruits peel extracts

Zaki S.¹, Abdelatif S.², Abdel Mohsen N.², and Ismail F.¹

¹Food Technology Department, Faculty of Agriculture, Cairo University, Giza, Egypt

²Hort. Crop. Proc. Res. Dept, Food Technol. Institute, Agric. Res. Center, Egypt, E-mail: drshafikazaki@gmail.com

The peel and seed fractions of some fruits possess high antioxidant activity. Methanol and water extracts of Peels from two pomegranate (*Punica granatum* L.) varieties grown in Egypt namely: Wardey and Manfalouty were screened for phenolic compounds and antioxidant

activities. A fine dried pomegranate peel powder of both cultivars was continuously extracted with methanol, or cold water. The obtained results demonstrated that the yields of methanol extract (% dry weight of pomegranate peels) from the two varieties were 48.2% for Manfalouty peels and 33.3% for Wardey peels. However, the yields of water extracts of Manfalouty and Wardey peels were 17.1% and 12.5%, respectively. Data for the pomegranate peel extracts indicated that the amount of phenolic compounds in the methanol extracts from the pomegranate peels were much higher (113.3 and 74.94 mg /100 g of dry Manfalouty and Wardey peels, respectively), than those in water extract (32.6 and 16.2 mg/100 g of the two varieties in respective order). The antioxidant activities of the two pomegranate peel extracts were evaluated by using β -carotene / linoleic acid method. Inhibition values both of peel extracts and standard (BHA) increased parallel with the elevation of concentration in the linoleic acid system. Inhibition values of both two pomegranate (Manfalouty and Wardey) peel extracts of methanol reached 45.50%, 80.21%, while those of water were 35.61% and 70.87%, respectively at a concentration of 100 ppm. BHA has 93.3% inhibition at the same concentration (100 ppm).

Extracts of Chilean native fruits inhibit inflammation, oxidative stress and insulin-resistance of adipocytes, induced by macrophage conditioned media treatment

Garcia-Diaz D.F.*, Reyes-Farias M., Ovalle-Marin A., Hernando C., Parra C., Jimenez P., and Quiral V.

University of Chile, Department of Nutrition, E-mail: dgarcia@med.uchile.cl

Obesity has been linked with the presence of a low-grade chronic pro-inflammatory state, which in turn has been related with the development of insulin-resistance. The aim of the present work was to evaluate whether extracts of Chilean native fruits, rich in polyphenols, have the ability to inhibit the presence of an inflammatory and pro-oxidant response, and of insulin-resistance, in adipocytes treated with conditioned media (CM) from macrophages. Ripe fruits of *Aristotelia chilensis* (ACh), *Berberis microphylla* (BM) and *Vaccinium corymbosum* (VC - as control) were dried, pulverized, extracted in methanol: water, and rota-evaporated. 100 μ M [polyphenols] of each extract were utilized. 3T3-L1 mouse adipocytes were treated with CM from previously LPS-activated macrophages in presence or absence of extracts for 24h/96h. Gene expression and secretion of inflammatory markers, antioxidant content, and glucose uptake were determined. CM induced higher and lower gene expression and secretion of MCP-1 and adiponectin, respectively, in adipocytes after 24h. Also, it induced lower reduced glutathione (GSH) content (after 24h) and glucose uptake (after 96h). ACh inhibited CM-induced higher MCP-1 expression, and all extracts slightly restore adiponectin secretory profile. All extracts restored GSH content; but only BM extract prevented adipocytes insulin-resistance. Summing up, extracts of Chilean native fruits have the ability to prevent the establishment of a pro-inflammatory/-oxidant environment in adipocytes, eventually preventing insulin-resistance development *in vitro*.

Antioxidant and anti-inflammatory effects of honeys and their phenolic extracts on adhesion molecules expression in L6 cells

Yusof H. M.¹*, and Pui C. S.²

¹School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

²School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia, E-mail: hayatimy@umt.edu.my

Free radicals and inflammation has been linked in the pathogenesis of cardiovascular disease (CVD), the world largest killer. The statin therapy used in CVD prevention may increase the risk of adverse effects in a long-term. Acacia and Kelulut honeys are natural products containing phenolic compounds which possess pharmacological effects, including antioxidant and anti-inflammatory actions. The aim of the present study was to study the antioxidant and cytotoxicity activity of Acacia and Kelulut honeys and their phenolic extracts as well as to determine the effects of quercetin, kaempferol and these two honey extracts on inflammatory markers, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) adhesion molecules in L6 cells. 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay and enzyme-linked immunosorbent assay (ELISA) had been used to investigate the antioxidant, cytotoxicity and anti-inflammatory activities of the samples. In the DPPH assay, Acacia honey extract (AHE) showed the highest inhibition percentage, 64.7% (IC₅₀=17.6 mg/mL) followed by Acacia honey, 42.9% (IC₅₀ =>500 mg/mL) and Kelulut honey extract (KHE), 40.2% (IC₅₀ =>50mg/mL). Acacia and Kelulut honeys and their extracts caused a mild or no toxicity due to more than 50% cell viability exhibited. In this study, LPS was used to induce the L6 cells. The results revealed that pre-incubation and treatment of quercetin and kaempferol exerted significant anti-inflammatory effects at 12 hours. Treatment of phenolic compounds (as to mimic secondary prevention) showed better anti-inflammatory effect than in pre-incubation of sample (as to mimic primary prevention). Thus, results showed that in order to treat CVD, quercetin and kaempferol are better than honeys. Pre-incubation and treatment of AHE and KHE exerted significant anti-inflammatory effects at 12 hours and 24 hours. Pre-incubation of honey extracts showed better anti-inflammatory effect than in treatment of sample at 12 hours. While for 24 hours, treatment of honey extracts showed better anti-inflammatory effect than in pre-incubation of sample. Thus, the present results suggest that for CVD prevention, honey is a better choice but for treatment, the use of honey requires longer duration to exhibit its beneficial effect.

Health claims in relation to active ingredients in food supplements

Winklerová D*

National Institute of Public Health, Prague, Czech Republic,

E-mail: winklerova@szu.cz

Health claims which are permitted in EU to be used on food labeling or in a presentation of food are laid down namely by general Regulation EU No. 1924/2008 and by specific regulations mentioning the wording of health claims. The most of permitted health claims are included in Regulation (EU) No. 432/2012. Comprised health claims not always concern active substances permitted for use in food supplements. For example the substance melatonin helping to fall asleep people who often undergo time shift when travel between continents. In the Czech Republic as well as in many other countries this substance is allowed only in medical preparation but not in food supplements. Another example can be red fermented rice containing monacolins. Red fermented rice was previously used in meat products for enhancing their color. This use is now forbidden in EU because of history of consumption of red fermented rice has not been documented as ingredient in traditional food. But in the case of food supplements the use of red fermented rice was proved before 1997 according to the Regulation (EU) No. 258/1997. Red fermented rice is obtained by fermentation process caused by mold *Monascus purpureus* which produces approximately 0,4% monacolins of which roughly half is monacolin K. Monacolin K has the same effect like group of substances called statins which are used as active ingredients in drugs intended for reduction of blood cholesterol level. Debatable matter is, a bit surprisingly, the use of the word "probiotics" because the word itself can represent health claim. The other time this word is considered to be nutritional claim but solely as information about presence of certain microorganisms. Until now EU member states have no unique opinion. Some other cases of active ingredients in relation to health claim will be discussed in the presentation.

An orthomolecular approach to mental disorders: Hoffer-osmond diagnostic test and nutritional supplementation

Johnson R.R.*, and Johnson A.

Johnson's Orthomolecular Clinic, Calgary, Canada, E-mail: dr-rj@telus.net

Background: Mental disorders - depression, anxiety, schizophrenia and addictive behaviours are common internationally. In Canada and the USA, one in five suffer from any of the category. It is a leading cause of disability. Every week, 500000 Canadians are unable to work. Yearly, this costs Canada \$51 billion. Objectives: To identify patients suffering from mental disorders using the Hoffer-Osmond Diagnostic test and to examine the effectiveness of Hoffer's nutritional approach on the correction of these disorders in an out-patient clinic. Method: The study was based on data extracted from our patient history database covering a period of five years. Using the DSM-IV, patients were identified as suffering from depression, anxiety and schizophrenia. Over 1500 patients were identified. Ten percent (150 patients) were randomly selected. Each patient had completed a Hoffer-Osmond Diagnostic (HOD) test at the first encounter. The HOD consists of 145 statements on visual, auditory, tactile, taste,

olfactory and time perceptions. A true or false response is required. Each patient had been tested for nutritional deficiencies. Hoffer's nutritional protocols (based on niacin, vitamin C, and proprietary blends) were administered based on the deficiencies identified. The patients completed the HOD test at periodic intervals to monitor the effects of the nutritional supplements. Results: We compared the HOD scores before and after the nutritional treatments for the three mental disorders: depression, anxiety and schizophrenia. HOD scores for the depressive group improved the most (76%), followed by anxiety (60%) and schizophrenia (45%). Low nutritional deficiencies (vitamin A,D, and B12) were predominant for all the groups. Hormonal deficiency of low thyroid was common among the depression group. All the groups were characterized by high toxic levels especially mercury and cadmium. High mercury level was dominant among the schizophrenic group. The lower the HOD scores the better the patients felt. Most of the patients had to be detoxified along with nutritional supplementation. Conclusion: Our data suggests that the HOD test is a very useful tool for primary care givers to quickly identify mental disorders. In the population studied, it is shown that by correcting the nutritional deficiencies through application of the Hoffer protocol, the mental disorders can also be corrected or alleviated. The limitations to Hoffer's protocols are: resistance to using nutritional supplements from orthodox medical community, patients compliance, and willingness to accept a diagnostic tool that appears to be too easy.

Ambivalent items of ema herbal monographs

Bažata V.*

Private Consultant, Prague, Czech Republic, E-mail: vaclav.bazata@email.cz

Traditional food use (25 years within EU) is evident from the classification of 165 items, where 27% (45) of it has food or food additive history with robust evidence of 20% (33) of significantly frequent use as spice supplement with health positive physiological effect and the remaining 7% (11) of spicy items on this list are having status as condiment, flavouring or colorant agents directly or via their derivatives/isolates (e.g. extracts, oleoresins, essential oils etc.). From that point it is clear that all plants/preparations which have been identified as having medicinal use by the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA) could not be forbidden in the manufacture of food supplements/ food fortification, and in the in light of this there is no background there for usable decision criterion for borderline products. Examples of established spices are given and reference to CODEX COMMITTEE ON SPICES AND CULINARY HERBS (CCSCH) new work is made.

Design and evaluation of nanolipid carrier as a vehicle for lipophilic bioactive component

Bostan A.*

Food Nanotechnology Department, Research Institute of Food Science & Technology, Mashhad, Iran, E-mail: arambostan@yahoo.com

Lipid carriers are potential delivery system in food formulations. A new type of lipid nanoparticles which offer the advantage of improved loading capacity and release behavior is nanostructured lipid carriers (NLCs). NLCs comprised of a blend of liquid and solid lipid. In this study we examined the effect of lipid phase composition and oil concentration on the particle size, zeta potential, thermal properties and physical stability of nanostructured lipid carriers (NLC). A series of fine-disperse oil-in-water emulsions was prepared at an elevated temperature (10°C upper than melting point) from lipid phases containing different amounts of a high melting lipid (Compritol/Percinol) and a low melting lipid (Wild sage seed oil). These emulsions were cooled to induce crystallization and form NLC suspensions. All particles were smaller than 130 nm one day after production. DSC measurements suggested that thermal properties and crystallinity of NLCs were influenced by the amount of oil incorporated and the lipid phase composition. In order to study the stability of nanocarriers NLCs were kept at ambient and refrigerator temperature. After 4 weeks mean diameter of all samples were less than 150 nm.

Combination of calcium chloride and hot water to control postharvest blue mould for production of safety and healthy apple fruit

Etebarian H. R.*, and Farhangian-kashani S.

Department of Agriculture, Yadegar-e- Imam Khomeini (RAH) Branch, Islamic Azad University, Tehran, Iran, E-mail: etebar@ut.ac.ir

Penicillium expansum Link ex Thom causes blue mould of apple, the most important disease of apple and produces the carcinogenic mycotoxin patulin in decayed fruit. Control of postharvest pathogens still relies mainly on the use of synthetic fungicides, but the development of fungicide-resistant pathogens and the public demand to reduce pesticides have increased the need for alternative control strategies. In this study the effect of calcium chloride alone and in combination with hot water at 40°C and 50°C for control of apple blue mould were investigated. Results *in vitro* studies showed that calcium chloride concentrations of 2, 4 and 6% reduced the growth of pathogen. Results *in vivo* experiments indicated that, when calcium chloride was applied at concentration of 2, 4 and 6% in wound of apple and then 24 hours later inoculated with *P. expansum*, the lesion area disease incidence and disease severity were less than that of pathogen control after 9 days incubation at 20°C. Results of experiment which carried out for incubation at 4°C indicated that when the apples immersed in hot water at 40°C or 50°C for 1, 2 or 3 min. after inoculation with pathogen, the lesion diameter, disease severity and disease incidence were significantly less than those of pathogen control after 21 days incubation at 4°C ($p < 0.05$). The lesion diameter, disease severity and disease incidence in the apple which were immersed in hot water at 40°C or 50°C after inoculation with pathogen and then treated with calcium chloride, were significantly less than pathogen control and in most cases no infection was occurred. In conclusion the results of this study showed that concentrations of calcium chloride especially combination with hot water affects apple responses to *P. expansum* and

could be an important method for control of apple blue mould.

Sugars, total carotenoids and phenols in microwave-vacuum drier processed vegetables and spices

Priecina L.*, Karklina D., Rakcejeva T., and Cinkmanis I.

Latvia University of Agriculture, Faculty of Food Technology, Latvia, Jelgava, Liela iela 2, E-mail: liga.priecina@gmail.com

There are many drying methods for food shelf-life extension. Microwave-vacuum (MV) drying is one of the newest known methods. In this research was analysed pre-treated with steam (1.5 and 3.0 min) and in microwave-vacuum drier processed nine spices and vegetables as celery, parsley, dill, leek, garlic, onion, celery root, pumpkin, carrot harvested in Latvia, Zemgale region in 2013. Total carotenoids and phenolic compounds (total phenol content, phenolic acids, total flavonoids, flavan-3-ols (proanthocyanidins), flavanols) and their antiradical scavenging activity (with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical solutions) were determined with spectrophotometric methods for all samples and major sugars (fructose, glucose, sucrose and maltose) by using of high performance liquid chromatography (HPLC). Dry matter content was calculated. Results of current experiments demonstrate that for all analysed samples dry matter content varies from 81.70% to 93.21%. The highest carotenoid content was determined in 1.5 min steamed and dried celery (286.22 ± 21.09 mg β -carotene equivalent (β -CE) per 100 g⁻¹ dry weight (DW)), lowest in dried celery roots (0.08 ± 0.01 mg β -CE 100 mg⁻¹ DW). It was determined, that steaming could provide bioavailability of carotenoids in most of analysed samples. The highest total phenol content was determined in spices, the lowest in vegetables. Steaming could promote increasing of phenolic acid and flavonoid content, and reduce flavan-3-ol content in spices; however phenolic acid content decreases and flavan-3-ol content increases in vegetables. It could be explained with reduce of phenolic compound from glycosides to more simple phenols by extra heat supply. Antiradical scavenging activity ranged from 287.38 ± 3.74 μ M Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents (TE) per 100g⁻¹ DW (3.0 min steamed and dried garlic) to 915.95 ± 0.67 μ M TE per 100⁻¹ DW (3.0 min steamed and dried pumpkin) using ABTS radical; from 127.38 ± 0.63 μ M (in dried celery roots) to 706.75 ± 1.25 μ M TE per 100g⁻¹ DW using DPPH radical. Differences could be explained with radical distinguish abilities to react with different phenolic compounds. Major sugars in spices are glucose and fructose, in vegetables – fructose, glucose and sucrose. The fructose content decrease, but content of glucose and sucrose increase in all analysed samples after steaming. Obtained sugar changes could be explained with possible oligosaccharides decreasing in mono or disaccharides.

The choices programme: 4 years experience in Czech Republic

Brát J.*

Board member of Vím, co jím a piju o.p.s., Czech Republic, E-mail:

j.brat@vimcojim.cz

Some food products fit better within a healthy diet than others, but how can consumers tell? The Choices programme (known as „Vím, co jím“ in the Czech Republic) is a simple and internationally-applicable programme to help consumers make a healthy choice on food and beverages and to stimulate industry towards healthy product innovation in all food groups. The essence of the programme is a front-of-pack logo on products that pass an evaluation against scientific criteria. Generic criteria have been established for levels of saturated fat, trans fat, sugars and sodium, which are based on international dietary guidelines (FAO / WHO). For some food categories there are specific criteria for these nutrients as well as for dietary fiber and energy content in the product. In this way the criteria are challenging without being impossible to meet. The Choices programme encourages food industry worldwide to develop healthy product innovations and to reformulate the nutritional content of existing products by using the Choices logo to highlight the efforts made by companies and caterers. For participating companies the Choices logo drives healthy innovation and reformulation activities. When a product is developed or reformulated to meet the Choices criteria, the logo can be used on packaging, in advertising and in marketing materials. This encourages consumers to select the product while setting new standards for food composition. In this way innovation can result in win-win situation for both consumers and food companies. Non-profit organization Vím, co jím a piju has been running the Choices programme in the Czech Republic since 2010. The Choices logo is currently found on approximately 7000 food and beverage products worldwide, of which 350 products are certified within the Czech Republic. There are more than 120 participating companies worldwide of which 23 are participating in the Czech Republic. These numbers continue to grow, making the Choices Programme a truly global initiative.

Methodology for the safety assessment of lupin in terms of alkaloids content

Vaněrková D.^{1*}, Marková L.¹, Portyčková L.¹, Horna A.¹, and Jelínek M.²

¹Institute of Nutrition and Diagnostics, RADANAL Ltd., Okružní 613, 530 03 Pardubice, Czech Republic ²University of Hradec Kralové, Faculty of Science, Czech Republic, E-mail: vanerkova@radanal.cz

Legumes, together with cereals, represent the main plant source of proteins in a human diet. They are generally rich in dietary fibre and carbohydrates, while minor compounds of legumes are lipids, polyphenols and bioactive peptides. Lupin is economically and agriculturally a very valuable plant. Its seeds are employed as a protein source for animal and human nutrition in various parts of the world, not only for their nutritional value, but also for their adaptability to marginal soils and climates. Furthermore, human consumption of lupins has rapidly increased in recent years. Nevertheless, lupins belong to the group of toxic plants due to their alkaloids content. Quinolizidine alkaloids are hepatotoxic and teratogenic, and represent hazard for livestock as well as for humans. The usage of lupins is

discussed considering lupine alkaloids from the nutritional and toxicological point of view. Therefore, modern analytical methods for a reliable alkaloid determination are highly required. The presented certified method reports the ultra – HPLC coupled to a triple quadrupole tandem mass spectrometry (UHPLC/MS/MS) method for the analysis of Lupanine, Lupinine and Sparteine in Lupinus species. A simple extraction with 70% (v/v) methanol in water was followed by the gradient separation on a C18 column with the 0.05% (v/v) formic acid in water and methanol. The mass spectrometer was operated in Multiple Reaction Monitoring (MRM) mode with the positive electrospray ionization (ESI) interface. The method was validated for linearity, sensitivity, accuracy, repeatability, robustness as well as precision; and then used to assess the seeds of different Lupinus species for their alkaloid content. Lupanine was present in all samples within a range from 0.01 to 0.06% (w/w), the amounts of Lupinine and Sparteine were below the limit of quantitation (LOQ). The LOQ values are 0.00026% (w/w) for Lupanine, 0.00033% (w/w) for Lupinine and 0.00014% (w/w) for Sparteine. The precision ranged from 4.4% to 15.2% and accuracy (recovery) ranged from 104% to 128%. The maximum limit of alkaloids content 0.02% (w/w) is fixed by the Health Authorities of Australia, New Zealand, Great Britain, and France.

Clinical and echocardiographic effects of hypocalcemia secondary to severe vitamin D deficiency (VDD) and effect of treatment

Soliman A. T.¹, Saeed S.^{2*}, and Khellah A.²

¹Hamad Medical Center, Endocrinology Department, Doha, Qatar

²Hamad Medical Center, Cardiology Department, Doha, Qatar, E-mail: saad_75_2000@yahoo.com

Introduction: Hypocalcemia (HC), without an underlying myocardial disease, is a relatively uncommon but reversible cause of congestive heart failure. Objective: We studied the cardiac functions (heart rate, blood pressure, ECG and Echocardiographic parameters (Fractional shortening (FR), left ventricular end diastolic diameter (LVEDD) of 14 children who presented with hypocalcaemia due to VDD before and 2 -4 weeks after treatment with an IM dose of vitamin D 3 (VD) (10,000 IU/kg). Results: Correction of HC and VDD was associated with marked improvement of the LVEDDSDS (3.2 +/- 4.4 to 1.1 +/- 2.8) and slowing of the heart rate (from 101 +/- 34 to 94.7 +/- 30/ min). The FS and QTc did not change. The LVEDDSDS was negatively correlated with serum calcium level ($r = -0.46$, $p = 0.03$) and PTH concentrations ($r = 0.44$, $p = 0.032$) but not with 25OHD level ($r = -0.2$, $p = 0.2$). Discussion: Improvement of the recorded changes in the heart rate and LVEDD after VD therapy implements an important role of VD through its effect on the concentration of the extracellular calcium ion that could modify the strength of the myocardial contraction through excitation-contraction coupling. Conclusion: These data strongly indicate that the maintenance of an optimal vitamin D status may be a promising approach for the prevention and/or therapy of myocardial diseases and in countries with high prevalence of VDD, vitamin D supplementation can prevent this risk of cardiac dysfunction.

Impact of vitamins B12, B6 and folate supplementation on the cardiovascular risk markers in an elderly community of sharpeville, SA

Grobler C.*, and Oldewage-Theron W.

Vaal University of Technology, South Africa, E-mail: christa@vut.ac.za

Background: In a vulnerable low income group with a confirmed high risk of cardiovascular disease, like the elderly in the Sharpeville care centre, an acute intervention is needed in order to improve their health profile. Previous studies suggested homocysteine lowering by vitamin B12, B6 and folate supplementation. The effect of vitamin B12, B6 and folate supplementation on the inflammatory response, thrombotic risk, lipid profile, hypertension, risk for metabolic syndrome and the homocysteine metabolism in an elderly, black South African population has never been reported. **Objectives:** The main aim of this interventional study was to assess the effect of vitamins B12, B6 and folate supplementation at 200% RDA for six months on cardiovascular risk markers of an elderly semi-urbanised black South African community. **Design:** This study was an experimental intervention non-equivalent control group study design (Welman and Kruger 2001:79) in 104 purposively selected samples of all the elders attending the day-care centre. **Setting and participants:** A homogenous group of respondents were included into the study. All subjects were equivalent in age (>60 years), race (black), unemployed / pensioner (socio-demographic) and 60 years and older attending a daycare centre in Sharpeville, situated in the Vaal region, Gauteng, SA. **Measurements:** The distinctiveness of this study is the broad panel of parameters evaluating the CVR, in correlation with the increased nutritional intake of vitamin B6, B12 and folate. These included: weight, height, waist, serum cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, blood pressure, fibrinogen, high sensitive C-reactive protein (HS-CRP), homocysteine, vitamin B12, folate, glucose, insulin, adiponectin and fibronectin. **Results:** A very high incidence (66.36%) of hyperhomocysteinaemia is present in the sample, the mean serum homocysteine level in hyperhomocysteinaemic individuals decreased statistical significantly from 25.00 ± 8.00 $\mu\text{mol/l}$ to 18.80 ± 12.00 $\mu\text{mol/l}$ after the intervention. The number of respondents with an increased homocysteine level decreased from 100% (baseline) to 67% (follow-up). The supplementation was beneficial (statistically significant changes) to all the individuals (independently of their homocysteine status) on their glucose levels, their fibrinolytic status, vitamin B6 serum levels, fibronectin levels and on their haemopoiesis (decrease macrocytosis). **Conclusion:** It is concluded that supplementation of vitamins B6, B12 and folate at 200% RDA for six months is an effective homocysteine lowering approach as a strategy to reduce hyperhomocysteinaemia in an elderly population, and thereby reduce the Cardiovascular risk (CVR). The mentioned supplementation intervention is not an effective multifactorial strategy to decrease CVR although beneficial effects were found with other CVR markers independent of homocysteine status.

Cardioprotection with polyphenols

Netticadan T.*

Canadian Centre for Agri-Food Research in Health and Medicine, Winnipeg, MB, Canada, E-mail: thomas.netticadan@agr.gc.ca

Despite the success of current therapies available for the prevention and treatment of cardiovascular disease (CVD), it remains to be one of the major causes of death worldwide. Accordingly, there is a dire need to explore new avenues to improve survival in patients with CVD. One such avenue may be exploring the potential of whole foods or food derived components. Over the course of time, the concept of food expanded beyond its' traditional role of survival and hunger satisfaction, to include a role in disease prevention and treatment. Polyphenols are a class of compounds that are synthesized by plants to serve a wide variety of function including growth, pollination and defence. These compounds have recently received increased attention for their potential health benefits. A prominent example being resveratrol, which is one of the most studied polyphenol, found predominantly in foods such as grapes, peanuts and berries. For the past eight years, we have studied in animal models of cardiovascular disease (CVD) the ability of resveratrol to prevent or reverse in vivo the development of abnormalities in cardiac structure and function. We also examined in vitro the effects of polyphenols in protecting diseased adult rat cardiomyocytes. The molecular mechanisms underlying the effects of resveratrol was studied in both diseased adult rat cardiomyocytes and heart tissues from the animal models of CVD. Our results showed that administration of 2.5 mg/kg/day of resveratrol was able to prevent/reverse abnormalities in cardiac structure and function in animal models of CVD such as hypertension (the spontaneously hypertensive rat) and obesity/type II diabetes (high fat fed rat). We also demonstrated that 30 micromolar resveratrol protected cardiomyocytes against chronic exposure to norepinephrine (NE), a potent hypertrophic and cell-death trigger. The beneficial effects observed with resveratrol were associated with its antioxidant and anti-inflammatory activities. The strong cardioprotective effects observed with resveratrol, led us to examine the potential of a polyphenol rich source – blueberry. Our results showed cardiomyocyte protection with total blueberry polyphenol enriched fraction in NE-exposed cardiomyocytes, similar to that observed with resveratrol. On the basis of the results from our animal studies, we conclude that polyphenols render strong cardioprotection, and may therefore have a potential in the prevention and treatment of CVD.

Stop wasting money on vitamins

Urbanová Z.¹, and Šamánek M.²

¹Dept. of Pediatrics, First Faculty of Medicine, Charles University, Prague, Czech Republic

²Kardiocentrum, University Hospital Motol, Prague, Czech Republic, E-mail: urbanova.zuzana@vfn.cz

The recent trials of single or paired vitamins supplementation concluded that there was no clear evidence of a beneficial effect of supplements on all-cause mortality, cardiovascular disease, or

cancer. Despite strong evidence of no benefit or possible harm, use of multivitamin supplements increased among U.S. adults from 30% between 1988 to 1994 to 39% between 2003 to 2006. Sales of multivitamins and other supplements have not been affected by major studies with null results, and the US supplement industry continues to grow, reaching \$28 billion in annual sales in 2012 which are growing at a 5% to 7% annual clip, (EU supplement industry 11% according to IMS Health). Longitudinal and secular trends show a steady increase in multivitamin supplements use and a decline in use of some individual supplements, such as carotene and vitamin E. The decline in use of carotene and vitamin E supplements followed reports of adverse outcomes in lung cancer and all-cause mortality, respectively. Similar trends have been observed in the United Kingdom and in other European countries. Most supplements do not prevent chronic disease or death, their use is not justified, and they should be avoided. This is especially true for the general population with no clear evidence of micronutrient deficiencies, which represents the majority of supplement users in the United States and in other countries. In conclusion, if you are generally healthy and eat a wide variety of foods, including fruits, vegetables, whole grains, legumes, low-fat dairy products, lean meats and fish, you likely do not need vitamin supplements. Eating a healthy food and exercise is the most cost-effective way to promote good health.

Vitamin and trace element deficiencies in industrial society - Clinical traps and pitfalls

Zadák Z.*

Charles University Prague, Faculty of Medicine and University Hospital Hradec Kralove, Czech Republic, E-mail: zadak@cmail.cz

Definition of vitamins: Essential complex micronutrients requiring permanent supplementation depending on the gender, body weight, age and health status. Deficit of micronutrients is characterized by following features: 1. Symptoms of deficit are reproducible. 2. Changes are progressive-morphological, biochemical, functional) 3. Normalization after adequate supplementation appears. Deficit of vitamins in undeveloped countries still kills huge number of people, even in industrial society in Europe is micronutrient deficit very frequent in some groups of population and increases mortality and morbidity in specific groups as in malnourished elderly people, seriously ill, critical patients in intensive care (ICU), homeless and very poor people, alcoholics etc. Typical example of unrecognized and misdiagnosed vitamin deficit with fatal consequences is deficiency of thiamine (B1). Thiamine, precursor of pyruvic acid decarboxylase is a key compound in glucose metabolism. Whole body stores are approximately 20-30 mg and daily requirement 2 mg and in critical condition in ICU this stores are exhausted in 7-10 days (in alcoholics, malnourished patients, old patients even earlier). B1 deficit results in serious acidosis (in absence of diabetes or renal failure), muscle weakness, heart failure, and neurological deficit. Only rational solution is early and correct diagnosis of vitamin deficit and treatment by means of pharmacological doses of Thiamine. Conclusion: Vitamin deficits

still kill in some developed European countries malnourished, frail and seriously ill patients (old patients, polytrauma patients, extensive operation, infections) due to incorrect or late diagnosis and inadequate micronutrient supplementation.

The vitamin K2 (Menaquinone-7) insufficiency in cardiovascular disease, osteoporosis and chronic inflammation

Maresz K.¹, and Badmaev V.²

¹The International Science and Health Foundation, Poland

²NattoPharma ASA, Norway, E-mail: katarzyna.maresz@nutricon.eu

Cardiovascular disease and osteoporosis are major age-related health concerns in Western society, and these two conditions are characterized by the presence of chronic inflammation. It has been recently postulated based on epidemiological studies that dietary insufficiency of vitamin K, especially menaquinone-7, maybe a leading factor in the multifactorial origins of the two conditions. We have hypothesized the molecular mechanism of menaquinone-7 insufficiency in chronic inflammation fueled by pro-inflammatory cytokines. Tumor necrosis factor- α (TNF- α) exemplifies the pro-inflammatory cytokine, implicated in the process of vascular calcification and osteoporosis. The goal of the following study was to test a hypothetical role of vitamin K2 in modulation of immune cells in vitro. High purity natural vitamin K2, 98% menaquinone-7 (MenaQ7[®]Crystals) was evaluated for its potential to inhibit production of pro-inflammatory markers. The efficacy of K2 was tested in tissue culture of human macrophages (HMDM- Human Monocyte-Derived Macrophage) and mouse macrophages (RAW 264.7 cell line) in four concentrations: 0, 1 μ M, 1 μ M, 10 μ M and 100 μ M. The collected results showed that vitamin K2 in concentration below the 10 μ M and short time pretreatment (6 hours), did not inhibit significantly the production of TNF- α and NO after the TLR activation. However, the 30 hours pretreatment of PBMC and RAW cell line with at least 10 μ M of vitamin K2 effectively inhibited the production of pro-inflammatory mediators by human and mouse macrophages, and the effect was dose dependent. The 10 μ M of vitamin K2 after 30 hours of PBMC pretreatment, resulted in 20%, statistically significant, inhibition of the TNF- α production after LPS activation ($p < 0.05$) and 22% inhibition of NO production by RAW cell line ($p < 0.05$). The 100 μ M of vitamin K2 inhibited the TNF- α production by HMDM after LPS activation by 63% ($p < 0.01$), when inhibition of NO production by RAW cell line was 20% ($p < 0.05$). In summary, our results show that Vitamin K2 inhibits the production of pro-inflammatory mediators by human and mouse macrophages in vitro. Thus supplementation with vitamin K2 should be beneficial for aging people.

Beneficial effects of dietary vitamin B6 as anti-disease and ergogenic factor via the modulation of amino acid metabolism in rats

Suidasari S*, and Kato N.

Graduate School of Biosphere Science, Hiroshima University,
E-mail: sofyasuidasari@hiroshima-u.ac.jp

We hypothesized that the beneficial effects of dietary vitamin B6 as an anti-disease and ergogenic factor are mediated via the modulation of amino acid metabolism. Accordingly, we examined the concentrations of free amino acids and related metabolites in the blood and tissues of rats fed a diet containing 1, 7 or 35 mg pyridoxine (PN) HCl/kg for 6 wk. Food intake and body weight gain were unaffected. The serum concentration of pyridoxal 5'-phosphate (PLP) was remarkably higher in the 7 and 35 mg PN HCl/kg groups than the 1 mg PN HCl/kg group ($P<0.05$). Plasma and colon concentrations of threonine, known to enhance intestinal barrier function, were significantly elevated with vitamin B6 supplementation compared to the 1 mg PN HCl/kg diet ($P<0.05$). The concentrations of carnosine and anserine (antioxidant, pH-buffering, anti-glycation and ergogenic factors) in gastrocnemius muscle were markedly higher in the 7 and 35 mg PN HCl/kg groups than the 1 mg PN HCl/kg group ($P<0.01$). The concentrations of these dipeptides in soleus muscle were also significantly higher in the vitamin B6 supplementation groups, albeit to lesser extents ($P<0.05$). Further, the concentrations of carnosine in heart and serum were significantly elevated by vitamin B6 supplementation compared to the 1 mg PN HCl/kg diet ($P<0.05$). Concentrations of citrulline (antioxidant) in the skeletal muscles and heart were significantly affected by dietary vitamin B6 supplementation ($P<0.05$), with the highest concentration in the 35 mg PN HCl/kg group. These findings indicate dietary vitamin B6 is a determinant of these amino acids and related metabolites that are considered as an anti-disease and ergogenic factor.

Is obesity associated with iron status in low-income black men and women? A case study from peri-urban qwa-qwa, South Africa

Oldewage-Theron W. H.^{1,2*}, Egal A. A.^{1,2}, and Grobler C. G.²

¹Centre of Sustainable Livelihoods, South Africa

²Vaal University of Technology, South Africa, E-mail: wilna@vut.ac.za

Objective: To investigate the relationship between overweight and/or obesity and poor iron status in low-income black women from peri-urban Qwa-Qwa in South Africa (SA). **Design:** Cross-sectional, observational baseline survey. **Subjects:** A sample size calculation determined a representative sample of 104 randomly selected women ($n=84$) and men ($n=20$), aged $\geq 20 < 60$ years old. Measurements included weight, height, biochemical and haematological parameters. Three types of double burden of malnutrition in an individual were considered for the analyses. These were the co-existence of (1) overweight with iron deficiency (ID), (2) obesity with ID and (3) high risk of abdominal obesity (waist > 88 cm in women and 102 cm in men respectively) with ID. **Results:** The anthropometric results showed 27.4% of the women had normal weight whereas 29.8% and 41.7% were overweight and obese respectively. A small percentage of the men were obese (10.0%) and overweight (15.0%) respectively with 45.0% being of normal weight. Regarding the double burden of malnutrition, 1.9% of the respondents

presented with both ID and overweight. In the obese category, 6.7% of the respondents were iron deficient. Regarding the high risk of abdominal obesity with ID, 8.7% presented with a double burden of malnutrition. The mean genders and statistically significant higher levels ($p=0.010$) were observed in the overweight and obese categories of the women.

Conclusions: A co-existence of overweight and obesity as well as poor iron status were observed in these women and men. The positive relationship between HS-CRP and BMI indicated chronic inflammation in the higher BMI groups, which is consistent with recent research 1-3. A relationship between obesity-related chronic, low-grade inflammation and poor iron status has been found in adults, but the significance of this study is that this relationship was also confirmed for low-income blacks in SA.

High fructose intake with maternal diet associated with obesity - preliminary look to fetal programming

Yuruk A.*, and Nergiz-Unal R.

Department of Nutrition and Dietetics, Faculty of Health Sciences, Hacettepe University, Turkey. E-mail: armaganyuruk@hacettepe.edu.tr

Introduction: Dietary changes during preconception, throughout pregnancy and lactation affect the development of tissues and organs of the fetus resulting in irreversible changes i.e. fetal programming. Therefore, the aim of this study is to investigate the effects of maternal high fructose intake on body weight, water and feed intake, plasma insulin levels of the mother rats and pups. **Methods:** This study was carried out on Sprague Dawley strain female rats ($n=10$). After a two weeks wash-out period the rats were randomly divided into two groups. Water including fructose or maltodextrin (0.2 g/mL (20% w/v)) was administered for 12 weeks. After mating, the dietary manipulation continued during pregnancy and lactation periods. At the end of lactation period, blood was isolated from the rats under anesthesia and animals were immediately sacrificed. Body weights, water and feed intakes were measured during the study. The plasma insulin levels were analyzed by colorimetric method with a microplate reader. **Results:** It was determined that mean body weight of mothers in the fructose group was 212.9 ± 20.25 g while in the maltodextrin group was 209.0 ± 20.51 g ($p<0.05$). Mean body weight of pups in the fructose group was 27.1 ± 5.38 g whereas 24.0 ± 4.96 g in the maltodextrin group ($p<0.05$). While, there was no significant difference of food intakes of mothers, daily average energy intake from fructose added water was 68.6 ± 3.73 kcal and from the maltodextrin added water was 82.5 ± 4.94 kcal ($p<0.05$). Mean plasma insulin levels of mothers in fructose group was 4.8 ± 0.38 ng/dL while in the maltodextrin group was 3.8 ± 0.37 ng/dL ($p<0.05$). In parallel, the mean plasma insulin level of pups in the fructose group was 4.5 ± 0.29 ng/dL while in the maltodextrin group was 3.8 ± 0.14 ng/dL ($p<0.05$). Briefly, body weights and plasma insulin levels were higher in the mother rats and pups exposed to fructose, although rats in the fructose group had lower energy intake compared to maltodextrin group.

Conclusion: Large amounts of maternal fructose intake during

preconception, throughout pregnancy and lactation periods, elevate body weight and blood insulin levels both in mothers and pups. Consequently, high amount of fructose, taken with processed foods, in maternal diet may increase the risk for chronic diseases i.e. obesity and type II diabetes mellitus.

P01. Higher morbidity and mortality in cirrhotic patients with diastolic dysfunction undergoing surgeries

Brotánek J.*

Thomayer University Hospital, Prague, Czech Republic, E-mail: jaroslav.brotanek@ftn.cz

Our research primarily focuses on patients with liver cirrhosis caused by alcohol and cirrhotic cardiomyopathy. It is a well known fact that liver cirrhosis is accompanied by many abnormalities of the cardiovascular system. However, they used to be assigned to other effects on the circulation (i.e. the impact of alcohol) than cirrhosis per se. Nowadays cirrhotic cardiomyopathy is defined as chronic cardiac dysfunction in patients with cirrhosis, characterized by impaired contractile responsiveness to stress and/or altered diastolic relaxation with electrophysiological abnormalities in the absence of other known cardiac disease. Major pathophysiological mechanisms underlying cirrhotic cardiomyopathy include the decrease in β -adrenergic receptor density, the reduction in Gs proteins and the attenuation of adenylate cyclase activity, which ultimately manifests itself as reduced cardiomyocyte contractility. Changes in the lipid composition of the cardiomyocyte plasma membrane likewise have negative effects on the function of β -adrenergic receptors. An experiment on rats in which cirrhosis was induced by bile duct ligation also revealed decreased muscarinic-receptor responsiveness. Likewise undeniable is the impact of cannabinoid CB1 receptors that lead to a negative inotropic effect via the activation of the inhibitory G protein. Both the muscarinic and cannabinoid receptors ultimately inhibit adenylate cyclase activity.

The production of nitric oxide (NO) amounts to another negative effect on the myocardium. Under normal circumstances NO has an important impact on the vasodilation of the systemic and coronary circulation and has cardioprotective effects via the inhibition of apoptosis. Cirrhotic patients have overproduction of NO. This results in the occurrence of the hyperkinetic circulation. NO is synthesized by nitric oxide synthase (NOS) which has three isoforms: endothelial, neuronal and inducible. The first two isoforms synthesize NO with a cardioprotective effect. The third one, inducible synthase (iNOS), whose function is induced by cytokines and a tumor necrosis factor (TNF), leads to the production of NO with a cardiotoxic effect (reduced contractility and induction of apoptosis). Cirrhosis also results in higher production of carbon monoxide (CO) which, too, causes decreased myocardial contractility through cyclic guanosine monophosphate (cGMP). Apoptosis also fundamentally contributes to the impaired myocardium in cirrhotic patients. There is no doubt that increased sympathetic activity together with increased inflammatory cytokines, such as Interleukin 6,8, TNF- α and transforming growth factor

(TGF- β), may, via mitogen-activated protein kinases (MAPK/P38- α), lead to the decreased growth and differentiation of cardiomyocytes and consequently to apoptosis. The clinical presentation of cirrhotic cardiomyopathy can be divided into three parts – systolic dysfunction, diastolic dysfunction and electrophysiological abnormalities. As is apparent from the above definition of cirrhotic cardiomyopathy, systolic dysfunction can occur too though it is not manifested at rest, but under stress. A number of studies have proved this in people and animals with cirrhosis. Intracardiac pressures measured at rest meet the norm in most patients. But pharmacologically induced stress or physical exertion leads to the enlargement of left-ventricular end-diastolic volume and decreased left ventricular ejection fraction (the cardiac output of healthy people increases three times during exercise or with submaximal exertion, while in cirrhotic patients, it does not even redouble). Angiotensin infusion increases the wedge pressure, which corresponds to the elevated filling pressure of the left ventricle, while the cardiac output does not change. A decrease in systolic function can contribute to the development of ascites and renal insufficiency as part of hepatorenal syndrome. Systolic dysfunction used to be assessed by ejection fraction echocardiography. These days it is possible to obtain more accurate assessment thanks to Tissue Doppler Imaging and magnetic resonance. Diastolic dysfunction in cirrhosis was first described in 1997. It is caused by a diminished left ventricular compliance and relaxation. The chief underlying pathology here is increased myocardial stiffness, which is due to a combination of factors – fibrosis, subendothelial edema and cardiac hypertrophy. QT-interval prolongation is among the chief electrophysiological abnormalities. Other abnormalities include an impairment of heart rate variability. Our previous research concentrated on a potential link between left ventricular diastolic dysfunction and alcoholic liver cirrhosis. It was to determine how often asymptomatic diastolic dysfunction occurs in cirrhotic patients. We included patients with Child-Pugh A and B cirrhosis. We have proved that there is a statistically significant higher incidence of asymptomatic left ventricular diastolic dysfunction in these patients. We have also proved that the incidence of diastolic dysfunction increases with the severity of the liver lesion. Another part of our research hypothesized whether people with alcoholic liver cirrhosis and asymptomatic left ventricular dysfunction in need of a planned surgical treatment had a worse post-operative prognosis and a higher morbidity and mortality. We observed a group of 11 patients who had to undergo intra-abdominal and thoracic surgeries. We compared them with a group of cirrhotic patients without diastolic dysfunction undergoing similar operations. The observation group consisted of 6 men and 5 women, the control group of 6 men and 6 women. The average age in the observation group was 66, and 64 in the control group. All persons in each of these groups had Child-Pugh A cirrhosis. Both groups underwent the standard echocardiographic examination as part of the pre-operative preparation. Diastolic dysfunction was detected by the Pulse Doppler echocardiography of the flow in the mitral valve and by the Tissue Doppler Imaging motion of the mitral annulus. The patients were observed throughout their hospitalization and had another check three, six and twelve months

after the operation. In the observation group, we detected post-surgical complications in four patients. Two male patients showed signs of left-sided cardiac decompensation which were eliminated by the standard treatment of heart failure. One female patient had, in the post-surgical stage, protracted hypotension which required catecholamine support. The last of the four patients had paroxysmal atrial fibrillation with signs of cardiac insufficiency. Arrhythmia was converted to sinus rhythm by the application of amiodarone; after the cardioversion the signs of heart failure disappeared. A recurrence of arrhythmia was however detected during the ensuing long-term observation of this patient problem with arrhythmia. Complications arose in two patients from the control group. One woman developed a pulmonary embolism which was, on examination, assessed as acute “non-massive”. The other patient, a man, showed light signs of both-sided heart failure, which was quickly treated by drug administration. During the subsequent observation one of the controls died. But this was caused by esophageal variceal bleeding.

Patients with cirrhosis undergoing surgery run a higher risk of morbidity and mortality. Studies differ in explaining the incidence and the cause of mortality in these patients. In-hospital mortality ranging from 8.3% to 25%, in contrast to the rest of the population (1.1%), has been reported. Operative mortality correlates with the severity of the liver lesion. The mortality of Child-Pugh grade A is about 14%. The score systems Child-Pugh and MELD (Model for End Stage Liver Disease) are used to classify the severity of liver damage prior to the operation. It is obvious that the post-operative progress and results are worse in patients who have to undergo an urgent operation (50% vs. 18%). Another non-negligible risk factor is of course the type of treatment. The highest morbidity and mortality are reported in patients undergoing cardiac surgeries (perioperative mortality is higher than 50% here (vs. 18%)) and intra-abdominal operations (where mortality oscillates from 17% to 54% depending on the type of treatment).

Our observation has revealed that there is a higher incidence of cardiovascular complications in patients with alcoholic cirrhosis and left ventricular diastolic dysfunction than in cirrhotic patients without signs of cirrhotic cardiomyopathy. The surgical treatment and post-operative period are a considerable strain on the cardiovascular system. During operation cardiac output diminishes due to the reduction of preload and decreased myocardial contractility. The operation can lead to hypovolemia as a result of bleeding, “third space” fluid loss and the development of ascites. Other indisputable factors with cardiodepressive effects are acidosis and ion imbalance (hypokalemia and hypocalcemia). Aggressive infusion therapy also leads to the deterioration of cardiac function. Our results yielded positive statistical evidence in the observed patients with diastolic dysfunction. It is true though that the observation cohort was not large, and statistically, this may have affected our study. Also, the examined cases had the lowest grade of liver damage (Child-Pugh A). On the other hand, patients with more serious liver damage (Child-Pugh B and C) have other, additional, complications (e.g. significant coagulation disorders, renal insufficiency) that increase operative risk, morbidity and mortality.

As is clear from our two studies and the data obtained from scientific literature, alcoholic liver cirrhosis is often accompanied by left ventricular diastolic dysfunction. This disorder causes higher operative morbidity and mortality. Therefore it is necessary to pay close attention to these patients prior to the planned surgical treatment, subject them to a detailed cardiac examination and conduct the operation as well as the treatment in the post-operative period accordingly.

P02. Effect of body composition intake the nano-lactic acid in rats

Sato Takeshi¹, Yasue Nakagawa¹, Takayuki Watanabe², Shoji Igawa³, and Oh Tae-Woong⁴

¹Jissen Women's University, Japan

²Hachinohe Gakuin University, Japan

³Nippon Sport Science University, Japan

⁴Matsumoto University, Japan, E-mail: sato-takeshi@jissen.ac.jp

Background: Prebiotic effect and probiotics lactobacillus were to one of the most famous effect-efficacy of the health conditions. It is important to reduce adult diseases risk for public health based politics. Especially in East Asian people, there were increasing the health problems related to being obsess by attributed to the Westernization of daily meals. The purpose of this study was to investigate the effect of probiotic pear feeding as lactobacillus in rat fed a high-fat diet, to prevention of increased body fat percentage. Methods: Male Wistar rats were individually housed in cages at temperature of 23 ± 1 degree centigrade, and 40-60% humidity. After basically 2 weeks breeding on pear feeding, 20 rats were divided 4 groups each 5 rats and were fed same calorific pear feeding diet and water, control (group: C) as high-carb meal, Japanese style, C with high levels lactobacillus (group: C plus), high-fat same calorific feed and water (group: F), F with high levels lactobacillus (group: F plus), all feed certain time once a day. It was delivered the lactobacillus 500 billion per 1cc by oral radio sonde tube. This animal experiment was carried out in accordance with the Japanese law, which allows experiments on laboratory animals in Nippon Sport Science University accordance to the principle of laboratory animal care. After experimental feed, it was measured lipid metabolism based in lean body mass. Results: It was showed that induced high level lactobacillus were to block the adipose tissue fat mass. There were significantly increasing the body fat percentage in F compare to C in spite of pear feeding same calorie. However there were no significant difference in body mass in between F and C. Conclusions: Consequently, it was recommended to preactive intake of the nano-lactobacillus because of inhibit to accumulated fat mass with high fat food. However it was inactive lactic acid because of heat-treated. Therefore, it is expected the highly-concentrated lactic bacterium as same as fiber rich diet for human.

P03 Grasshoppers *Sphenarium purpurascens* Ch source of proteins and essential amino acids

Melo V.¹, Quirino T.¹, Diaz R.¹, Garcia, L.¹, and Schettino B.²

¹Universidad Autónoma Metropolitana Unidad Xochimilco, Mexico

²Instituto Nacional de Nutrición "Salvador Zubirán", Mexico, E-mail: vmelo@correo.xoc.uam.mx

Protein energy malnutrition is quite common in the world as a whole; deficiency has adverse effects in all organs, since cellular functions are the fundamental components of them. Edible insects traditional cultural food, commonly consumed in rural communities, now intake is also being incorporated into other countries of the world. Grasshoppers, valued as a cheap source of fresh protein and essential amino acids nowadays become part of the regular diet of different social groups. Proteins comprise by the building blocks of amino acids are one of the five classes of complex biomolecules found in cells and tissues and play an important role in human nutrition and metabolism. This research was conducted to determine proximal composition of grasshoppers *Sphenarium purpurascens* Ch, focus on proteins and amino acids. Sampling was carried during winter season 2012/2013 at Oaxaca State. Dry adult insects were ground and pass through a 30 mesh screen to get a uniform powder and proceed to protein analysis by kjeldahl AOAC method, and essential amino acids in a Beckman 6300 equipment. Data obtained was: total proteins 71.56%; essential amino acids (mg/16g): isoleucine 4.5; leucine 8.1; lysine 5.4; methionine + cysteine 3.1; phenylalanine + tyrosine 15.1; threonine 3.5; tryptophan 0.6; and valine 5.5. Essential amino acids requested according FAO 1973 are in similar proportion, of the grasshoppers studied, however tryptophan is the limitant one. Grasshoppers *Sphenarium purpurascens* Ch, represent a good source of proteins and essential amino acids, that can grow almost everywhere, available mostly all year round and affordable to all social groups, therefore can be an option to improve human health.

P04. Consumption of Escamoles *Liometopum apiculatum* M source of vitamins A and E enhance immunity system of population

Melo V.¹, Calvo C.², Quirino T.¹, and Díaz R.¹

¹Universidad Autónoma Metropolitana Unidad Xochimilco, Mexico

²Instituto Nacional de Nutrición "Salvador Zubirán", Mexico, E-mail: vmelo@correo.xoc.uam.mx

The immune system is the body defense against all kinds of unwanted illness causing by substances that can include not only microbes, otherwise known as pathogens, but also toxic molecules and the sick or damaged cells. Through the immune system the human body can build up resistance to respond more efficient to repeated attacks, therefore maintaining a good nutrition status and adequate fat-soluble vitamins A and E stores in the human body is essential for building up an effective immune response. Particular foods are associated with good health, therefore nutrition is considered to be an important fact in the human body resistance to infection. Escamoles, ant eggs *Liometopum apiculatum* M are consumed by population in

Mexico and due to its fat-soluble vitamins A and E composition, can enhance their immune system. The aim of this study is to assess fat-soluble vitamins A and E in escamoles, and inform population the benefits in the immune system the intake of this edible insect can provide in human health. Fat-soluble vitamins A and E of Escamoles collected at Hidalgo State on spring season 2013 were analyzed by liquid chromatography. Results obtained were: Vitamin A, Retinol: UI/100g, 1008 and Vitamin E, alpha tocopherol: mg/100g, 3.29. The functions of fat-soluble vitamins are: for Vitamin A, immune processes related for normal functioning of phagocytes and activation of T- and B-lymphocytes. For Vitamin E, protection cell walls from the damaging reactive molecules associated with the response of the innate immune system. Escamoles consumption, as a source of vitamins A and E will enhance the immunity system of population, improving their health.

P05. Analgesic and antipyretic activity of Tri-sa-maw recipe

Nanna U.¹, Sireeratawong S.¹, and Jaijoy K.²

¹Division of Pharmacology, Department of Preclinical Science, Faculty of Medicine, Thammasat University, Rungsit Campus, Pathumthani 12120, Thailand

²McCormick Faculty of Nursing, Payap University, Chiang Mai 50000, Thailand, E-mail: assist.prof.ae@gmail.com

Tri-sa-maw recipe is composed of equal proportions of the three fruits including *Terminalia chebula* Retz., *Terminalia* sp. and *Terminalia bellirica* Roxb. In Southeast Asia, these fruits are used as both food and medicine. In Thai traditional medicine, Tri-sa-maw recipe is well known for treating fever, expectorant, periodic maintenance, and tight stomach relief. In this study, anti-inflammatory, analgesic and antipyretic activities of Tri-sa-maw recipe extract were evaluated using animal models in comparison with reference drugs. The anti-inflammatory study was conducted by two experimental models; ethyl phenylpropionate-induced ear edema and carrageenan-induced paw edema. For analgesic activity, the pain was induced by acetic acid or heat. In addition, yeast-induced hyperthermia was performed for the study of antipyretic activity. The results showed that Tri-sa-maw recipe extract did not inhibit acute inflammation in the carrageenan-induced paw edema. However, the extract was able to inhibit the acetic acid-induced writhing response, but not the heat-induced pain. This result suggests the peripheral effect of its analgesic activity which inhibits the biosynthesis and/or release of some pain mediators. Finally, oral administration of the extract at the dose of 1200 mg/kg body weight effectively reduced the hyperthermia, which might be due to the inhibition of prostaglandins. In conclusion, the present study has clearly demonstrated both analgesic and anti-pyretic activities of Tri-sa-maw recipe.

P06. Effects of *Cymbopogon citrates* Stapf water extract on rat antioxidant defense system

Somporn N.¹, Saenthaweeuk S.¹, Naowaboot J.¹, and Thaeomor A.²

¹Department of Preclinical Science, Faculty of Medicine, Thammasat University, Rangsit Campus, Pathum Thani, 12120, Thailand

²School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand, E-mail: nuntiya_tom@hotmail.com

Cymbopogon Citratus, Stapf (CCS) is a commonly known as lemon grass. Previous studies showed that it has a strong antioxidant property. The present study was conducted to investigate the effects of CCS water extract on rat antioxidant defense system. In the study, the extract was screened for its phytochemical contents and antioxidant activity in vitro. The extract contained flavonoids (496.17 mg gallic acid/g CCS extract) and phenolic compounds (4,020.18 mg catechin/g CCS extract). The scavenging activity (DPPH assay) of the extract was demonstrated by EC₅₀ of 917.76 + 86.89 mg/ml whereas the EC₅₀ of the potent antioxidant, vitamin C, was 31.22 + 1.84mg/ml. Moreover, the extract was also studied in rats to evaluate its effects in vivo. Male Sprague-Dawley rats were orally administered with CCS at 250, 500 and 1,000 mg/kg/day for one month. In the animals, the protein expression of antioxidant enzymes, γ -glutamylcysteine ligase and heme oxygenase, was significantly increased in the high dose-treated animals (1,000 mg/kg/day). This was consistent with elevation of serum total antioxidant capacity. Taken together, the present study provides evidence that CCS exhibits direct antioxidant properties and can induce cytoprotective enzymes in vivo.

P07. Effect of broccoli intake on antioxidants in the liver and kidneys tissues of hyperglycemic rats

El-Hendy H. A. R.¹, and Owyed Al- Gemeai H. A. R.²

¹Department of Home Economics, Alexandria University, Egypt

²Department of Home Economics, Altaif University, Saudi Arabia, E-mail: elhendy99@yahoo.com

The objective of this study was to investigate the effect of broccoli intake on hyperglycemic rats. The Broccoli was bought from the local market of Saudi Arabia and the chemical analyses were conducted. After steam boiling of broccoli for 4-5 minutes, it was dried at 50 centigrade and added to the diet. The research sample included 50 male adult Albino rats, its weight ranged from 200-210 grams. After the rats adapted to their new environmental conditions, they were divided into seven equal groups composed of 10 rats in each group. The first group was fed the standard diet (negative control group), whereas the 40 rats were injected with Streptozotocin 60 mg/kg and the rats became diabetic. The treated animals were divided into groups of: the positive control group and the second group was fed with the standard meal; while the third, fourth, and fifth groups were fed with the standard meal mixed with 10, 20, 30% of dried broccoli respectively, for two months. At the end of the experiment, the results showed improved all biochemical parameters in the serum and antioxidants in each of the liver and kidneys of rats fed on broccoli.

P08. Evaluation nurses knowledge regarding drug food

interactions in a teaching hospital

Abbasinazari M.*

Department of Clinical Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran, E-mail: farshadpharm@yahoo.com

Introduction: Drug-food interactions can increase or decrease drug effects, resulting in therapeutic failure or toxicity. Drug food interactions have given rise to many issues, one of which is whether nurses are competent when administering oral medications. This study was planned and performed in order to determine nurse's knowledge regarding drug food interactions in a teaching hospital in Tehran, Iran. **Methods:** The subjects comprised nurses working in a teaching hospital located in the north city of Tehran (Taleghani hospital). Nurses' knowledge of drug food interactions was evaluated with a questionnaire that included 20 true or false questions. The questionnaire was developed from literature and expert input and validated by subject experts (content validity). Questionnaires were distributed between 300 nurses working in the Taleghani hospital. The data collected through questionnaire were evaluated by means of the package software SPSS as well as decimal number and Chi-square tests. **Results:** A total of 284 nurses participated in the study and achieved an average overall correct answer rate of 50.9%. Most of the respondents, 63.5%, had a score of less than 60. Their knowledge of drug food interactions came mainly from consultation with colleagues and in-hospital continuing education.

Discussion: The findings suggest that ongoing education about drug food interactions should be provided to nurses, but also that standardized evaluation of adequate knowledge, skills and competencies' regarding safe practices is warranted. More fundamentally, however, nurses need more education about drug food interactions in nursing school and through in-hospital continuing education. Future research should focus on effective mechanisms to ensure that nurses educate regarding drug food interactions.

P09. Assessment of nutritional status in Iranian cirrhotic patients due to hepatitis B or hepatitis C

Sarafzadeh F.¹, and Abbasinazari M.²

¹Department of Internal Medicine, Infectious Control Ward, Afzalipour Educational and Medical Centre, Kerman, Iran

²Department of Clinical Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran, E-mail: f_sarafzadeh@yahoo.com

Introduction: A cross sectional investigation was done to evaluate the clinical and paraclinical parameters, related to nutritional status in cirrhotic patients due to hepatitis B and C, and determine the prevalence of protein-calorie malnutrition. **Methods:** Nutritional assessment including anthropometric and biochemical measurements in 50 hospitalized patients with diagnosis of cirrhosis due to hepatitis (36 hepatitis B and 14 hepatitis C) were evaluated during 7 months at GI and Liver diseases ward at a teaching hospital in Tehran, Iran. Anthropometric measures consist of weight, height, Triceps Skinfold

Thickness (TSF), Mid Arm Circumference (MAC), Mid Arm Muscle Circumference (MAMC), Ideal Body Weight (IBW) and Body Mass Index (BMI). Biochemical parameters consist of albumin and prealbumin were determined in the samples. Results: In 18% of patients Actual Body Weight/Ideal Body Weight ratio was less than 70% (malnourished). 8% of patients had BMI below 18 Kg/m². Non-caloric stores, evaluated by TSF were extremely low in 36% of the cases studied (TSF<25%), while muscular stores evaluated by MAMC were depleted in all 50 patients (MAMC <65%). There was a correlation between Mid Arm Circumference and etiology of cirrhosis (p=0.074). There were some correlations between anthropometric parameters. TSF values were correlated with BMI (p=0.001) and IBW (p=0.038). Mean values of albumin and prealbumin plasma concentrations showed significant decrease compared with normal levels. There was no correlation between plasma concentration of these proteins and etiologies of cirrhosis (hepatitis C, B). Discussion: This study showed that PCM is a common complication of cirrhosis. Protein malnutrition was found more frequently than calorie malnutrition. The results showed that the anthropometric and biochemical markers which were used in this study can be used as sensitive and valuable markers of presence and severity of PCM in cirrhosis.

P10. Evaluation of enteral nutrition and phenytoin interaction in head trauma patients

Sohrevardi S. M.¹, and Ahmadi nejad M.²

¹Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

²Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran, E-mail: smsohrevardi@yahoo.com

Background and Objective: Seizure is common after head trauma and neurosurgery. Phenytoin is the most common anti-convulsant drug used in epileptic patients and for prevention of seizure in patients with head trauma and stroke. This drug has unique pharmacokinetic and pharmacodynamic characteristics. Phenytoin administration along with enteral nutrition in ICU patients may be accompanied by decreased phenytoin absorption and inadequate therapeutic concentration. The present study was performed to assess the effect of enteral nutrition on the pharmacokinetic therapeutic parameters of phenytoin given to our patients. **Methods:** In a clinical trial, the study group was divided into two groups of 15 patients each. After obtaining steady-state phenytoin serum concentration, two blood samples were obtained from each patient on 2 consecutive days and then analyzed. The mean was assessed on the basis of serum albumin level of the patient. Clearance and maximum metabolic capacity were also calculated. **Results and discussion:** Serum phenytoin level was below the therapeutic range (10- 20 mg/l) in 70% of patients in group 1 and was higher than the therapeutic range in 70% of patients in group 2 who received oral phenytoin (by dissolving in water) 2h after enteral nutrition. Mean phenytoin concentration was 6.3 ± 4mg/l and 24.7 ± 9.4mg/l in group 1 and group 2, respectively. **Conclusion:** We found oral phenytoin administration with enteral nutrition (gavage solutions) to

result in a significant decrease in absorption and blood concentration of phenytoin. We recommend administration of phenytoin with water only. In addition, monitoring of phenytoin serum concentration is necessary for assessment of therapeutic concentration and prevention of side effects.

P11. Continuous glucose monitoring (CGMS) versus oral glucose tolerance test (OGTT) and glycated hemoglobin (HbA1C) in the evaluation of glycemic abnormalities in an obese adolescent before versus after partial gastrectomy

Soliman A. T.^{*}, Sabt A., Alyafei F., Aldarsi N.

Hamad General Hospital, Doha, Qatar, E-mail: ATSOLIMAN@yahoo.com

We compared CGMS (Medtronic) to OGTT and HbA1C in the follow-up of glycemic abnormality in an adolescent girl with morbid obesity and glycemic abnormalities before and after 2 months of partial gastrectomy. **Case:** This 16 year old adolescent girl presented with obesity (weight 98 kg, height 158 cm, BMI=39.2 kg/m²), acanthosis nigricans and nocturnal polyuria and polydipsia. Trials to reduce weight through dieting, exercise and use of Metformin was not successful; (Patient lost 3 kg in 4 months). Her fasting BG=102 mg/dl but 2h BG after oral glucose (75 g)=225 mg/dl. She underwent partial gastrectomy surgery. 2 months after surgery her weight=70 kg and BMI=28 kg/m². **Conclusion:** Before surgery this obese patient with morbid obesity had normal FBG and her diabetic state was diagnosed using OGTT and confirmed with CGMS criteria. After surgery CGMS evidenced details of glycemic correction during both basal and postprandial states during real life and assured correction of her glycemic abnormalities.

P12. Resveratrol derivative, 3,5-dimethoxy (4-methoxyphenyl) benzamide suppress adipogenesis in 3T3-L1 cells

Choi Sang Yoon¹, Sanghee Kim², Jin Taek Hwang¹, and Inwook Choi¹

¹Korea Food Research Institute, Seongnam 463-746, Republic of Korea

²College of Pharmacy, Seoul National University, Seoul 152-742, Republic of Korea, E-mail: sychoi@kfri.re.kr

Resveratrol is a naturally occurring ingredient found in grape and peanut that shows potential as an anti-obesity agent. In this study, the adipogenesis-suppressing effect of 3,5-dimethoxy (4-methoxyphenyl) benzamide (DMPB), a derivative of resveratrol, was measured in 3T3-L1 cells. The results show that DMPB effectively suppressed the hormone-induced differentiation of 3T3-L1 cells, compared to resveratrol at the same concentration, and reduced the protein expression of fatty acid synthase and acetyl-CoA carboxylase. In addition, DMPB was observed to decrease the PPAR-γ transcription activity, which was increased by rosiglitazone, in a concentration-dependent manner. From the above

results, it is considered that DMPB shows strong potential as an anti-obesity substance.

P13. Isolation and identification of coagulase-negative cocci (CNC) from Turkish dry fermented sausage (sucuk) and detection of their antibiotic susceptibility

Tuncer Y.*, and Geniş B.

Faculty of Engineering, Department of Food Engineering, Süleyman Demirel University, Isparta, Turkey, E-mail: yasintuncer@sdu.edu.tr

Sucuk is a popular dry fermented sausage in Turkey and can also be found in countries located in Balkans, Middle East and Caucasus. The aim of this study was to isolate coagulase-negative cocci (CNC) in Turkish dry fermented sausage (sucuk) produced by different manufacturers without using starter culture and to analyse isolates for their biodiversity and antibiotic susceptibility. CNC strains were isolated by Mannitol Salt agar (MSA). We isolated 61 CNC strains from 20 sucuk samples. Isolates were identified to the species level by 16S rDNA homology using polymerase chain reaction (PCR). *Staphylococcus saprophyticus* was found the dominant species (36.1%) followed by *S. epidermidis* (26.2%), *Macrococcus caseolyticus* (19.7%), *S. xyloso* (4.9%), *S. warnei* (3.3%), *S. hominis* (3.3%), *S. cohnii* (1.6%), *S. pasteurii* (1.6%), *S. sciuri* (1.6%) and *S. vitulinus* (1.6%). The antibiotic susceptibility patterns of CNC strains were detected by the disc diffusion method onto Muller-Hinton agar using 14 commercially distributed discs according to the recommendation of Clinical and Laboratory Institute (CLSI, 2012). All strains were found complete (100%) sensitive to linezolid (30 µg), chloramphenicol (30 µg), quinupristin-dalfopristin (15 µg) and trimethoprim-sulfamethoxazole (25 µg). Only 5 strains *S. saprophyticus* BYS4, *M. caseolyticus* BYS16, BYS22 and BYS67 and *S. xyloso* BYS35 were found sensitive to all antibiotics. The CNC strains were found mostly resistant to penicillin (70.5%) and ampicillin (70.5%) followed by tetracycline (59.0%), erythromycin (50.8%), cefoxitin (37.7%), kanamycin (21.3%), cephalotin (14.8%), gentamicin (13.1%), clindamycin (8.2%) and ofloxacin (3.3%). The most of the CNC isolates were found resistant to one or more antibiotics. In total 82.0% of the strains exhibited multiple antibiotic resistance patterns. The results of this study indicated that CNC strains isolated from Turkish dry fermented sausages can be a potential reservoir of antibiotic resistance gene exchanges between staphylococci and other species of bacteria.

P14 Antibiotic susceptibility of lactic acid bacteria isolated from sucuk, a Turkish dry fermented sausage

Özden Tuncer B.*, and Yüceer Ö.

Faculty of Engineering, Department of Food Engineering, Süleyman Demirel University, Isparta, Turkey, E-mail: banutuncer@sdu.edu.tr

The aim of this study was to isolate of lactic acid bacteria from sucuk and to determine their antibiotic susceptibility. 20 sucuk

samples produced without starter culture were obtained from different manufacturers in Afyonkarahisar province located in Aegean region of Turkey. A total of 65 presumptive lactic acid bacteria were isolated by M17 and MRS (de Man, Rogosa and Sharpe) agar medium. Species were molecularly identified as *Pediococcus acidilactici* (47.70%), *Enterococcus faecium* (36.93%), *Lactobacillus sakei* subsp. *carnosus* (4.61%), *Lactobacillus sakei* subsp. *sakei* (4.61%), *Pediococcus pentasaceus* (3.07%), *Enterococcus faecalis* (1.54%) and *Weissella viridescens* (1.54%) by sequencing 16S rDNA. Antibiotic susceptibility of strains were determined by disc diffusion method onto Muller-Hinton agar medium against 18 and 16 clinically important antibiotics for Enterococcus and other bacteria, according to recommendation of Clinical and Laboratory Institute (CLSI, 2012) and Charteris et al. (1998), respectively. All Enterococcus strains were found 100% sensitive to doxycycline (30 µg), vancomycin (30 µg), chloramphenicol (30 µg), minocycline (30 µg), streptomycin (300 µg), ampicillin (10 µg), quinupristin-dalfopristin (15 µg), tetracycline (30 µg), gentamycin (120 µg) and teicoplanin (30 µg). On the other hand, Enterococcus strains were found mostly resistant to rifampicin (72%), ciprofloxacin (52%) and nitrofurantoin (44%) followed by norfloxacin (16%), penicillin (12%) and erythromycin (4%). Most of the Enterococcus strains (68%) showed multiple antibiotic resistance patterns. Many strains of the other lactic acid bacteria were classified as resistant to ofloxacin (86.7%), streptomycin (86.7%), norfloxacin (82.2%), vancomycin (80%), ciprofloxacin (80%), kanamycin (77.8%) and cefoxitin (71.1%), while all of them were susceptible to chloramphenicol (30 µg), rifampicin (5 µg), erythromycin (15 µg) and ampicillin (10 µg). Results of this study suggested that lactic acid bacteria in sucuk may play a role to spread of antibiotic resistance between other bacteria including pathogens and thus they may have potential risk to consumer health by indirectly.

P15. Effect of temperature, carbon source and PH on exopolysaccharide (EPS) production and bacterial growth of *Streptococcus thermophilus* ST8.01 in M17 medium

Akpınar D.*, Özden Tuncer B., and Tuncer Y.

Faculty of Engineering, Department of Food Engineering, Süleyman Demirel University, Isparta, Turkey, E-mail: ddmakpinar@hotmail.com

Streptococcus thermophilus is one of the most important lactic acid bacteria in the dairy industry. Many *S. thermophilus* strains synthesize exopolysaccharide (EPS) and these are commonly used for the manufacture of fermented dairy products such as yoghurt and cheese. EPS plays a major role in the rheological behaviour, mouth-feel and texture of fermented products without the use of additives. Production of EPS by *S. thermophilus* is generally growth rate associated, and the influence of environmental factors such as temperature, pH and carbon source, is strain-dependent. In this study, the effect of different temperatures (30, 37, 42 and 45 °C), pHs (4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0) and carbon sources (glucose, sucrose and lactose) on bacterial growth and EPS production of *S. thermophilus* ST8.01, isolated from

home-made yoghurt, in M17 medium were investigated. Higher EPS productions were measured at 24th h in all different incubation temperatures. Maximum EPS production was detected 141.33 ± 4.63 mg/L at 42°C. Number of viable cells at 42°C were counted $8.17 \log$ cfu/mL. In pH experiments, the maximum EPS production was obtained at pH 6.5. The highest EPS level was determined 155.27 mg/L in M17 basal medium supplemented with 2% glucose. On the other hand, maximum cell numbers were detected as $7.51 \log$ cfu/mL in M17 medium supplemented with 2% lactose. This study indicated that EPS production of *S. thermophilus* ST8.01 strain was increased by optimizing environmental factors. Thus, this strain can use for improvement of physical properties of fermented milk products.

P16. Hypolipidimic effect of *Cymbopogon citratus* extract in streptozotocin-induced diabetic rats

Saenthaweek S.¹, Somparn N.¹, Naowaboot J.¹, and Thaeomor A.²

¹Department of Preclinical Science, Faculty of Medicine, Thammasat University, Rungsit Campus, Klong Loung, Pathum Thani, 12120, Thailand

²School of Biology, Institute of Science, Suranaree University of Technology, 111 University Avenue, Suranaree Subdistrict, Muang District, Nakhon Ratchasima 30000, Thailand, E-mail: suphaket18_s@hotmail.com

This study aims to examine the level of insulin and histological changes of pancreatic β -cell in diabetic rats after administration of *Cymbopogon citratus* extract (CC). Diabetes was induced by injection of streptozotocin (STZ, 50 mg/kg) intraperitoneally. Diabetic rats were divided into 5 groups, consisting of 6 rats. Group I reserved as diabetic control was administered distilled water and group II reserved as positive control was administered glibenclamide (10 mg/kg BW/day) throughout the duration of the experiment. Those in group III, IV and V were administered 250, 500 and 1,000 mg/kg BW/day of the extract, respectively for 28 days. For the results, treated with CC did not alter the insulin secretory activity and had no effect on the histopathological changes of pancreatic β -cell in comparison with diabetic control rats. Nevertheless, treatment with 500 and 1000 mg/kg BW/day of the extract resulted in reduction of serum AST, ALT, serum cholesterol, triglycerides and LDL, whereas HDL was found to be increased compared with diabetic control rats. Although, CC did not process antidiabetic potential, however, the extract could improve lipid profile that may lower cardiovascular disease risk and others complications related to hyperlipidemia in diabetic patients.

P17. Effect of *Lagerstroemia speciosa* L. aqueous extract on histological changes of the liver in streptozotocin-induced diabetic rats

Thuppia A.¹, Rabintossaporn P.¹, Saenthaweek S.¹, Sireeratawong S.², and Ingkaninan K.³

¹Division of Anatomy, Department of Preclinical sciences, Faculty

of Medicine, Thammasat University, Pathum Thani, 12120 Thailand

²Department of Preclinical sciences, Faculty of Medicine, Thammasat University, Pathum Thani, 12120 Thailand

³Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Science, Naresuan University, Phitsanulok, 65000 Thailand, E-mail: phungamornnat@hotmail.com

The purpose of this study was to investigate hepatic histological changes in the livers of normal and streptozotocin (STZ)-induced diabetic rats after receiving various doses of aqueous extract of *Lagerstroemia speciosa* L. (LSL extract). Diabetes Mellitus (DM) was induced in rats by intraperitoneal injection of STZ at a dose of 45 mg/kg. Then, the vehicle, glibenclamide (3 mg/kg), and LSL extract (500, 1000 and 2000 mg/kg) were administered orally for 12 days. At the end of the experiment, the rats were sacrificed, and their livers were collected for histological examination. In diabetic control rats, the hepatocytes possessed shrinking nuclei, vacuolated and granular in cytoplasm, and poorly defined cell boundaries. On the other hand, the hepatocytes of diabetic rats receiving all doses of LSL extract showed their clear cell boundary and regular cell size. Moreover, the vacuolated and granular appearance in cytoplasm was reduced when compared to diabetic control rats. The results demonstrated that administration of LSL extract could improve the histological appearances of the livers of diabetic rats.

P18. The glycemic indexes of guava, tangerine and pineapple in the forms of whole fruit, pureed fruit and fruit-juice extract in type 2 diabetes

Meewan S.¹, and Komindr S.²

¹Department of Nutrition and Dietetics, Prasat Neurological Institute, Bangkok, Thailand

²Faculty of Medicine at Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, E-mail: katesunee@yahoo.com

Fruits are an important source of vitamins, minerals, fibers and carbohydrate which are necessary for good health. Several fruits are consumed by the diabetic patients, while the low glycemic index (GI) fruits are being recommended. A considerable number of diabetic in Thailand are elderly with dental problem, which inevitably forces them to consume soft fruits. If pureed fruit drink and whole fruit do not differ in their glycemic responses, people with diabetes, especially the elderly and those with chewing problem will be able to consume a greater variety of fruit. Thus, this study is aimed to determine the GI of certain fruits in 3 different forms which are whole fruit, pureed fruit and fruit-juice extract. Eleven participants with type 2 diabetes were included. Each participant underwent 10 tests consisting of consumption of 3 different physical forms (whole fruit, pureed fruit and fruit juice extract) of guava, tangerine and pineapple and a glucose drink. The test fruit contained 25 grams of available carbohydrate. Each test was undertaken every 2 weeks. On the test day, fasting blood specimen was taken for baseline glucose and additional blood glucose samples were collected at 30, 60, 90, 120, 180 and 240 minutes after

the first bite. The Glycemic indexes of guava in the forms of whole fruit (GW), pureed (GP), and juice extract (GE) were 31, 38 and 46, respectively. Whole fruit of tangerine (TW), pureed tangerine (TP) and tangerine-juice extract (TE) had GI of 43, 54 and 58; in addition, GI of whole fruit of pineapple (PW), pureed pineapple (PP) and pineapple-juice extracted (PE) were 50, 51 and 54. TE was classified as having moderate GI whereas all the others had low GI. Peak incremental plasma glucose of all fruits were seen at 60 minutes after ingestion except for that of PE which was present at 30 minutes. At 30 minutes, GW and GP showed significantly lower incremental plasma glucose ($p < 0.005$) than the glucose drink. Although, most forms of these 3 fruits were demonstrated as having low GI, TE had moderate GI. The Glycemic index tended to increase in ascending order according to the intactness of fiber in the fruit from whole fruit, pureed fruit and fruit-juice extract, respectively. Moreover, PE has produced rapid excursion (within 30 minutes) of plasma glucose similar to the glucose drink. These results suggest that guava could be consumed in all forms without worsening the glycemic response, while it is better to consume tangerine and pineapple in the forms of whole fruit and pureed fruit.

P19. *Aronia melanocarpa* fruit juice attenuates oxidative damage of phospholipid polyunsaturated fatty acids in female handball players

Petrovic S.¹, Arsic A.¹, Vucic V.¹, Cikiriz N.², Valdevit Z.³, Jakovljevic V.⁴, and Glibetic M.¹

¹Centre of Research Excellence in Nutrition and Metabolism, Institute for Medical Research, University of Belgrade, Belgrade, Serbia

²Department of Sports Medicine, Military Medical Academy, Belgrade, Serbia

³Faculty of Sport and Physical Education, University of Belgrade, Belgrade, Serbia, and ⁴Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia, E-mail: snjezana5.imr12@yahoo.com

Aronia melanocarpa fruit juice (AMFJ), rich in flavonoids and phenolic antioxidants, has been shown to affect plasma lipids content in experimental animals, as well as in humans. Other studies have reported that chronic intensive training, such as in elite athletes, induce alterations in plasma phospholipid fatty acid (FA) composition. This study was aimed to estimate the influence of AMFJ on plasma phospholipid FA composition in young (18-19 years) active female handball players, and was conducted at the beginning of the handball season. The subjects (N=20) were randomized in two groups (N=10). The intervention group received 100 ml/day AMFJ for 4 weeks, and placebo group received the same doses of placebo, along with their usual diet. Blood samples before and after the treatments were collected. Fatty acid profiling by gas chromatography identified high within-group homogeneity. As observed, treatment with AMFJ produced no significant alternations in proportion of individual FA. At the same time, in the placebo group significantly lower proportion of n-6 polyunsaturated FA (PUFA) and total PUFA was found, when

compared to baseline values. Considering that intense and prolonged exercises provoke an increase in oxidative stress, that could induce oxidation of PUFA, our findings suggest that treatment with AMFJ can attenuate oxidative damage of plasma phospholipid PUFA, as detected in the placebo group. However, additional research of longer duration is needed to confirm beneficial effects of AMFJ consumption on plasma lipids in active female handball players.

P20. Glycemic index of three pre-germinated brown rice consumed in Thailand: The study in type 2 diabetes

Boonpattararuksa J.¹, Songchitsomboon S.², Akkaravessapong P.³, Wongpiyachon S.⁴, and Komindr S.⁵

¹Division of Nutrition and Dietetics, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

²Postgraduate Program of Nutrition, Faculty of Medicine at Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

³Bureau of Rice Research and Development, Rice Department, Ministry of Agriculture and Cooperatives, Bangkok 10900, Thailand

⁴Pathumthani Rice Research Center, Bureau of Rice Research and Development, Rice Department, Ministry of Agriculture and Cooperatives, Pathumthani 12110, Thailand

⁵Division of Nutrition and Biochemical Medicine, Department of Medicine, Faculty of Medicine at Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand, E-mail: jariya_boon22@yahoo.com

The glycemic responses to three types of Thai pre-germinated brown rice [Khao Dawk Mali105 (KDML105), Sangyod and glutinous rice RD6 (GRD6) grown in Surin, Phatthalung and Chiang Mai Province, respectively] were compared with glucose standard (50 g in drinking water) in a single-blind, randomized block design. Eleven subjects with type 2 diabetes (5 females, 6 males) were recruited to participate in four separate 4 hour meal tolerance test. All the rice samples contained 50g of available carbohydrates. Each previous day the subjects were requested to eat same standard menu all day. On test day, they ingested each test rice sample with steamed garlic-chicken and 150 mL of drinking water within 15 min. Venous blood sample were collected in fasting(0) subjects and at 30, 60, 90, 120, 180 and 240 min after starting to eat. The incremental areas under the curve (IAUC) of postprandial plasma glucose (PPG) and serum insulin response, glycemic index (GI) and insulin index (II) values were determined. Results showed that PGBR of KDML105 and Sangyod had mean values of PPG at 30 and 60 min significantly lower ($p < 0.05$) than glucose at the same time whereas PGBR-glutinous rice RD6 had mean values of PPG only at 60 min significantly lower ($p < 0.05$) than glucose. Mean value of IAUC-glucose of PGBR-KDML105 and PGBR-Sangyod were also lesser than PGBR-glutinous rice RD6 but not significant. Data showed that PGBR-Sangyod and PGBR-KDML105 had medium GI=65.4 and 66.0 while PGBR- glutinous rice RD6 had high GI =71.5. However, no significant difference in GI of these 3 rice tests was found. There were no significant differences in mean values of IAUC-insulin, II and lipid

profiles among rice tests too.

P21. Long-term effect of high antioxidant rice bran supplement on postprandial glucose and antioxidant status in type 2 diabetic patients

Songchitsomboon S.¹, Ae-uam P.², Viroonudomphol D.³, and Komindr S.⁴

¹Graduate Program of Nutrition, Faculty of Medicine at Ramathibodi Hospital and Institute of Nutrition, Mahidol University, Thailand

²Nursing Department, Faculty of Medicine at Ramathibodi Hospital, Mahidol University, Thailand

³National Institute of Metrology, Pathum Thani, Thailand

⁴Department of Medicine, Faculty of Medicine at Ramathibodi Hospital, Mahidol University, Thailand, E-mail: sriwatana.son@mahidol.ac.th

Riceberry is a cross-bred unmilled rice possessing dark violet grain, which is a combination of Hom Nin Rice, with high antioxidant properties, and KhaoDawk Mali 105. Randomized crossover clinical trial was performed to study the effect of rice bran of Riceberry with high antioxidants (30 g of rice bran/d contained insoluble fiber 6g/d and total antioxidants equal to 8.7 mmole TE/day) supplement before dietary meals on glucose, lipid profile, antioxidant status and oxidative stress in 26 patients with type 2 diabetes over a 8 weeks period, compared to cellulose (6 g/d as placebo) with a 8-wks washout period. They were advised to consume an American Diabetes Association (ADA) diet as baseline diet for 4 wks (run-in period) and still advised to follow baseline diet throughout the study. Result showed that after Riceberry rice bran with high antioxidants supplemented 8 weeks, diabetic patients had significantly better postprandial glucose control and antioxidant status (serum oxygen radical absorbance capacity, ORAC) throughout 180 min than only fiber supplement (mean changes of IAUC of glucose; -2500.6 vs 3415.6 mg/dL x min, $p=0.029$ and mean changes of IAUC of ORAC; 550 vs 300 umole TE/ml x min, $p=0.000$). But there were no significant differences in lipid profiles (total cholesterol, LDL-C, HDL-C and triglyceride) after both periods. Data suggested that not only fiber but also high antioxidants content in Riceberry rice bran which had more health benefit to type 2 diabetic patients than cellulose without antioxidants. However, there were no significant differences in superoxide dismutase (SOD) and glutathione peroxidase (GPX), both are antioxidant enzymes after both periods. Although, after Riceberry rice bran supplemented, conjugated dienes was significantly increased but malondialdehyde was trendily decreased too, both are by-product of lipid peroxidation occurred in the body.

P22. Prevalence of vitamin D deficiency and relation with body composition in adult Serbian women

Vucic V.¹, Petrovic S.¹, Ristic-Medic D.¹, Petrovic-Oggiano G.¹, Cubrilo D.², Popovic-Kuzmanovic D.³, and Glibetic M.¹

¹Centre of Research Excellence in Nutrition and Metabolism,

Institute for Medical Research, University of Belgrade, Serbia

²Department of Physiology, Faculty of Sport Science, University EDUCONS, Novi Sad, Serbia

³Medical Center "Bezanijska Kosa", University of Belgrade, Belgrade, Serbia, E-mail: vesna.vucic.imr@gmail.com

Low vitamin D status have been associated with increased risk of chronic diseases, including osteoporosis, cancer, cardiovascular diseases, type 2 diabetes and autoimmune diseases. Recent studies have reported relationship between low levels of serum vitamin D and obesity. The aim of this study is to determine the prevalence of vitamin D deficiency among Serbian women and to analyze the relationship between serum 25-OH-vitamin D (25-OH-D) levels and body composition. A cross-sectional study was conducted in 87 apparently healthy women aged 18-68 years, 21 post menopausal women and 66 who reported regular menstrual cycle (26-32 days), with body mass index (BMI) 20-40. Women were grouped according to BMI as normal weight (BMI 20-25), overweight (BMI 25.1-30) and obese (BMI>30). Serum 25-OH-D was related to measures of body fat and BMI. Average concentration of serum 25-OH-D was 21.57 ± 8.62 ng/mL. Almost half of the study participants (46%) exhibited a vitamin D deficiency (<20 ng/mL). Significant differences were found in vitamin D status in normal weight group (23.84 ± 9.23 ng/mL), overweight (22.82 ± 7.65 ng/mL) and obese women (17.69 ± 7.93 ng/mL). Body fat was inversely related to 25-OH-D ($r=-0.234$, $p=0.029$). Our findings showed high prevalence of vitamin D deficiency in Serbian women and implicate that vitamin D supplementation becomes important especially in overweight/obese women.

P23. Neuroprotective effect of Korean wild radish, *Raphanus sativus* var. *hortensis* f. *raphanistroides* in SH-SY5Y cells via regulation of microglial activation

Hur J.^{1,2*}, and Kim M.¹

¹Korea Food Research Institute, Seoungnam, 463-746, Republic of Korea

²Division of Food Biotechnology, University of Science and Technology, Daejeon, Republic of Korea, E-mail: jyhur@kfri.re.kr

Activation of microglia cells induced by external stimulation plays an important role in cell growth and survival; however, overactivated microglia cells destroy neuronal cells. In our current research, we investigated the efficacy of Korean wild radish (WR), *Raphanus sativus* var. *hortensis* f. *raphanistroides*, a root vegetable from the Brassicaceae family, to examine whether it could protect neuronal cells by controlling microglia cells that had been overactivated by lipopolysaccharide (LPS). Cells were treated with different concentrations (1, 10, and 50 mg/ml) of WR extract, and concentration-dependent decrease of NO production was confirmed, as was the decrease in the expression of iNOS. The phosphorylation of p38 kinase associated with the LPS-induced inflammatory response was inhibited by WR extracts. Additionally, while the number of SH-SY5Y cells decreased in the conditioned media treated with LPS alone, cell viability in the

conditioned media treated with both LPS and WR were significantly increased, thus demonstrating the protective effect of WR on neuronal cells. Taking these results together, we believe that the WR extract will prove effective as a neuroprotective agent by controlling overactivated microglia cells.

P24. Determination of S-Allyl-L-cysteine in rat plasma by liquid chromatography-tandem mass spectrometry method: Application to pharmacokinetic study

Lee S.*, Chang N. I., Yoo M., and Shin D.

Korea Food Research Institute, Republic of Korea, E-mail: shlee@kfri.re.kr

S-Allyl-L-cysteine (SAC), the most abundant organosulfur compound derived from garlic, has multifunctional biological activities that occur via different mechanisms. A sensitive, rapid and simple LC-MS/MS method using a mixed-mode reversed-phase and cation-exchange column containing C18 silica particles and sulfonic acid cation-exchange particles has been developed and validated for the analysis of SAC in rat plasma. The mobile phase was optimized at 2 mM ammonium acetate buffer (pH 5.35) and acetonitrile (75:25, v/v). The assay utilized 0.6% acetic acid in methanol to achieve simple and rapid deproteinization. Quantification was conducted using multiple reaction monitoring (MRM) of the transitions of m/z 162.0->145.0 for SAC. The standard curve for SAC was linear ($r^2 > 0.999$) over a range from 5 to 2,500 ng/mL. The intra- and inter-day precision (relative standard deviation) of the method was not >6.0% at three quality control levels. The limit of quantification (LOQ) was 5.0 ng/mL. After being fully validated, the method was successfully applied to the pharmacokinetic monitoring of SAC in rat plasma.

P25. Conflicting views on vitamin D and food fortification in Denmark: An understanding of viewpoints from the civil society, the private and the public sector

Jürgensen A.*, and Valentin G. S.

Metropolitan University College, Copenhagen, Denmark, E-mail: agnete.jurgensen@hotmail.com

Objectives: Food fortification as a tool to address the vitamin D deficiency in Denmark is controversial. This study tries to investigate the main viewpoints and interests for the key stakeholders to support a vitamin D fortification policy in Denmark. Material and Methods: Four interviews were conducted using convenience sampling; one personal interview and three e-mail correspondences. A documentary method was applied. Hearing statements and memorandums with statements from key stakeholders were collected. Articles published between the 30th of October 2013 and 12 months back were retrieved using Infomedia search using the following terms: Food fortification and fortified food. Documents had to contain at least one statement from one key stakeholder. Twenty-seven relevant documents were identified. This approach was used to support a discourse analysis.

Results: A food fortification policy is interlinked with opposing views: ethics; economy; evidence; politics; health; consumer; deceptiveness; promotion; trade and competition. Based on these views four food fortification discourses were identified: Consumer rights; Health promotion; Competition in economy and Evidence in nutrition. The civil society and the public sector advocate for a mandatory regulation. In contrast the private sector advocates for voluntary regulation. Key Findings: Depending on their position, stakeholders have different viewpoints concerning food fortification. The civil society, the private sector and the public sector have opposing views concerning voluntary vs. mandatory regulations. These opposing views reflect their different vested interests. This makes the entire policy process controversial and complicated. Further investigation is required to inform government policy.

P26. The functional role of tomato and carrot on some antioxidants' enzymes and histopathological lesions of brain, small intestine and prostate in mice treated with acrylamide

Zaki S.*, Amin W., and Nagi H.

Food Technology Department, Faculty of Agriculture, Cairo University, Egypt, E-mail: drshafikazaki@gmail.com

Lycopene is among the major carotenoids and one of the protective factors in vegetable-rich diets. Extensive research has been shown that cancer could be a preventable disease requiring major changes in life style and diet. Much research attention has been focused on discovering the role of nutrition in disease prevention. The present investigation aimed to study the protective effect of tomato and carrot against acrylamide on antioxidants enzymes and histopathological sections of some organs in mice. A total of 28 adult male Swiss albino mice (25 ± 2 g) were divided into four groups, 7 each. First group was considered as negative control. The remaining mice were subjected for daily oral administration of acrylamide (40 µg/kg body weight) for 8 weeks. Second group was considered as positive control. Both negative and positive groups were fed on basal diet. The other groups were given basal diets with 20% of dried tomato and carrot (Groups 3, and 4, respectively). At the end of experiment, the relative organs' weights were calculated. Brain, prostate and small intestines were histopathologically examined. Results showed insignificant differences among feed intakes, initial, final & gain% of body weight, feed efficiency ratios and organs' weights. Groups fed on diets with carrot and tomato showed significant improvement in almost all the parameters studied compared to the positive control. No alteration and normal histopathological structure in different organs (small intestine, prostate and brain) of the normal group (negative control) was observed. Very severe alterations were noticed in small intestine, prostate and brain of the positive control treated with acrylamide. The other groups detected slight alterations such as moderate and mild (in small intestines and prostate gland) and showed a normal histopathological structure (in brain) organs.

P27. Radical scavenging activities of plant food using

ESR spin trap method

Ukai M.^{1,2*}, Kameya H.², and Suzuki T.³

¹Hokkaido University of Education, Japan

²National Agriculture and Food Research Organization Japan

³Hokkaido University, Japan, E-mail: ukai.mitsuko@h.hokkyodai.ac.jp

The radical scavenging activity against alkyl-oxy and superoxide radicals was examined using a new reagent 5-(2,2-dimethyl-1,3-propoxycyclophosphoryl)-5-methyl-1-pyrroline N-oxide (CYPMPO). CYPMPO, colorless crystalline, is very soluble in aqueous solution. For careful detection of spin adducts, we used borosilicate ESR flat cells. We examined plant specimens from teas, vegetables and fruits. We generated very pure alkyl-oxy radicals by illuminating phosphate buffer solution containing 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) and CYPMPO with Hg-Xe arc lamp. Superoxide radicals were similarly generated using hypoxanthine (HPX), diethylenetriamine-pentaacetic acid (DTPA), CYPMPO and xanthenes oxidase (XOD). The spin adducts by CYPMPO were sensitive and very stable. CYPMPO successfully trapped alkyl-oxy and superoxide radicals in the plant extracts. The scavenging activity against alkyl-oxy and superoxide radicals was measured by 2-amino-5-[[2-[(carboxymethyl) amino]-1-(mercaptomethyl)-2-oxoethyl]amino] 5-oxopentanoic acid (GSH) and superoxide dismutase (SOD) equivalents, respectively. We concluded ESR spin trap method with CYPMPO is useful for determine the radical scavenging activities against alkyl-oxy and superoxide radicals of plant food.

P28. Korean traditional rice wine and its constituent farnesol induce apoptosis by induction of PTEN in AGS human gastric adenocarcinoma cells

Hwang Jin Taek*, Eun Ju Shin, Sung Hee Kim, Jae Ho Kim, and Jae Ho Ha

Korea Food Research Institute, E-mail: jthwang@kfri.re.kr

This study was to examine the effects of Makgeolli, a Korean traditional rice wine and its constituent farnesol on cancer cell growth. Dealcoholized Makgeolli (MK) exhibited anti-angiogenic effect in human umbilical vein endothelial cells (HUVEC) without cytotoxicity. Treatment of MK reduced the proliferation of AGS human gastric adenocarcinoma cells in a dose dependent manner and increased the sub G1 population. Next we evaluated whether MK can induce apoptosis in AGS cells using TUNEL or Annexin V methods. Treatment of MK at 500 and 1000 µg/ml increased the TUNEL positive cells in AGS cells. Under same condition, 500 and 1000 µg/ml MK significantly induced the early or late apoptosis in comparison to the untreated cells (None). In addition, MK also induced PTEN (Phosphatase and tensin homolog) expressions in AGS cells. However, p53 was not changed by MK in AGS cells. Furthermore, we also examined whether farnesol a constituent of MK can induce apoptosis in AGS cells. In our condition, farnesol exhibited anti-angiogenic effect in HUVEC in a dose

dependent manner. In addition, farnesol increased Sub G1 population, TUNEL positive cells, and PTEN expressions. Taken together, MK and its constituent, farnesol may be useful for the prevention of cancer cell growth by targeting PTEN.

P29. High-risk drinking is positively associated with a higher risk of diabetes mellitus in Korean men, based on the 2010-2012 KNHANES

Hong Sung-Won^{1*}, Hee-Taik Kang¹, John A. Linton², Yon-chul Park³, Mi-Na Lee⁴, Jae-Yong Shim⁵, and Hye-Ree Lee⁵

¹Department of Family Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea

²International Health Care Center, Severance Hospital, College of Medicine, Yonsei University, Seoul, Republic of Korea

³Department of Family Medicine, Wonju Severance Christian Hospital, Yonsei University Wonju College of Medicine, Wonju, South Korea

⁴Department of Family Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea

⁵Department of Family Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, E-mail: swhong82@yuhs.ac

Aims: We examined the association between alcohol-drinking pattern and diabetes mellitus (DM) in Korean adults.

Methods: This cross-sectional study included 12,486 participants (5,551 men and 6,935 women) who participated in the 2010-2012 Korean National Health and Nutrition Examination Survey. We categorized alcohol-drinking pattern based on the alcohol use disorders identification test (AUDIT) into three groups [low-risk (0-7), intermediate-risk (8-14), and high-risk (≥15)]. DM was defined as participants with fasting plasma glucose ≥126 mg/dL or on glucose-lowering medication including insulin therapy.

Results: The percentage of high-risk drinkers is 25.2% in men and 4.7% in women. DM prevalence is 9.2% in men and 5.4% in women. DM prevalence is 9.0% and 5.7% in low-risk drinking group, 7.6% and 4.1% in intermediate-risk drinking group, 11.2% and 3.5% in high-risk drinking group in men and women, respectively. Compared with low-risk drinking group, odds ratios (95% confidence intervals) are 1.043 (0.779-1.396) and 1.139 (0.712-1.824) in intermediate-risk drinking group and 1.480 (1.133-1.933) and 0.827 (0.296-2.311) in high-risk drinking group in men and women, respectively, after adjusting for age and other confounding factors.

Conclusions: High-risk drinking is positively associated with the risk of DM in men, not in women.

P30. Crude polysaccharides isolated from Makgeolli (traditional Korean rice wine) exert immunostimulatory activities in normal and immune-suppressed mice

Cho C. W.¹, Han C. J.², Rhee Y. K.¹, Lee Y. C.¹, Shin K. S.³, and Hong H. D.¹

¹Korea Food Research Institute, Seongnam, Republic of Korea

²Yanbian University, Yangji, China

³Kyonggi University, Suwon, Republic of Korea, E-mail: cwcho@kfri.re.kr

In this work, we examined in vivo immunostimulatory activities of crude polysaccharides from Makgeolli (traditional Korean Rice wine). The crude polysaccharide (PSM) was isolated from Makgeolli by hot water extraction, ethanol precipitation and ultrafiltration. The major constituents in PSM were neutral sugar (87.3%). PSM was composed of 5 different sugars, glucose, mannose, galactose, xylose, and arabinose. Immunostimulatory actions of PSM were investigated in normal and immune-suppressed mice. In normal mice model, male Chinese Kun Ming (KM) mice were treated by oral administration with PSM into two dosages (100 mg/kg and 200 mg/kg body weight) for 4 weeks. In the splenocytes proliferation test, T and B lymphocyte proliferation abilities of PSM treated groups were significantly increased than that of control group. We also investigated the immunostimulatory activities of PSM in cyclophosphamide-treated immunosuppressed KM mice. The results showed that PSM stimulated proliferation of splenic lymphocytes (T and B cells) in immunosuppressed mice. PSM also markedly increased the natural killer-cell activity and peripheral white blood cell number. These results suggested that PSM could enhance immune functions in normal mice model and also restore declined immune functions in immunosuppressed mice. In conclusion, Makgeolli (traditional Korean Rice wine) may have immunostimulatory activities beneficial to human health.

P31. Vitamin D3 status in children with acute diarrhea

Mileva S.¹, Galunska B.², Gospodinova M.¹, Gerova D.³, and Svinarov D.⁴

¹Department of Infectious diseases, Medical University of Varna, Bulgaria

²Department of Pharmaceutical Sciences, Medical University of Varna, Bulgaria

³Department of General Medicine, Medical University of Varna, Bulgaria

⁴Central Laboratory of TDM and Clinical Pharmacology, Alexander Hospital, Sofia, Bulgaria, E-mail: sevdamileva@mail.bg

Introduction: Vitamin D deficiency is highly prevalent among children worldwide. It includes impaired immune response to infection and decreased activity of gut antimicrobial peptides. Elucidating the impact of vitamin D deficiency for the severity of acute diarrhea among children may be helpful for the disease management.

Objective: Determination of vitamin D status in toddlers with acute diarrhea and evaluation the relationship with diarrhea severity. **Patients and methods:** 77 children (1.0 – 3.5 years) with acute diarrhea,

hospitalized in the Department of Infectious diseases were enrolled in the study. The patients were divided into 2 groups: with risk factors for severe diarrhea (group A, n=30) and group B without risk factors (n=47). The severity of diarrhea was assessed by the number of stools. The levels of circulating vitamin D were assayed by liquid chromatography with tandem mass-spectrometric detection. One way ANOVA and Kruskal Wallis statistics were used for statistical analysis.

Results: Patients in group A were vitamin D insufficient (median 53,63nmol/L), compared to group B (median 66,09nmol/L), p<0.05. Vitamin D deficiency (median 49,20nmol/L) was detected in children with severe diarrhea (> 20 stools) vs vitamin D in children (median 64,93nmol/L) with less intensive diarrhea, p<0,05. An inverse relationship between vitamin D levels and diarrhea intensity and outcome was detected. For both groups considerable seasonal variations in vitamin D levels were established.

Conclusion: VitaminD deficiency may impact on the severity of acute diarrhea in children. VitaminD supplementation could be favorable for prevention of acute diarrhea in vitamin D deficient toddlers.

P32. Hyptis suaveolens: New plant oligosaccharides and their prebiotic activity

Čavarkapa A.*, Müller M., Praznik W., Unger F., and Viernstein H.

Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Vienna, Austria, E-mail: andrea.cavarkapa@univie.ac.at

The mucilage extracted from the seeds of *Hyptis suaveolens* contains acidic and neutral polysaccharides and shows a great water binding capacity. According to the molecular size and chemical composition of this heterobiopolymer, it could be used for both industrial and pharmaceutical applications. Small amounts of the neutral polysaccharide were dissolved in water and incubated separately with different enzymes. Oligosaccharides produced by enzymatic cleavage were detected by thin layer chromatography and fractionated by size exclusion chromatography. In order to investigate their prebiotic activity with selected *Lactobacillus* spp., oligosaccharide mixtures were analysed in vitro by Bioscreen C MBR, based on optical density measurements.

The recent interest and permanent search for the natural renewable sources of the manifold applicable raw materials requires further investigation steps for the more detailed characterisation of these new oligosaccharides.

P33. Comparison of anthocyanin component in fruits of blueberry cultivars and wild vaccinium SPP. originating from Japan related to antioxidant activity

Takashi S.*, Yokota R., and Tanaka N.

Research Faculty and Graduate School of Agriculture, Hokkaido University, Japan, E-mail: suz-tak@res.agr.hokudai.ac.jp

Many recent researches have focused on the prevention against oxidation-related human diseases by taking in berry anthocyanins as natural antioxidants in foods. It is well known that most *Vaccinium* fruits like blueberry contain amounts of anthocyanins. To clarify yearly variations and species differences, component of anthocyanins and antioxidant activity in fruits were examined with 8 cultivars of highbush blueberry (*Vaccinium corymbosum*) and 5 wild *Vaccinium* spp. originating from Hokkaido, Japan in 2008 through 2010. Anthocyanins were determined using MALDI-TOF MS and antioxidant activity was evaluated by DPPH radical scavenging method using 96-well microtitration plate. Malvidin-3,5-diglucoside was added to each sample as an internal standard for quantification of anthocyanins. Fifteen anthocyanin peaks, including their aglycones i.e. cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin, were detected from the fruits. The kind and amount of anthocyanins in blue-colored wild berries (*V. smallii*, *V. praestans* and *V. oldhamii*) was the same as and a bit greater than those in blueberry cultivars. However, they were rather smaller in red-colored wild berries (*V. japonicum* and *V. ovalifolium*). Since no yearly variation but clear varietal difference was observed in the present study, the anthocyanin component in fruits could be an index to distinguish blueberry cultivars. The fact that positive correlation coefficient ($r=0.86$, $n=29$) was confirmed between total content of anthocyanins and DPPH radical scavenging activity indicates that antioxidant activity of *Vaccinium* fruits strongly depends on the amount of anthocyanins.

P34. Databank requirement for polyamines in foods

Buyukuslu N.*

Istanbul Medipol University, Turkey, E-mail: nbuyukuslu@medipol.edu.tr

Putrescine (diamine), spermidine (triamine) and spermine (tetramine) are most abundant polycationic natural amines. Putrescine and spermidine are found in nearly all organisms, while spermine is mainly found in eukaryotic cells. They are involved in regulation of gene expression, translation, cell proliferation and differentiation, DNA, RNA and protein synthesis in mammal cells. They can be supplied by the endogenous synthesis inside the cell or by the intake from exogenous sources. Polyamines are critical for all types of cellular proliferation and for the continuation of life in all cell types. An increased need of dietary polyamines is suggested during rapid growth, such as during the neonatal period, wound healing and after surgery. However, it was also shown that the high levels of intracellular polyamines correlate with various human cancers. The limited exogenous polyamines emerges as a promising strategy in tumour therapy. There is a growing body of literature related the effects of bioactive amines on health and diseases, but limited information about polyamine content of foods and human milk is available for diets. Reliable information on polyamine content in foods is needed for dieticians. Create a databank for polyamines might be crucial to evaluate the nutritional status of individuals, especially in the case of cancer care.

P35. Continuous glucose monitoring system (CGMS) and new era of early diagnosis of diabetes in high risk groups

Soliman A. T.¹, Desanctis V.², Sabt A.³, and Aldarsi N.¹

¹Hamad Medical Center, Doha, Qatar

²Quisisana Hospital, 44121 Ferrara – Italy

³Hamad General Hospital, Doha, Qatar, E-mail: ATSOLIMAN@yahoo.com

Continuous glucose monitoring (CGM) systems are an emerging technology that allows frequent glucose measurements to monitor glucose trends in real time. Their use as a diagnostic tool is still developing and appears to be promising. Combining intermittent glucose self-monitoring (SGM) and CGM combines the benefits of both. Significant improvement in the treatment modalities that may prevent the progress of prediabetes to diabetes have been achieved recently and dictates screening of high risk patients for early diagnosis and management of glycemic abnormalities. The use of CGMS in the diagnosis of early dysglycemia (prediabetes) especially in high risk patients appears to be an attractive approach. In this presentation we searched the literature to investigate the value of using CGMS as a diagnostic tool compared to other known tools, namely oral glucose tolerance test (OGTT) and measurement of glycated hemoglobin (HbA1C) in high risk groups. Those categories of patients include adolescents and adults with obesity associated with family history of type 2 diabetes mellitus, polycystic ovary syndrome (PCO), cystic fibrosis, thalassemia major, gestational diabetes, and after renal transplantation. Conclusion: The use of CGMS in the diagnosis of early dysglycemia (prediabetes) especially in high risk patients appears to be promising and in many occasions superior to other known diagnostic modalities namely oral glucose tolerance test and measurement of HbA1C. Its use in combination with intermittent glucose monitoring adds to its accuracy and reliability.

P36. Identification of modulators for the corticotropin releasing factor receptor from a natural product library

Lee S.*, and Noh H.

Department of Biomedical Technology, Sangmyung University, Cheonan, Republic of Korea, E-mail: sunghou.lee@gmail.com

Recently, efforts for the search of various physiological modulators have been intensively developed through variety of research groups to utilize them for cosmetics and/or foods in addition to the conventional therapeutics. Therefore, drug development schemes have been expanding their application territories towards the development of healthy foods. As an example of these possibilities, the screening of corticotropin releasing factor (CRF) modulator with a natural product library was processed. CRF and urocortin analogs have been suggested to control various neuroendocrine system through two high-affinity CRF receptors: CRF1 and CRF2. Recent studies associated with CRF receptor system demonstrate that, in the CRF over-expressing

mice with alopecia, blockade of both CRF1 and CRF2 receptors with intraperitoneal or subcutaneous injection of CRF1 and CRF2 antagonist, astressin-B, induces a robust skin pigmentation and hair re-growth. Based on the existing evidence that chronic stress impairs hair growth and that major components of the CRF system are expressed in the mouse and human skin, the ability of CRF receptor antagonists to influence hair loss/re-growth was widely proposed. Here, we presented the performance of conventional screening system for the search of CRF antagonists using a pilot natural product library. The aequorin parental cells (ES-000-A30, PerkinElmer Life and Analytical Sciences, Boston, USA) were transfected with cDNA for CRF1 receptor (GenBank Acc# AY457172) and alpha 16 promiscuous G protein (GenBank Acc# AF493904) to establish HEK293-aeq-a16/hCRF1, a stable cell line for the human CRF1 receptor. In HEK293-aeq-a16/hCRF1 cells, 3 μ M of sauvagine stimulated calcium mobilization reached a peak of about 50,000 relative luminescence units within 20 s after agonist addition and then rapidly returned to baseline levels of 2,500. A pilot natural product library composed of 1,040 extracted materials was used to screen for CRF1 receptor antagonists. The overall screening results presented the z' value of 0.5055 and the signal to noise ratio reached to 160.57, suggesting that the assay window was very high and control values were quite stable. As a result of this screening operation, three final hits were identified to proceed next step of studies. Therefore, the current screening trials can be a model system for the development of CRF1 antagonists that can have a clinical efficacy on hair growth and pigmentation and furthermore, these materials can be starting agents for the development of functional foods for the hair growth.

P37. Green tea in cancer prevention?

Zoubková H.^{*}, and Polívková Z.

Department of General Biology and Genetic, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic, E-mail: hana.demova@lf3.cuni.cz

Bioactive substances, also called phytochemicals, have been identified in fruits, vegetables and other crops in addition to vitamins and minerals. Conclusions about effects of phytochemicals on human are miscellaneous, but data showing preventive actions against tumor initiation, growth and other diseases predominate. Epigallocatechin gallate (EGCG), the phytochemical from green tea, has been tested in Ames, micronucleus test and Comet assay. Results of experiments confirm the preventive effects of this phytochemical against a mutagenicity of carcinogenic substances.

P38. Intake of *Lepidium meyenii* enhances the gene expression of thyroid stimulating hormone beta in female rats

Uchiyama F.^{1,2*}, Jikyo T.¹, and Ogata M.¹

¹Institute of Preventive and Medicinal Dietetics, Nakamura Gakuen University, Fukuoka 814-0198, Japan ²Graduate School of Nutritional Sciences, Nakamura Gakuen University, Fukuoka 814-0198, Japan, E-mail: uchiya-f@nakamura-u.ac.jp

Lepidium meyenii is an herbaceous biennial plant of the Brassicaceae family that is native to the high Andes of Peru. The root of *Lepidium meyenii*, known as Maca, has been used as a nutritious and medicinal food source. Native Indians in high Andes are known to elevate hand and finger temperatures in chronic cold exposure and to intake Maca before going to outside work places during a cold day. We have studied whether Maca has a characteristic of thermogenesis through the activity of the hypothalamic-pituitary-thyroid axis in the rat and have just found that Maca has a characteristic of elevating body temperature within the normal range in female rats. To address the mechanism of the Maca action, we investigated the gene expression of TSH (thyroid stimulating hormone) in female rat pituitary. Two groups of female Sprague-Dawley rats, named a Maca group and a control group, were provided normal feed with or without Maca powder ad libitum for 7 weeks, respectively. The rats were euthanized at 1800h, and pituitary organs were collected. To investigate the alteration of whole gene expression in the pituitary, we measured differential gene expression levels by microarray analysis for 14,658 genes. In the gene expression profiling, the average of the total gene expression levels was no significant difference between the control and the Maca groups. The high expression hormone genes such as growth hormone (Gh1), proopiomelanocortin (Pomc), chorionic gonadotropin alpha (Cga), and prolactin (Prl) in pituitary were in the respective same expression levels between the control and the Maca groups. In significant differences ($P < 0.05$), twelve genes were up-regulated in more than 20% increase and one gene was down-regulated in less than 20% decrease by Maca intake. The gene expression of thyroid stimulating hormone beta (Tshb) was a 1.7-fold increase by Maca intake. To confirm to increase the expression levels of Tshb gene, we measured Tshb gene expression levels by qRT-PCR. We found that the expression level of Tshb gene significantly increased with a 1.7-fold high level by Maca intake ($n=3$, $P < 0.01$). These results show that Maca has a characteristic of enhancing the gene expression of Tshb gene. Cold exposure is known to affect the hypothalamic-pituitary-thyroid axis and to increase the secretion of TSH. Therefore, the traditional use of Maca for cold exposure may be related to maintain higher average body temperature through Tshb gene expression in the rat.

P39. Correlation between plasma levels of lutein and oxidized low density lipoproteins: A short human intervention study

Bacchetti T.^{1*}, Tullii D.², Brugè F.³, Silvestri S.³, Orlando P.³, Tiano L.³, and Ferretti G.³

¹Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Italia

²Italsur s.r.l., Notaresco (TE), Italia

³Dipartimento di Scienze Cliniche Specialistiche e Odontostomatologiche, Università Politecnica delle Marche, Italia, E-mail: t.bacchetti@univpm.it; g.ferretti@univpm.it

The development of vegetable functional food products that

provide benefits beyond their traditional nutritional value raised increasing interest. In fact several studies have shown that diet plays a role in the development of human diseases associated with oxidative damage (diabetes, cardiovascular disease and metabolic syndrome). Aim of the study was to evaluate the effects of daily intake of a frozen vegetable product containing red and green spinach, red and green chicory, red and green leaf chard (Italsur srl Notaresco, Italy) on plasma lipids and oxidized low density lipoproteins (ox-LDL). Furthermore, the bioavailability of lutein and β -carotene contained in the vegetable products, was investigated. The study included healthy volunteers (n=49, age ranged from 23 to 73 years), who consumed a portion of the vegetable product every day for 2 weeks. The significant increase of plasma lutein (mean value from 0.27 ± 0.13 to 0.40 ± 0.17 $\mu\text{g/ml}$, $p < 0.001$) and β -carotene (mean value from 0.31 ± 0.12 to 0.56 ± 0.17 $\mu\text{g/ml}$, $p < 0.001$) after intake of vegetable product demonstrated that these phytonutrients are highly bioavailable. A significant reduction of plasma levels of total cholesterol (TC) (mean percentage reduction -6.4% with respect to baseline, $p < 0.05$) and LDL-cholesterol (LDL-C) (mean percentage reduction -9.4% with respect to baseline, $p < 0.05$) was observed after dietary intervention. Our results demonstrated also a significant decrease in plasma concentration of oxidized LDL (ox-LDL) after treatment (mean percentage decrease -21.9%, with respect to baseline) ($p < 0.05$). Evaluation of ox-LDL represents a useful biochemical marker of lipid peroxidation, therefore the results demonstrated a decrease of lipoprotein peroxidation after the daily intake of the vegetable product. A significant negative correlation has been established between plasma levels of lutein and of ox-LDL ($r = 0.69$, $p < 0.001$). Our results suggest that lutein may play a role in the protective effect against oxidation of LDL. In conclusion the increase of plasma carotenoids after dietary treatment for two weeks is associated with physiological effect against lipid peroxidation of lipoproteins.

P40. HPLC-UV determination of 25-hydroxy-vitamin D in human plasma: Comparison with ID-LC-MS/MS and immunoassay

Galunsk B.¹, Gerova D.², Todorov S.³, and Svinarov D.⁴

¹Department of Pharmaceutical Sciences, Medical University of Varna, Varna, Bulgaria

²Department of General Medicine, Medical University of Varna, Bulgaria

³Medical Diagnostic Laboratory „Status“, Varna, Bulgaria, and ⁴Central Laboratory of TDM and Clinical Pharmacology, Alexander Hospital, Sofia, Bulgaria, E-mail: bistra.galunsk@gmail.com

Introduction: Twenty five-hydroxy-vitamin D (25OHD) levels are accepted as an indicator for Vitamin D status and their measurement is still a challenge. **AIM:** To develop a robust and easy to use HPLC-method for simultaneous analysis of 25OHD₂ and 25OHD₃ in human plasma and to assess its analytical performance.

Methodology: Protein precipitation followed by liquid-liquid extraction with n-hexane was used for separation from the matrix

substances. A HPLC-system with UV detector (Thermo Scientific Spectra System, USA) was used. HPLC-UV analysis was performed on a Synergy hydro-RP column 4,6x250 mm, 4.0 μm particle size with mobile phase consisting of methanol/acetonitrile/water (70/25/5) under isocratic elution at a flow rate of 1.2mL/min and UV-detection at 265nm. Our HPLC-method was compared with a validated ID-LC-MS/MS method and with a commercial immunochemical method. Linear regression was used for data analysis.

Results: Linearity of the HPLC-assay was between 10-250nmol/L for each analyte ($r^2 = 0.997$) with lower limit of detection 4nmol/L, and limit of quantification 10nmol/L. Intra-assay precision was determined by extracting and quantifying five replicates of 3 QC levels (RSD \leq 8%). Inter-assay precision was determined over five consecutive days analyzing five replicates of all QC samples (RSD $<$ 10%). All results were within 14.2% deviation of the 25OHD LC-MS/MS method mean. Method comparison demonstrated good agreement between HPLC and LC-MS/MS ($r = 0.85$) and disparity with the commercial immunoassay ($r = 0.60$).

Conclusion: This assay demonstrates excellent linearity, acceptable accuracy and precision, a good agreement with a validated ID-LC-MS/MS method. The simple sample preparation and ease of determination make it useful for the routine clinical laboratory.

P41. Evaluation of multiple radical scavenging capacities using ESR

Kameya H.^{*}, Watanabe J., Ishikawa Y., and Todoriki S.

NARO, National Food Research Institute, Japan, E-mail: hkameya@affrc.go.jp

Reactive oxygen species (ROS) are considered to cause many health problems. Medicinal foods contain large quantities of antioxidants. These antioxidants inhibit ROS-induced decomposition of biological molecules by scavenging the radicals. To evaluate radical scavenging capacities, the oxygen radical absorbance capacity (ORAC) method has been reported. However, the ORAC method is based on the measuring of alkoxy radical scavenging capacity. It is necessary to evaluate the multiple ROS scavenging capacities. Electron Spin Resonance (ESR) method is very useful for identifying and quantifying of free radicals directly. It is reported ESR spin trapping method with an in situ photolysis system is a promising approach for enabling a comparative assessment of the scavenging capacity of multiple ROS. We have succeeded to synthesize the new spin trap reagent 5-(2,2-dimethyl-1,3-propoxy cyclophosphoryl)-5-methyl-1-pyrroline N-oxide (CYPMPO), a cyclic DEPMPO-type nitron. Both the solid and diluted aqueous solution of CYPMPO is very stable, and any ESR signals from CYPMPO were not detected during 1 month storage at ambient conditions. This long shelf-life of CYPMPO is very useful for the practical use. The optimal ESR spin trap protocol of multiple radicals; hydroxyl, superoxide, alkoxy, peroxy, methyl radical, and singlet oxygen scavenging capacity were determined. The ESR adduct spectrum of CYPMPO were stable over 15 minutes. Quantification of radical adduct signals by CYPMPO was highly reproducible with

variations of less than 4%. We concluded that ESR spin trapping method with CYPMPPO is very useful for the evaluation of multiple radical scavenging capacities.

P42. *Lepidium meyenii* (MACA) increases body temperature with the normal scale in female rats

Jikyo Tamaki¹, Misato Ogata², and Fumiaki Uchiyama^{2,3}

¹Department of Biological Sciences, Institute of Preventive and Medicinal Dietetics, Nakamura Gakuen University, Japan

²Department of Molecular Biosciences, Institute of Preventive and Medicinal Dietetics, Nakamura Gakuen University, Japan

³Graduate School of Nutritional Sciences, Nakamura Gakuen University, Japan, E-mail: tjikyo@nakamura-u.ac.jp

Lepidium meyenii (Maca) has been traditionally used for cold exposure in the Andean region. However, the influence of body temperature on Maca intake has not been reported. We investigated effects of Maca on the tail temperature in female rats. Maca powder was made from the tubers of *Lepidium meyenii* Walp collected, dried, and reduced to powder at the plantation in Junín Plateau and was purchased from Yamano del Perú SAC. The Maca powder was identified by chemical profiling and taxonomic methods. Two groups of female Sprague-Dawley rats, named a Maca group and a control group, were respectively provided normal feed with or without 50% Maca powder ad libitum for 7 weeks. No significant differences in feed intake or growth rate were observed among the rats. We measured the tail temperature every day during 15 days after 5 weeks. The tail temperatures had been significantly increased in Maca intake after the 10th day of monitoring. The average of tail temperature was 33.4 ± 0.3°C in the control group (n=10) and, 34.7 ± 0.2°C in the Maca group (n=9). The results show that Maca has a continuous effect of thermogenesis in the range of normal body temperature in female rats. These findings support that the thermogenesis on Maca intake may be effective for cold exposure stress by rising body temperature and it may be rational for the traditional use in the high-altitude Andes.

P43. A novel antioxidative phytochemical detected from *Cardamine fauriei*, a wild edible brassicaceae herb in Japan

Abe K.*, Matsuura H., Shimura H., and Suzuki T.

Research Faculty and Graduate School of Agriculture, Hokkaido University, Japan, E-mail: kabe@res.agr.hokudai.ac.jp

Cardamine fauriei Maxim. (Ezo-wasabi in Japanese) is a wild edible herb originated from Hokkaido, Japan and has a different flavor from *Wasabia japonica* (Wasabi in Japanese). Because it is unknown about characteristics of *C. fauriei* as functional food ingredients, we here examined any antioxidant phytochemicals in *C. fauriei*. We analyzed antioxidant activities of *C. fauriei* and other vegetables in Brassicaceae by DPPH radical scavenging activity and found that *C. fauriei* showed higher antioxidant activities than broccoli, cabbage, watercress and potherb mustard. To identify phytochemicals contributing to high

antioxidant activity of *C. fauriei*, 80% methanol extract of lyophilized *C. fauriei* powder was separated using Diaion HP-20, Sephadex LH-20 and C18 Sep-Pak cartridge, and antioxidant activities of each fraction were evaluated by DPPH radical scavenging activity. The fraction 3 with the highest antioxidant activity was then subjected to MALDI TOF-MS, and a single peak with high resolution and strong ion intensity was detected at 555 m/z. In analyses using reverse phase HPLC equipped with UV detector, the fraction 3 was separated to three peaks but each peak showed the same m/z of 555.3177 by High Resolution ESI-MS analysis, and the estimated molecular formula was C₃₀H₄₂O₆N₄. According to the results from fragment ions by MS/MS and 1H-NMR, the antioxidant phytochemicals in *C. fauriei* was identified as N1, N14-di-feruloyl-sperimine. This compound is symmetric structure containing two olefinic double bonds, and thus three stereoisomers (EE, EZ and ZZ form) were separately detected in the fraction 3 by HPLC. This unique phytochemical with high antioxidant activity seems to be specific to *C. fauriei* in Brassicaceae.

P44. Physicochemical and microbiological characteristics of probiotic yogurts produced with freeze dried immobilized cells on casein

Dimitrellou D.¹, Kandylis P.², and Kourkoutas Y.¹

¹Department of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupolis, 68100, Greece

²Department of Chemistry, University of Patras, 26500 Patras, Greece, E-mail: dimitrellou@gmail.com

The effect of freeze dried immobilized *Lactobacillus casei* ATCC 393 cells on casein, on physicochemical and microbiological characteristics of probiotic yogurts during 4 weeks storage at 4°C were studied. There was a decline in pH with the storage time and the use of immobilized cells led to lower pH values and subsequently higher values of titratable acidity. The immobilized biocatalysts led to reduced values of syneresis during storage.

More specifically the values of syneresis were 44.1% and 42.6% for control and free cells respectively while this with immobilized cells was 41% at the end of storage. Similar results were observed also in the case of water holding capacity, where the immobilized cells led to higher values compared to control and free cells yogurts. The moisture content was not affected and varied in all cases from 87.5% to 88.7%. In all cases the sum of yogurt bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. bulgaricus was above the requirement of 107 viable microorganisms per gram. The numbers of all microorganisms were declined during storage. Microbiological and strain-specific multiplex PCR analysis showed that *L. casei* ATCC 393 were detected at levels required to confer a probiotic effect (at least 6 log cfu/g) even after 4 weeks of storage. The sensory evaluation ascertained the overall quality of the probiotic yogurts that scored similar values with the commercial sample.

P45. Effects of 6-month weight loss programme on metabolic profile

Jenko-Pražnikar Zala*, Mojca Bizjak, Ana Petelin, Maša Černelič-Bizjak, Mihaela Jurdana, and Tadeja Jakus

University of Primorska, Faculty of Health Sciences, Polje 42, SI-6310 Izola, Slovenia, E-mail: zala.praznikar@fvz.upr.si

The hypothesis of the present study was put forward that significant alterations in inflammatory markers and adipokines would occur over multidisciplinary intervention and that these changes may also be important for improvement of cardiovascular risk factors. 33 overweight and obese adults completed a 6-month intervention trial to evaluate the effects of an individual dietary programme based on individual's resting metabolic rate on anthropometry, metabolic profile, and inflammation. According to the % of fat mass loss, subjects were divided into two groups; weight loss group and weight stable group. Weight loss group, compared to weight stable group, significantly decreased body weight, trunk fat, waist circumference, serum insulin, insulin resistance score, total cholesterol and inflammatory markers, but increased anti-inflammatory adiponectin. The important findings of the present study were that reduction of sugars and saturated fatty acids in diet, and on the other hand increase in exercises significantly correlated with reduction of waist circumference, trunk fat and body mass index. Moreover, positive correlations between reduction in waist circumference and body fat and cardiovascular risk factors were demonstrated. In conclusion, weight loss programme resulted in health benefits.

P46. Red gooseberry fruit as in vitro pancreatic lipase inhibitors

Sosnowska D.*, and Redzynia M.

Institute of Technical Biochemistry, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Stefanowskiego 4/10, 90-924 Łódź, Poland, E-mail: dorota.sosnowska@p.lodz.pl

Obesity is a one of the main worldwide health problems, and has been implicated in the development of major chronic diseases, such as cardiovascular disease, diabetes and cancer. One of the therapeutic approaches to preventing obesity is to retard absorption of fatty acid by the inhibition of lipase in the digestive tract. Various plant sources, including fruits, are reported to have an inhibitory effect on pancreatic lipase activity in vitro. Aim of the present work is to determine the inhibitory effects of red gooseberry fruit different extracts on pancreatic lipase activity. The gooseberry fruits (cultivars "Hinnonmaki Rot" and "Tryumf") were extracted with 70% acetone, 70% ethanol and water with constant mixing for 1 h. Crude extracts were obtained through the removal of the organic solvents during evaporation. Pancreatic lipase activity was measured by a fluorimetric assay using 4-methylumbelliferyl oleate as a substrate, and by titrate methods using triolein, tributyrin, sunflower oil and rapeseed oil as substrates. Anti-lipase activity of gooseberry fruits was expressed as IC50 value. This ratio expresses the amount of fruit needed for 50% inhibition of enzyme. Additionally, the total phenolic and anthocyanin contents were estimated. Content of phenolic compounds in analyzed fruits and their anti-lipase activity varied depending on the variation

of gooseberry and an extraction agent. Total polyphenol content ranged from 130 (cv. Hinnonmaki Rot; water extract) to 187 mg/100g (cv. Tryumf; acetone extract). Gooseberry fruit cv. Hinnonmaki Rot exhibited approximately 5 times higher content of anthocyanins than fruit cv. Tryumf, regardless of the applied extraction agent. The acetone extracts were the most potent inhibitors with IC50 0.6-0.9 mg of fruits per ml in fluorimetric method. The concentration of the acetone extracts required for 50% inhibition of the enzyme in the lipid substrate emulsions was from 106 to 281 mg of fruit/ml.

P47. Comparative study of biological activity of black currant fruit and commercial products in vitro assay models

Podsędek A.*, Majewska I., and Anders B.

Institute of Technical Biochemistry, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Stefanowskiego 4/10, 90-924 Łódź, Poland, E-mail: anna.podsedek@p.lodz.pl

Black currant (*Ribes nigrum* L.) fruits are rich in phenolic compounds, especially anthocyanins. In Poland, most of the production of black currant berries is for use in processed products, such as nectar, drink, jam, wine and cordial. Fruits processing may have influence on chemical composition of the final product, thus affecting its beneficial health effect. It is well-known that polyphenols are susceptible to degradation during processing. The aim of the present study was to compare the composition, antioxidant capacity and anti-lipase activity of fresh and processed black currant berries. Total phenolics, individual anthocyanins, antioxidant properties and inhibitory activity against porcine pancreatic lipase were evaluated in black currant fruit (cv. Tiben and Ores) as well as in commercially obtained nectars (7 samples) and jams (3 samples). Phenolic compounds from fruit and jams were extracted with 70% acetone, while nectars were analyzed directly. Antioxidant capacity was determined by both ABTS radical-scavenging and ferric reducing antioxidant power (FRAP) assays. The effects of fruit and products on the porcine pancreatic lipase activity were measured in vitro on 4-methylumbelliferyl oleate as substrate. Anthocyanin composition was determined by HPLC and total phenolics content was measured with Folin-Ciocalteu reagent.

In vitro, the black currant fruits which have highest phenolics and anthocyanins contents showed stronger antioxidant capacity and higher pancreatic lipase inhibitory activity when compared with different black currant products. Phenolics content among samples varied from 67.26 to 841.74 mg per 100 g. Anthocyanins comprised 33-38% of total phenolics in fruit and 1-12% in processed berries. A strong correlations of antioxidant capacity and anti-lipase activity with total phenolics and anthocyanins contents were noticed. The present study suggests that phenolic and anthocyanin level may be used to predict the in vitro biological activity of black currant products.

P48. Processed cheese sauces with different preservative systems

Saad Suhila A.¹, Laila D. EL-mahdi¹, Awad R. A.², and Hassan Z. M. R.²

¹Food Technology Research Institute (FTRI), Agricultural Research Center, Giza, Egypt

²Food Science Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, E-mail: suhilasaad@yahoo.com

Five novel Formulations of processed cheese sauces with different preservatives systems were achieved in this study. The incorporated ingredients used to introduce shelf life stable processed cheese sauces with formulations consists of: Ras cheese, UF- retentate curd, butter fat, corn starch & guar gum, skim milk powder, emulsifying salt, nisin and natamycin or potassium sorbate as preservative systems. All formulated processed cheese sauces blends were adjusted to contain 25% TS, 40% F/DM, in the processed cheese sauce as a finished product. Addition of the preservatives in cheese sauce formula has a significant effect on the keeping properties. nisin + potassium sorbate mixture has no undesirable effect on the chemical, physical and sensory properties even after 3 months of storage. Resultant processed cheese sauces were evaluated when fresh and periodically during storage at (25 ± 2°C) for chemical composition, pH, oil separation index and viscosity. All treatments were also examined for microbiological and sensory quality attributes when fresh and during storage up to 3 months. Three replicates were carried out for each treatment and the data obtained were statistically analyzed at $p \leq 0.05$.

P49. Interaction between whey protein nanoparticles and fatty acids

Hassan Z. M. R.¹, Awad R. A.¹, El-Sayed M. M.², Foda M. I.², Otzen, D.³, and Salama H. H.²

¹Food Science Department; Faculty of Agriculture; Ain Shams University; Cairo; Egypt

²Dairy Science & Technology Department, National Research Center, Egypt

³Interdisciplinary Nanoscience Center, Department of Molecular Biology; Faculty of Science; University of Aarhus; Denmark, E-mail: hebasalama11@yahoo.com

The cytotoxicity of formulated nanoparticle complexes of different fatty acids (oleic, eliedic, Cis-vaccenic, Trans-vaccenic, and linolenic acids) in the presence or absence of whey protein isolate (WPI) was investigated in this study. Nanoparticle complexes formed with WPI was examined for surface tension, circular dichroism (CD), turbidity, isothermal titration calorimetry (ITC) and Cytotoxic activity. Surface tension values were decreased with adding fatty acid to WPI. This would indicate that WPI can bind greater amount of fatty acid. Cis-fatty acids such as oleic, cis-vaccenic and linolenic caused higher decrease in the surface tension of WPI nanoparticles than that of trans-fatty acids (eledic and trans-vaccenic acids). The tertiary structure of protein (WPI) was lost and changed from fold to unfold after binding with fatty acids. The changes in protein structure would be correlated to exhibit a cytotoxic activity to tumor cells. All formed protein WPI/fatty acid complexes presented lower turbidity measurements compared to the fatty acid only at same concentration. The turbidity

values for nanocomplexes of WPI/fatty acids were lower confirming higher ability in binding fatty acids. All nano complexes formed of WPI/fatty acids exhibited a cytotoxic ability as a lysis in erythrocytes. The cytotoxic activity of WPI/fatty acid complexes was almost as found with α -LA complexes. Nanocomplexes can be formed from WPI with good cytotoxic effect to tumor cells using cis-vaccenic and linolenic fatty acids comparable to oleic acid. It was a new interesting observation being that the nanocomplexes formed of WPI with fatty acids has a comparable cytotoxicity to that of α -LA and β -lg and can be used in tumor therapy.

P50. *Lentinula edodes* mushroom as a functional food: safety assessment of its consumption through biochemical measurements

Grotto, D.*¹, Bueno, D. C. R., Costa, S. R., and Ramos, G. K. A.

University of Sorocaba, São Paulo, Brazil, E-mail: denise.grotto@prof.uniso.br

Edible mushrooms are excellent food. They are rich in proteins, essential amino acids, vitamins, minerals, β -glutamic compounds, antioxidants, and also have low lipid levels. That compounds provide to the edible mushrooms healthy properties. *Lentinula edodes*, or Shiitake, is the second most consumed mushroom in the world. It has high nutritional content and has been used as an antioxidant. Moreover Shiitake has been studied in prevention - or as an adjunct - in several illnesses such as hypertension, hypercholesterolemia, diabetes, and cancer. However, besides knowing its functional properties, it is important to guarantee its safety consumption. The doses of Shiitake intake reported in experimental studies are much higher than those actually consumed. Thus, the aim of this study was to establish effective and safe doses of Shiitake intake through the measurement of biochemical parameters to verify lipid and glycemic profile and antioxidant status. Shiitake samples in nature were dehydrated in an oven at 40°C and the dried mushroom were ground in a mill. A homogeneous powder Shiitake (such as flour) was obtained. Healthy male Wistar rats were exposed via gavage daily to water or Shiitake dissolved in water in different concentrations, as follow: Group I - Control, water; Group II - Shiitake at 100 mg/kg; Group III - Shiitake at 400 mg/kg; Group IV - Shiitake at 800 mg/kg. National and International guidelines from Care and Use of Experimental Animal Resources Committee were adopted (Process Approbation: 008/2012). Serum cholesterol (total and fraction) and triglycerides, representing the lipid profile, were performed based on colorimetric determinations, using commercial kits. Serum glucose and fructosamine were assessed to evaluate glycemic profile. Those parameters were also performed by colorimetric determinations, using commercial kits. Finally blood reduced glutathione, an important endogenous antioxidant, was determined by the method of Ellman (1959). Data were analysed by one-way ANOVA and post hoc Duncan and p-values < 0.05 were considered significant. Regarding lipid profile, total cholesterol, high density lipoprotein (HDL) and triglycerides levels did not show differences among all Shiitake groups compared to

the control. Moreover there was no difference in glucose levels among all groups. On the other hand, fructosamine was significantly decreased in Shiitake at 100 and 400 mg/kg compared to Shiitake at 800 mg/kg. Fructosamine is a glycated serum protein test that evaluates long-term glycemic level. Regarding antioxidant status, Shiitake at 100 and 400 mg/kg increased reduced glutathione levels compared to control. In summary, Shiitake is a healthy nutriment and its intake demonstrated to be safe in lowest doses (100 and 400 mg/kg). At these Shiitake doses increased the antioxidant status and caused hypoglycaemic effect. Shiitake intake at 100 and 400 mg/kg represents from 0.5 to 2% of the total intake, which amount represents a reasonable intake.

P51. Consumption of *Cymbopogon citrates* alters the interplay of glucose level and blood pressure in male rats

Thaeomor A.¹, Saenthaweesuk S.², and Somparn N.³

¹School of Physiology, Institute of Science, Suranaree University of Technology, Muang Nakorn Ratchasima, Nakorn Ratchasima, 30000, Thailand

²Division of Anatomy, Department of Preclinic Science, Faculty of Medicine, Thammasat University, Rangsit Campus, Khlong Luang, Pathum Thani, 12120, Thailand

³Division of Pharmacology, Department of Preclinic Science, Faculty of Medicine, Thammasat University, Rangsit Campus, Khlong Luang, Pathum Thani, 12120, Thailand, E-mail: thawmor@yahoo.com

In adult rats, high fructose consumption induces diabetes mellitus and hypertension. The aim of this study was to determine the effects of *Cymbopogon citrates* to prevent fructose-induced hypertension in adult male rats. Male Sprague-Dawley rats were fed normal rat chow and drank water alone (C), 10% fructose in water (F), 20% *Cymbopogon citrates* extract in water (CC) or 10% fructose plus 20% *Cymbopogon citrates* extract in water (FC) for 8 weeks. At 14 weeks of age, body weight, heart rate, baroreflex sensitivity, blood urea nitrogen, plasma creatinine, plasma sodium, plasma potassium and plasma chloride were not significantly different among the four groups. Compared to C group, high fructose intake (F group) significantly increased mean arterial pressure (C, 104 ± 3 mm Hg versus F, 116 ± 4 mm Hg; p<0.05), decreased fasting blood sugar (C, 91 ± 3 mg/dl versus F, 77 ± 5 mg/dl; p<0.05) and displayed glucose intolerance. These fructose effects were all abolished by *Cymbopogon citrates* extract treatment (FC group). In addition, the *Cymbopogon citrates* extract treatment alone had no any significant effect on measured parameters. This experiment indicate that *Cymbopogon citrates* extract prevents fructose-induced hypertension in adult male rats.

P52. In vitro antiplasmodial activity of water extracts of *Breynia glauca* Craib., *Capparis micracantha* DC. And *Piper chaba* Hunter

Torudom S.¹, Hiriole W.¹, Pinmai K.¹, and Sireeratawong S.²

¹Division of Microbiology and Immunology, Department of

Preclinical Sciences, Faculty of Medicine, Thammasat University, Thailand

²Division of Pharmacology, Department of Preclinical Sciences, Faculty of Medicine, Thammasat University, Thailand, E-mail: siripentt@gmail.com

Malaria, a tropical disease caused by protozoa genus *Plasmodium*, is one of the leading fatal infectious diseases in the world. According to an increasing incidence of multidrug resistance *Plasmodium*, there is a need to find a new combat strategy that is in expensive, widely accessible especially for people in the affected area. Plants have always been considered to be a potential source of new medicine. This study aims to evaluate the in vitro antiplasmodial activity of *Breynia glauca* Craib., *Capparis micracantha* DC. And *Piper chaba* Hunter, herbs traditional used to treat malaria in Thailand. The water extracts of these Thai medicinal plants were tested for their antiplasmodial activity by assessing their ability to inhibit the uptake of [³H] hypoxanthine into the multidrug-resistant strain *Plasmodium falciparum* K1. The antiplasmodial activity was expressed by the concentration that inhibited 50% of parasite growth (IC50). *Piper chaba* Hunter was the only one of three plants that showed in vitro antiplasmodial activity (IC50=494.58 µg/ml) whereas *Breynia glauca* Craib. And *Capparis micracantha* DC. did not show antiplasmodial activity (IC50>1,000 µg/ml). Further study is needed to evaluate the in vivo antiplasmodial activity of *Piper chaba* Hunter extract.

P53. Lipid composition of the black sea macroalgae *Gelidium crinale*

Stancheva M.*., Panayotova V., Dobрева D., and Merdzhanova A.

Department of Chemistry, Medical University - Varna, Bulgaria, E-mail: mona_stancheva@abv.bg

Red alga *Gelidium crinale* is widespread in the Black Sea. Also known by the common name “tengusa”, it is traditionally consumed as food in Japan. There is limited information about lipid composition of this species from Bulgarian Black Sea coast. The aim of this study was to determine fat soluble vitamins, pigments, total lipid and fatty acid (FA) composition of *Gelidium crinale*. Fat soluble vitamins (vitamin E and D2) and pigments (β-carotene and astaxanthin) were analyzed simultaneously using HPLC/UV/FL system equipped with RP analytical column. Sample preparation procedure includes alkaline saponification, followed by liquid-liquid extraction. Red seaweeds *Gelidium crinale* contained high amounts of α-tocopherol (7.74 ± 0.89 mg.100g⁻¹ d.w.) and β-carotene (17.73 ± 0.68 mg.100g⁻¹ d.w.). Total lipids, phospholipids, sterols and sterol esters composition were determined. In addition, the specific distribution of fatty acids among the lipid classes was elucidated. Although total lipid content was generally low *Gelidium crinale* was rich in eicosapentaenoic (C20:5n3) and arachidonic acid (C20:4n6). Phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine were the main phospholipids. Cholesterol was the main sterol component. Palmitic acid (C16:0) was the major fatty acid of the phospholipids and palmitoleic acid (C16:1n7) dominated in the sterol ester fraction. High

levels of α -tocopherol correlated with high levels of polyunsaturated fatty acids (32.28% of total FA). As an antioxidant α -tocopherol preserves tissue polyunsaturated fatty acids from oxidation. High concentrations of α -tocopherol, β -carotene, polyunsaturated fatty acids and the presence of the powerful antioxidant astaxanthin demonstrate possible application of *Gelidium crinale* species as supplements for use in food, pharmaceutical industry and cosmetics.

P54. Reduction of aflatoxin M1 from bio yoghurt by *Bifidobacterium bifidum*

Sarabi M.¹, Tajalli F.^{2,3}, and Adibpour N.⁴

¹Research Institute of Food Science and Technology (RIFST), Department of Food Biotechnology, Mashhad, Iran

²Molecular Medicine Research Department, Iranian Academic Centre for Education, Culture and Research (ACECR)-Mashhad Branch, Iran

³Food Quality & Safety Research Department, Iranian Academic Centre for Education, Culture and Research (ACECR)-Mashhad Branch, Iran

⁴Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan 8415683111, Iran, E-mail: m.sarabi@rifst.ac.ir

Aflatoxin M1 (AFM1) is the hydroxylated metabolite of aflatoxin B1 and can be found in milk and subsequently in other dairy products when lactating animals are fed with contaminated feedstuffs. AFM1 is classified as the most important chronic and noninfectious foodborne risk factor. Biological detoxification is a way to avoid harmful effect of contamination of food and feed caused by aflatoxins. A few strains of lactic acid bacteria have been reported to bind AFM1 in contaminated media or in a food model and several studies have suggested that the antimutagenic and anticarcinogenic properties of probiotic bacteria can be attributed to their ability to non-covalently bind hazardous chemical compounds such as aflatoxins in the colon. The aim of this study was to investigate the ability of *Bifidobacterium bifidum* to remove AFM1 from probiotic yoghurt. In order to prepare bio yoghurt, reconstituted milk was heated at 90-95°C for 5 min and then cooled to 42°C. The pasteurized milk was contaminated with AFM1 working solution in three levels (0.1, 0.5 and 0.75 $\mu\text{g/L}$). Later than, the prepared bacterial pellets (3×10^8 cfu/g) were inoculated into the contaminated milk. All treatments were incubated at 42°C until medium acidity (amount of lactic acid) reached to 0.6%. The samples were stored at 4°C for 21 days and they were sampled each 7 days to determine non bounded AFM1. The concentration of AFM1 was determined in the supernatant fraction using ELISA kit and the results were randomly confirmed by High Performance Liquid Chromatography. There was significant difference in reduction percent of AFM1 among samples contained various concentration of AFM1. The result showed significantly higher decrease in AFM1 content at 0.1 $\mu\text{g/L}$ than other concentrations (0.5 and 0.75 $\mu\text{g/L}$) tested in this study. The binding abilities of AFM1 by *Bifidobacterium bifidum* ranged from 93.40 to 95.42%.

bifidum reduced the level of AFM1 to 95.42% at the zero time of storage period.

P55. A new approach to delay staling in bakery products: part-baking

Karaoğlu M. M.*

Food Engineering Department, Faculty of Agriculture, Ataturk University, Turkey, E-mail: mmurat@atauni.edu.tr

Bakery products such as especially bread, cake, rolls, muffin, pastry and similar products have a limited shelf life in terms of consumer quality. Physicochemical changes such as staling and firming, and microbiological spoilage such as yeast, mould and bacterial growth, opines reduce the shelf life of these products. Staling in these products results in decreased consumer acceptance and great economic losses. Because bakery products are an important part of the diets of people in many countries, the economic losses arising from staling are extremely important. For this reason, considerable attention has been focused on staling of bakery products and many researches have been made to extend shelf-life, to improve quality of baked products by retarding staling. Consumers want to buy and eat all day wide variety of baked products that is fresh. For this reason, partial baking process is currently a convenient alternative for many bakery products. Fresh bread and similar soft bakery products usually presents an appealing brownish, crispy and crunchy crust, besides a pleasant aroma and a soft an elastic crumb texture, which are an important sensory characteristic for the consumer's appreciation. Terms such as "part-baked", "part-baking", "partially baked" and "par-bake" are used to describe a method of bread manufacture which involves two stages of baking. Part-baking is a method of baked products production involving two stages of baking with an intermediate storage step. First, the proofed dough pieces are partially baked under the defined oven condition. In this stage, the minimum crust color development, the maximum moisture retention, starch gelatinization and gluten coagulation occur in partially baked bread. During the prebaking or par-baking step, the products should be baked until a structure sets and crumb formation that makes the products suitable for storage conditions. Next, the partial-baked breads are rapidly cooled, wrapped and stored until the final baking at the point of sale or consumption. Final baking process reverses certain product properties typically developed during storage, generating the characteristics of a freshly baked product. Part-baking has a market potential, because this process provides a chance to supply fresh baked product with a simple baking stage at home or at the point of sale.

P56. Neuroprotective effect of dibenzocyclooctadiene lignans from *Schisandra chinensis* fruit in primary neuronal cells

Park Sun Young¹, Seon Yeong Chae¹, Young Hun Kim¹, Young-Whan Choi², Hong-Joo Son³, and Geuntae Park⁴

¹Bio-IT Fusion Technology Research Institute, Pusan National University, Busan 609-735, Korea

²Department of Horticultural Bioscience, Pusan National University, Busan 609-735, Korea

³Department of Life Science and Environmental Biochemistry, Pusan National University, Busan 609-735, Korea

⁴Department of Nanomaterials Engineering, Pusan National University, Busan 609-735, Korea, E-mail: gtpark@pusan.ac.kr, sundeng99@pusan.ac.kr

Dibenzocyclooctadiene lignans have been isolated from *Schisandra chinensis* fruit, which has been used for centuries in Southeast Asia as a food and for its anti-inflammatory, anti-viral, and neuroprotective effects. Several investigators have shown that the major constituents of this fruit, which have biological and medicinal properties, are lignans containing dibenzocyclooctadiene skeletons with (S) or (R)-biphenyl configurations. However, the various biological and pharmacological activities of dibenzocyclooctadiene lignans remain to be elucidated. In this study, a total of 13 dibenzocyclooctadiene lignans were isolated from *Schisandra chinensis*. The aim of our study was to determine the signaling pathways associated with the anti-neuroinflammatory and neuroprotective responses induced by dibenzocyclooctadiene lignans in primary microglia and cortical neurons. We found that dibenzocyclooctadiene lignans suppress TLR 2/4 agonist-induced pro-inflammatory cytokines and chemokines, PGE₂, nitric oxide (NO), reactive oxygen species (ROS), and MMP-9 enzymatic activity through the suppression of MAPK, NF- κ B and JAK-STAT activation. We next demonstrated that dibenzocyclooctadiene lignans induced the expression of Phase II detoxifying/antioxidant enzymes and suppressed the iNOS and ROS activation induced by TLR 2/4 agonists. Interestingly, we also found that dibenzocyclooctadiene lignans induced PKA/CREB/Nrf2 activation in microglia and that activation of Phase II detoxifying/antioxidant enzymes via stimulation of the PKA/CREB/Nrf2 pathway attenuated TLR 2/4 agonist-induced iNOS and ROS activation. Furthermore, dibenzocyclooctadiene lignans protected primary cortical neurons against microglia-mediated neurotoxicity. Based on the results presented herein, we propose that their neuroprotective properties occur via the activation of PKA/CREB/Nrf2 mediated Phase II detoxifying/antioxidant enzymes.

P57. Changes in negative cognitions and nutrient intake in asymptomatic adults predicts reduction in inflammation biomarkers over multidisciplinary intervention

Maša Černelič Bizjak*, and Zala Jenko-Pražnikar

University of Primorska, Faculty of Health Sciences, Slovenia, E-mail: masa.cernelic@gmail.com

Objective: The aim of the present study was to explore interactions between food, cognition and biological processes in relation to health. Therefore, we assess dietary composition and nutrient intake, negative cognition about body image and inflammation biomarkers before and after 6-month of multidisciplinary intervention. Design: 33 overweight and obese adults completed a 6-month intervention trial to evaluate the

effects of an individual dietary programme based on individual's resting metabolic rate on anthropometry, metabolic profile, and inflammation. Results: Pearson's correlations were performed to investigate the possible associations between reductions in obesity, inflammation, dietary intakes with decrease in body dissatisfaction. Furthermore, hierarchical multiple regression analyses revealed that relative changes in obesity indicators accounted for 23% of the variation in reduction of inflammation biomarker C-reactive protein, changes in composition of diet 13% of variation and changes in negative cognition explained an additional 8% of the variation in inflammation level of CRP. Together the independent factors accounted for 44% of the variance in inflammation level of CRP. Conclusions: The important findings of the present study were that reduction in carbohydrate intake and increase in protein intake in diet, with more positive cognition about body image, significantly predicted a reduction in level of inflammation biomarker, measured with CRP. Changes in energy intake and total fat intake and physical activity did not predict reduction of inflammation.

P58. Skin Whitening activity of Kimchi extracts following extraction condition and fermentation period

Yang Ji-Hee*, Ji-Hyun Lee, Mi-Ai Lee, and Eung Soo Han

World Institute of Kimchi, Korea, E-mail: jhyang@wikim.re.kr

To develop the skin whitening agent, we investigated effects of Kimchi extracts on their extraction condition and fermentation period. Baechu Kimchi prepared on water, ethanol and methanol extraction was investigated for 56 days at 4 °C. To evaluate the activity as a whitening agent, cytotoxic effects, DPPH radical scavenging activity and inhibition of tyrosinase activity of the extracts samples were measured. In cytotoxic effects (MTT assay), all the B16F10 melanoma cells were treated with water, ethanol and methanol extracts (0 day, 28day, 56day) at various concentrations had shown a no significant difference compared with control. The DPPH radical scavenging activity of the water, ethanol and methanol extracts (0 day, 28day, 56day) were 41.46% to 50.53%, 52.12% to 63.48% and 57.93% to 69.11%, respectively. Inhibition of tyrosinase effects of all sample 67.66 to 92.77%, especially, the highest at 89.03% methanol extracts and 92.77% ethanol extracts of 1000 μ g/mL in the 56days. The results would be useful for understanding of the antioxidant and inhibition tyrosinase activity of Kimchi extracts. Therefore, Kimchi may be useful as a skin whitening material in cosmetics.

P59. Polychlorinated biphenyls and organochlorine pesticides in marine fish species from Black Sea, Bulgaria: levels, estimated human intake and risk assessment

Stancheva M.*, Georgieva S., and Makedonski L.

Department of Chemistry, Medical University - Varna, Marin Drinov 55, 9002 Varna, Bulgaria, E-mail: stanislavavn@mail.bg

Concentrations of persistent organic pollutants including polychlorinated biphenyls (PCBs), and organochlorine pesticides were

measured in muscle tissue of marine fish, collected from different parts of Bulgarian Black Sea coast in order to evaluate the status of pollution in these areas. The fish species: goby (*Neogobius cephalargoides*), sprat (*Sprattus sprattus sulinus*), horse mackerel (*Trachurus Mediterraneus ponticus*) and grey mullet (*Mugil cephalus*) were selected because of their characteristic feeding behavior and importance to human consumption in Bulgaria. Samples were collected during 2007 – 2011. Human health risk assessment was undertaken to evaluate potential risks associated with the consumption of the marine fish.

The fifteen congeners of PCBs, DDT and its metabolites DDE and DDD were determined by capillary gas chromatography system with mass spectrometry detection. The sum of the six Indicator PCBs ranged from 7.22 to 10.86 ng/g wet weight (in goby and horse mackerel, respectively) and did not exceed the European maximum limit. Concentrations for total DDTs ranged from 21.01 to 65.69 ng/g wet weight (in goby and grey mullet, respectively). Detection of DDT and its metabolites in the fish species indicated that Black Sea was predominantly contaminated by metabolites DDE and DDD. Dioxin-like PCBs are used in order to estimate the toxicity potential (TEQs) of PCB exposure. TEQs of the “dioxin-like” PCB congeners were calculated from 0.03 pg TEQ/g ww to 0.08 pg TEQ/g ww and did not exceed the limit of 3 pg TEQ/g ww, according to European Commission. The results for contaminants in fish species were used to evaluate daily intake of organochlorine compounds by human through fish consumption. The current dl-PCBs intake through consumption of fish by a standard adult male of 70 kg body weight was 5.8% of the tolerable daily intake (TDI) for these compounds, which has been established by the WHO in 1–4 pg WHO-TEQ/kg body weight/day. Estimated daily intake and risk quotient (RQ) in this study indicating that consumption of fish does not represent a human health risk.

P60. Changing and developing Turkish food law within the framework of European Union membership process and the new Turkish food law

Inan E.*, and Yuruk A.

Department of Nutrition and Dietetics, Faculty of Health Sciences, Hacettepe University, Turkey, E-mail: elif.inan@hacettepe.edu.tr

Food Safety is an important issue in today's world as a result of the food crisis in both European Union and in the world. Within the framework of EU harmonization process in Turkey, various laws are put into force and new regulations are started about food safety. In this process, Chapter 12 on Food Safety, Veterinary and Phytosanitary was opened on June 30th, 2012 for negotiations. In order to close this chapter, closing criteria was determined for Turkey and Turkey started to work on that issue. The purpose of this study is to examine the Turkish food law from past to present and to expose the priorities of the requirements of EU food legislation on the process of negotiations by examining the Turkish food law in comparison with EU food legislation. In this context, the legal framework of EU Food Safety Policy was primarily determined, the food safety history of the EU was analyzed and the organizations and institutions on food safety in the

EU were declared. Then, the history of Turkish Food Law was stated and the new Turkish Food Law was deeply analyzed. And then, Turkey-EU relations on food safety were reviewed and the opening process of this chapter was examined. In the final part of the study, by finding the differences between new Turkish Food Law and EU Food Law, the advantages and disadvantages of Turkish Food Law were detected and suggestions regarding the activities in next period were given. In order to find answers of afore-mentioned questions, the EU General Food Law, Hygiene Package Regulations, the green and white books were deeply analyzed in the website of www.europe.eu; and Turkish Food Laws were found in the website of the Ministry of Food, Agriculture and Livestock, www.tarim.gov.tr. Finally, it was stated that Turkey made significant advances in order to make production on EU standards by amending the laws and regulations on food safety. However, it is not possible to say that these efforts are sufficient. In order to have food law and food safety in EU standards, the last food law should be revised, risk assessment units like EFSA should be set up, arrangements should be made about the deterrent effect of fines, educations about HACCP should be increased and consumer education should be made for prevention of misleading information.

P61. Food taboos of Malay pregnant women attending antenatal check-up at the maternal health clinic in Kuala Lumpur

Maznorila M.*, and Chong Y. L.

International Medical University (IMU) Malaysia, E-mail: maznorila_mohamad@imu.edu.my

Introduction: Food taboos are commonly practice during pregnancy in many cultures. Certain food or activities are not allowed during pregnancy in order to safeguard the unborn child and prevent any negative outcomes to the mother. Passed on from one generation to another, most of these taboos are based on learned behavior, either acquired mostly by instruction from parents and family members or observation from close relatives and friends who practices it. Objectives: The aims of this study were to determine the prevalence and types of food taboos with its reason for avoidance and also to determine its association with weight gain status of the pregnant women. Methods: A cross sectional study was conducted among 105 Malay pregnant women ranging from age 20 to 46 years. The subjects were interviewed using a structured questionnaire to gather information on their socio-demographic and food taboo practices. Their weight gain status was assessed by measuring current weight and compare with pre-pregnancy weight obtained from their medical records. Results: This study found that 52.4% avoid more than one food due to food taboos. Pineapple and sugar cane drinks were regarded as taboo foods by more than half of the subjects (74.3% and 60.0% respectively), followed by hot foods (47.6%), carbonated drinks (39.0%) and cold foods (12.4%). Most common reason for avoiding foods were fear of abortion (95.2%), followed by fear of excessive bleeding during labour (34.3%), baby born with deformity (27.6%) and other reasons (19.0%) such as difficulty during labour, unnecessary sickness, induce vomiting and edema. Among

few socio-demographic characteristics studied, multipara women with 3 pregnancies or more shown to be associated with the practice of food taboos ($p > 0.05$). 71.6% of women who practice food taboo had an inadequate weight gained. However, there was no significant association between prevalence of food taboos with weight gain status of the pregnant women ($p < 0.05$). Conclusion: The finding of this study shown that the prevalence of food taboos was high among Malay pregnant women and the main reason for adhering to food taboo was fear of abortion. Multipara women with higher number of pregnancy (>3) seem to observed food taboo more than those with lower number of pregnancy. There was no association between the prevalence of food taboo with status of weight gained in pregnant women.

P62. Dietary intake and nutritional knowledge of pregnant mothers in comparisons with RNI and food pyramid guidelines and association with their weight gain

Normah H.¹, Chin, S. W.¹, and Azlinda H.²

¹Department of Nutrition and Dietetics, International Medical University Malaysia, Bukit Jalil, 57000, Kuala Lumpur, Malaysia

²Health Clinic Putrajaya, Ministry of Health Malaysia, 62250, Putrajaya, Malaysia, E-mail: normah_hashim@imu.edu.my

Background: Appropriate nutrients intake and acceptable gestational weight gain are very crucial during pregnancy. Low pre-pregnancy body mass index (BMI) and suboptimal gestational weight gain are directly associated with poor maternal and foetal outcomes. In order to maintain optimal health while avoiding deficiency or toxicity, pregnant women are recommended to consume according to the Recommended Nutrient Intake (RNI) and Malaysia Food Pyramid Guidelines. Based on the recommended weight gain range issued by Institute of Medicine (IOM) in 2009, optimal weight gain can be achieved by pregnant women.

Objectives: To determine the correlation between the dietary intakes of pregnant women and gestational weight gain, and also to compare the dietary intakes of pregnant women in Malaysia with the Recommended Nutrient Intake (RNI) for Malaysian 2005, and the Food Pyramid Guideline.

Methods: A cross-sectional study was conducted in a district health clinic in Malaysia in 2011. A total of 60 pregnant women who were in their third trimester of pregnancy were recruited by convenience sampling. Anthropometric assessment, self-administered questionnaire and 2-day dietary recall were used for data collection. Due to attrition, the data from only 55 pregnant women were included in the analysis.

Results: Mean pre-pregnancy BMI of the subjects was 23.2kg/m². Majority (41%) of the pregnant women did not meet the RNI levels with the exception of protein and the number of servings as recommended by Food Pyramid Guidelines. A total of 65.5% of the pregnant women did not gain weight within the recommended range. The dietary intake

of pregnant women was not correlated with the gestational weight gain ($p > 0.05$). Conclusion: Nutritional guidance should be considered among the pregnant women to promote dietary intake in meeting the RNI and Food Pyramid guidelines.

P63. Vitamin A, D3 and E contents and polyunsaturated fatty acid composition of turbot (*Psetta maxima*), red mullet (*Mullus barbatus*) and goby (*Neogobius rattan*) from Bulgarian Black Sea

Dobрева D.¹, Galunska B.², Merdzhanova A.¹, and Stancheva M.¹

¹Department of Chemistry, Medical University of Varna, 55 Marin Drinov Str., 9002 Varna, Bulgaria ²Department of Pharmaceutical Sciences, Medical University of Varna, 55 Marin Drinov Str., 9002 Varna, Bulgaria, E-mail: didobрева@gmail.com

Introduction: Fish have always been seen as a food necessary for good health being a rich source of fat soluble vitamins as well as n-3 and n-6 long chain polyunsaturated fatty acids (PUFA). There is a lack of information for fat soluble vitamins and PUFA contents of traditionally consumed Black Sea fish species from Bulgarian waters.

Aim: To determine and compare the contents of retinol, cholecalciferol and alpha-tocopherol, and PUFA composition in some commercially important fish species of Bulgarian Black sea coast.

Material and methods: Edible tissue of three fishes - turbot (*Psetta maxima*), red mullet (*Mullus barbatus*) and goby (*Neogobius rattan*), were used for evaluation the fat soluble vitamins and PUFA content. All-trans-retinol (vitamin A), cholecalciferol (vitamin D3) and alpha-tocopherol (vitamin E) were analyzed simultaneously using HPLC/UV/FL, equipped with RP analytical column ODS2 Hypersil™ 250 x 4,6 mm, 5u, with mobile phase composed of 97:3=MeOH:H₂O. The sample preparation procedure includes alkaline saponification, followed by liquid-liquid extraction. The fatty acid (FA) composition was analyzed by Gas Chromatography with MS detector. Lipid extraction was done according to the Bligh and Dyer method.

Results: The content of fat soluble vitamins in fresh edible fish tissue were in the range: 0.9 ± 0.1 µg/100g – 15.7 ± 1.2 µg/100g for all-trans-retinol; 4.6 ± 0.5 µg/100g–4.9 ± 0.3 µg/100g for cholecalciferol, and 308.0 ± 23.4 µg/100g- 2836.8 ± 96.0 µg/100g for alpha-tocopherol. The PUFA content ranged from 0.41 g/100g (turbot) to 1.065 g/100g (red mullet). n- 3 PUFA contents were higher compared to n-6 FAs for all analyzed species. The DHA level was always found higher than that of EPA. The sum EPA+DHA ranged from 0.22 ± 0.03 g/100g (turbot) to 0.396 ± 0.020g/100g (red mullet) due to its higher lipid content.

Conclusion: The present work indicates that 100 g portion of studied Black Sea fish species meets the recommended daily intake for cholecalciferol and EPA+DHA.

P64. High fat diets elevate excretion of bloody stool in mice without receiving colitis-inducing chemicals

Yoshida S.^{*}, Suidasari S., and Kato N.

Hiroshima University, Japan, E-mail: m131902@hiroshima-u.ac.jp

Administration of colitis-inducing chemicals such as dextran sulfate (DSS) and 2, 4, 6-trinitrobenzene sulfonic acid (TNBS) can induce bloody stool in mice. Recently, we have serendipitously found that consumption of 30% beef tallow diet for several weeks caused excretion of blood red-color substance in a small part of feces of male ICR mice without receiving colitis-inducing chemicals. Western blotting analysis revealed that the substance is hemoglobin, implying fecal excretion of blood stool. Subsequent study has indicated that 5% beef tallow diet (low-fat diet) caused a significant reduction in the excretion of bloody stool compared with 30% beef fat diet (high-fat diet) ($p < 0.05$). Experiment was conducted to compare the excretion of fecal bloody stool in some mouse strains, including male ICR, ddY and BALB/c strain mice. The results showed that the ddY mice had highest excretion of bloody stool, while the BALB/c mice had lowest excretion. Further study was performed to examine the effect of 30% beef tallow diet, 30% lard diet and 5% beef tallow diet on the bloody stool and gene expression in colon of ddY mice. The results showed that 5% beef tallow diet both markedly reduced excretion of bloody stool compared with 30% beef tallow diet and 30% lard diet ($p < 0.05$), with no difference between 30% beef tallow and 30% lard diet. Real-time PCR analysis showed no positive association between the gene expression of iNOS and COX-2 and the excretion of bloody stool. These results suggest that high-fat diet can elevate fecal bloody stool of mice through mechanisms not involving inflammation.

P65. Comparison of a trained sensory panel and an electronic tongue in the assessment of hot taste in Kimchi

Mi-Ai Lee*, Kyung-Lae Cho, and Ji-Hee Yang

World Institute of Kimchi, Korea, E-mail: leemae@wikim.re.kr

The hot taste elicited by Kimchi is a well-known issue for their acceptability by consumers and therefore incorporation into foods. The traditional method of assessment of taste in foods is by sensory analysis but this can be problematic due to the overall unpleasantness of the samples. Thus, there is a growing interest into the use of electronic tongues (e-tongues) as an alternative method to quantify the hot taste in such samples. In the present study the response of the e-tongue to the standard hot taste agent capsaicinoid contents and a rage of red pepper were compared to that of a trained sensory panel. There was an excellent correlation between the predicted concentration assigned by the e-tongue and the capsaicinoid contents resulting in a high R2 value of 0.94. Also, a PLS regression was constructed using the hot taste intensity values as the independent variables. The R2 value from the regression was 0.92, showing an excellent correlation between the e-tongue and the sensory panel. This study exhibits potential for the e-tongue to be used in hot taste screening in Kimchi.

P66. Optimization of high hydrostatic pressure enzymatic hydrolysis from flatfish byproducts using response surface methodology

Kim Y. S.*

Korea Food Research Institute, E-mail: kimyus@kfri.re.kr

High hydrostatic pressure enzymatic hydrolysis was applied to develop the functional food materials from flatfish byproducts. Flatfish protein hydrolysate (FPH) was produced with 100 MPa pressure and ProtamexTM. Proximate composition, cholesterol, minerals, and vitamin content of FPH were determined. Crude protein content, which could be converted to bioactive peptide, was 20.4%. The degree of hydrolysis (DH) of FPH was mainly depended on the process parameters such as temperature, s/e ratio and pH. Response surface methodology (RSM) was applied to optimize FPH production conditions and results showed that parameters for optimal production were S/E ratio of 50.65 (w/w), pH 6.898 and temperature of 56.97. Under the optimal condition, the DH of FPH was predicted as 32.09%. To separate FPH fractions with more bioavailability, a series of ultrafiltration through membrane with molecular weight cut-off (MWCO) 10, 5 and 3 kDa was applied. Permeates were analyzed for their amino-acid compositions and tested for their functionality. From the results, high hydrostatic pressure enzymatic hydrolysis was effective processing method to produce FPH as functional food materials.

P67. Wild sage seed oil: Novel herbal source of α -Linolenic Acid

Bostan A.*

Food Nanotechnology Department, Research Institute of Food Science & Technology, Mashhad, Iran, E-mail: arambostan@yahoo.com

In this study, the oil yield, fatty acid (FA), sterol, tocopherol and phenolic composition and the physicochemical and quality characteristics of *Salvia macrosiphon* seed oil were determined. The main fatty acids ranked in the following order of abundance: α -Linolenic Acid > Linoleic Acid > Oleic Acid. The n3 /n6 FA ratio and polyunsaturated FA/ Saturated FA ratio were 1.6 and 7.23, respectively. The FA composition revealed that the oil had great potential to use as nutritional dietary component and also was very susceptible to oxidation. The main sterol was β -sitosterol which comprised 52.75% of total sterol content. The two next major components were Δ -5-avenasterol and campesterol which comprised 22.32 and 12.60%, respectively. β and γ tocopherols were the major tocopherol isomers. 20 phenolic compound was identified and tyrosol was the major one. The physicochemical properties such as Iodine (168.7 gI2/100g oil), Acidic (0.5) and Peroxide (1.9) Value, color (L: 59.22, a: -5.09, b: 22.94) and refractive index (1.4723) were also determined. Total phenol, tocopherol and sterol content were 165.22 mg GA/kg oil, 629.59 and 2540.6 ppm. In addition Oxidative Stability Index was 3.94 h.

P68. Modern UHPLC stationary phases for separation of important antioxidants

Červinková B.^{1,2*}, Kujovská Krčmová L.^{1,2}, Solichová D.², and Solich P.¹

¹Department of Analytical Chemistry, Charles University in Prague Faculty of Pharmacy, Heyrovského 1203, 500 05, Hradec Králové, Czech Republic

²3rd Internal Gerontometabolic Clinic, University Hospital, Sokolská 581, 500 05, Hradec Králové, Czech Republic, E-mail: barbara.cervinkova@gmail.com

Free radicals are highly reactive species having the ability to damage vital biological structures, this fact could cause various diseases, including cancer. Free radicals are scavenged by antioxidants, especially by vitamin E. Association between levels of vitamin E isomers and cancer risk is highly possible, particularly in population with inadequate vitamin E nutrition. Vitamin E forms two groups of chemical entities - tocopherols and tocotrienols, each of them consists of four isoforms - α , β , γ , δ . In last decades mainly α and γ tocopherols were studied. Anti-inflammation and proliferative effect of α tocopherol in prevention and treatment of cancer was described. The effect of α and γ tocopherols were compared and it was shown that γ tocopherol had generally better behaviour in tumor growth inhibition. Moreover, effect of δ tocopherol on apoptosis of cancer cells was studied and results showed that it has stronger influence on inhibition of tumor growth than other tocopherols. In this work, the novel method for analysis of tocopherol isomers was developed. Modern Ultra High Performance Liquid Chromatography (UHPLC) technique was selected for separation of α , β , γ , δ tocopherols and various modern UHPLC stationary phases were tested to achieve the best results: a) 2nd generation of monolith (Chromolith® High Resolution RP-18e, 100 x 4.6 mm, Merck, Germany) with improvement of peak shape, b) sub-2- μ m pentafluorophenyl (PFP) phase (Aquity UPLC (CSH) PFP, 1.7 μ m, 100 x 2.1 mm, Waters, USA) with high efficient separation of wide range of compounds, c) Aquity UPLC Beh Amide in HILIC mode (1.7 μ m, 150 x 3 mm, Waters, USA), d) fused core particle phase Kinetex (C18, 1.7 μ m, 150 x 3 mm; 1.7 μ m 100 x 3 mm, Phenomenex, USA) based on porous particles with solid core, which allow very rapid separations

Development of this new chromatographic method for the determination of tocopherol isomers might help to extend the knowledge of the role of each isomer in various metabolic pathways.

P69. Effect of *Annona squamosa* L. aqueous leaves extract on the liver of streptozotocin-induced diabetic rats

Rabintossaporn P.¹, Saenthaweek S.¹, Thuppia A.¹, Sireeratawong S.¹, and Ingkaninan K.²

¹Department of Preclinical Science, Faculty of Medicine, Thammasat University, Rangsit Campus, Klong Luang, Pathum Thani, 12120 Thailand

²Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Science, Naresuan University, Muang, Pitsanuloke, 65000 Thailand, E-mail: PornrutRabintossaporn@gmail.com

The present study aimed to investigate histological characters of livers in normal and streptozotocin (STZ)-induced diabetic rats after receiving various doses of *Annona squamosa* L. aqueous leave extract. Diabetes was induced by intraperitoneal injection of STZ (45 mg/kg). After that the diabetic rats were orally administered the vehicle, glibenclamide and leaves extract (125, 250 and 500 mg/kg) for 12 days. Their liver tissues were collected for histological examination. The histological study of liver showed that control diabetic rats possessed many hepatocytes with shrunken nuclei, vacuolated granular cytoplasm, poorly defined cell boundaries and dilated sinusoids. The diabetic rats received the extract revealed some improvements of liver tissue in dose dependent manner in which hepatocytes possessed regular size of nuclei, well defined cell boundaries, reduced vacuoles and granulated characters as well as narrowed sinusoids when compared with those of control diabetic rats. This present data suggests that the aqueous leaves extract of *Annona squamosa* L. could improve the histological appearances of the livers of diabetic rats.

P70. Leucine and lysine intakes are highly associated with serum adiponectin levels in asymptomatic adults: Amino acid intervention to ameliorate inflammation?

Ana Petelin¹, Nina Mohorko², Tadeja Jakus¹, and Zala Jenko Pražnikar¹

¹University of Primorska, Faculty of Health Sciences, Slovenia, and ²University of Primorska, Science and Research Centre of Koper, Slovenia, E-mail: ana.petelin@fvz.upr.si

Adiponectin has anti-inflammatory, anti-atherosclerotic properties and it is involved in metabolic regulation, especially by sensitising tissues for insulin. Adherence to the Mediterranean diet has been shown to have a positive effect on adiponectin levels. Therefore, our aim was to assess asymptomatic general population of the Mediterranean part of Slovenia and to evaluate the correlations between serum adiponectin levels and diet components, metabolic syndrome components and inflammatory markers. Healthy adults aged 25-49 participated in the cross sectional study. All participants underwent standard anthropomorphological measurements of body composition, aerobic capabilities assessment, dietary intake evaluation, and fasting serological measurements of adiponectin, visfatin, interleukin-6, tumor necrosis factor- α , glucose, insulin, triacylglycerol's, total cholesterol, low- and high-density lipoprotein cholesterol and C-reactive protein (CRP). Linear correlation analyses were used to examine serum adiponectin levels on diet components and all risk factors. A positive correlation between the ratio of leucine: lysine intake and adiponectin and a negative correlation between adiponectin and already 2 components of metabolic syndrome was found. Moreover, we confirmed the negative correlations between adiponectin and CRP, but found a negative correlation between adiponectin and visfatin. Furthermore, hierarchical multiple regression analyses confirmed that besides lower HOMA-IR, CRP, homocysteine and body mass index, also higher ratio of leucine:lysine contribute to the prediction of higher level of adiponectin. Our results indicate that high leucine:lysine intake

ratio may promote the increase in serum adiponectin levels in clinically asymptomatic adults of Mediterranean part of Slovenia. On the other hand, lower serum adiponectin levels are due to obesity, inflammation and presence of already 2 components of metabolic syndrome.

P71. Neuroprotective effects in HT22 cells and memory enhancement by C-Kyungohkgo on scopolamine-induced memory deficit rats

Choi I. Y.¹, Cho H. W.¹, Kim J. H.¹, Pang E. M.¹, Cho J. H.¹, and Jung S. Y.²

¹Jeonnam Development Institute for Traditional Korean Medicine, Jangheung-gun, Jeonnam, Republic of Korea

²Clinical Trial Center for Functional Foods, Chonbuk National University Hospital, Jeonju, Republic of Korea, E-mail: ciy76@naver.com

The impairment of learning and loss of memory are the main symptoms of Alzheimer's disease (AD). Kyungohkgo (KOG) has been used as a medicine for anti-fatigue and anti-aging in Korea and China. But nowadays it has been used as functional food because of its safety and efficacy. We modified the original KOG and tested its effects. CKOG (C-Kyungohkgo) is composed of Citrus unshiu immature peel, Honey, Rehmannia root, Ginseng, and Hoelen. Herein, we evaluated neuroprotective effects of hydrogen peroxide-stimulated HT22 cells and the memory enhancing effect of CKOG on the rats with scopolamine-induced memory deficit, by using morris water maze tool. The CKOG prevented HT22 cytotoxicity from the hydrogen peroxide-induced injury by inhibiting the caspase-3 activation in in vitro model. For in vivo model, when CKOG was administered in rats via oral route once a day for 20 days, we found that the time taken to arrive at the island under the water significantly decreased compared to the rats treated with scopolamine. Moreover, the CKOG treated rats showed memory improvement in the passive avoidance test. In conclusion, these results suggest that the CKOG could prevent the cell death by imposing oxidative stress in HT22 cells and ameliorating scopolamine-induced memory impairments in rats.

P72. Analysis of 10 systemic pesticide residues in various baby foods using liquid chromatography-tandem mass spectrometry

Shim J. H.^{1*}, Yang A.¹, Abd El-Aty A. M.^{1,2}, Park J. H.¹, Goudah A.², Rahman M. M.¹, and Do J. A.³

¹Biotechnology Research Institute, College of Agriculture and Life Science, Chonnam National University, 77 Yongbong-ro, Buk-gu, Gwangju 500-757, Republic of Korea

²Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211-Giza, Egypt, and ³Department of Food & Cooking Science, Sunchon National University, 413 Jungangno Sunchon, Jeollanam-do 540-742, Republic of Korea, E-mail: jhshim@jnu.ac.kr

Ten systemic pesticides, including methomyl, thiamethoxam, acetamiprid, carbofuran, fosthiazate, metalaxyl, azoxystrobin, diethofencarb, propiconazole, and difenoconazole were detected in 13 baby foods (cereals, boiled potatoes, fruits, and milk) using the QuEChERS method for sample preparation and liquid chromatography tandem mass spectrometry for analysis. The matrix-matched calibration curves showed good linearity with determination coefficients (R^2)>0.992. The limits of detection and quantitation were 0.0015–0.003 and 0.005–0.01 mg/kg, respectively. Mean recoveries of three different concentrations ranged from 69.2–127.1% with relative standard deviations<20. The method was successfully applied to 13 actual samples collected from a local market, and none of the samples contained pesticide residues. In sum, this method is suitable to accurately identify and quantify systemic pesticides with matrix-matched standards in various baby foods.

P73. Effect of processing methods on the physico-chemical properties of pomegranate juice (*Punica granatum L.*) extracted from two Egyptian varieties

Zaki S.¹, Ismail F.¹, Abdelatif S.², and Abd El-Mohsen N.²

¹Food Technology Department, Faculty of Agriculture, Cairo University, Egypt, and ²Hort. Crop. Proc. Res. Dept. Food Technol. Institute, Agricultural Res. Center, Egypt, E-mail: drshafikazaki@gmail.com

Pomegranate fruits are widely consumed fresh or processed into juice, jams, syrup and sauce. The edible portion of fruit consists of about 75-85% juice. The present investigation was carried out to study the effect of processing on the physico-chemical properties of pomegranate juice extracted from two Egyptian varieties (Manfalouty and Wardey) using pressing or blending. Two clarification methods (adding 0.2% gelatin or centrifugation 4000 rpm/15 min) and pasteurization (20 min in boiling water) were applied. The obtained results showed no significant differences between the effects of clarification and pasteurization methods, and between extraction juice methods within the same clarification and pasteurization methods of pomegranate varieties on the physico-chemical of pomegranate juice, on the total soluble solids, pH, total acidity, total sugars and maturity indexes of tested juices. Concerning turbidity of the obtained juice, data indicated that there were significant differences between the two varieties of pomegranate and between the two extraction methods of juice (from whole fruit and / or seeds) as well as between the clarification and pasteurization methods. The clarified pomegranate juices had much lower levels of turbidity compared to that of control sample. Generally, results indicated that the best juice was found for the juice obtained by adding 0.2% gelatin (juices of two varieties). Data showed that the pasteurization of these samples gave a high degree of clarification. Results also demonstrated that clarification and pasteurization methods caused some changes in fresh juice properties obtained from two Egyptian pomegranate cultivars. The two methods of clarification decreased tannins content of pomegranate juice samples, and the decrease were significant between the two varieties, clarification

methods, and extraction methods. Both treatments caused noticeable decreases in the contents of ascorbic acid, pectin, polyphenols, and anthocyanins as well as antioxidant activity.

P74. Determination of kresoxim-methyl and its thermolabile metabolites in pear utilizing pepper leaf matrix as a protectant using gas chromatography

Shim J. H.¹, Rahman M. M.¹, Abd El-Aty A. M.^{1,2}, Choi J. H.¹, and Kim S. W.¹

¹Biotechnology Research Institute, College of Agriculture and Life Sciences, Chonnam National University, 300 Yongbong-dong, Buk-gu, Gwangju 500-757, Republic of Korea

²Department of Pharmacology, Faculty of Veterinary Medicine, Cairz University, 12211-Giza, Egypt, E-mail: jhshim@jnu.ac.kr

Kresoxim-methyl and its two thermolabile metabolites BF 490-2 and BF 490-9 were analyzed in pear using a pepper leaf matrix protection to maintain the metabolites inside the gas chromatography system. Samples were extracted with a mixture of ethyl acetate and n-hexane (1:1, v/v) and purified and/or separated using a solid phase extraction procedure. The pepper leaf matrix was added and optimized with cleaned pear extract to enhance metabolite sensitivity. Matrix matched calibration was used for kresoxim-methyl in the pear matrix and for metabolites in the pear mixed with pepper leaf matrix. Good linearity was obtained for all analytes with a coefficient of determination, $r^2 \geq 0.992$. Limits of detection (LOD) and quantification (LOQ) were 0.006 and 0.02 mg.kg⁻¹ and 0.02 and 0.065 mg kg⁻¹ for kresoxim-methyl and the metabolites, respectively. Recoveries were carried out at two concentration levels and were 85.6–97.9% with a relative standard deviation <2.5%. The method was successfully applied to field incurred pear samples, and only kresoxim-methyl was detected at a concentration of 0.03 mg.kg⁻¹.

P75. Association between serum bilirubin levels and mets components in overweight middle-aged population

Jurdana M.¹, Petelin A.¹, Žiberna L.², and Jenko Pražnikar Z.¹

¹Faculty of Health Sciences, University of Primorska, Izola, Slovenia

²Department of Life Sciences, University of Trieste, Italy, E-mail: mihaela.jurdana@fvz.upr.si

It has been suggested that bilirubin is not only a waste end-product but also an antioxidant that may protect against diseases associated with oxidative stress. Low levels of bilirubin have recently been associated with some pathological conditions; cardiovascular disease (CVD), coronary heart disease, cancer mortality, as well as a number of inflammatory, autoimmune and degenerative diseases. Bilirubin serum levels are determined, by genetic factors, and by external factors like dietary status, fasting, age, fitness level and general health status. Overweight status can be considered as a pre-disease state that can compromise antioxidant defense system on a long term basis. Recently we showed that serum bilirubin levels were already altered in overweight asymptomatic apparently healthy middle-aged individuals

before full development of the metabolic syndrome (MetS)¹. Healthy adults aged 25-45 participated in our study. We showed that serum bilirubin levels were decreased in overweight healthy individuals in both sexes, and were negatively associated with abdominal obesity, insulin resistance, fasting glucose, fasting insulin, fasting triglycerides, total cholesterol, low-density lipoprotein cholesterol, fatty acid consumption and C-reactive protein levels; and positively associated with aerobic body capabilities, vitamin C intake and serum folic acid concentration. Therefore, our findings suggest that serum bilirubin levels have the potential to be used as an early biomarker for indicating asymptomatic individuals at increased risk of developing metabolic syndrome and other chronic oxidative stress-induced pathologies.

P76. Acyl serotonin endocannabinoids protect against glutamate-induced oxidative damage of hippocampal cells via Nrf2 induction

Kim M. R.¹, Jin M. C.², Kim H. J.¹, Shin S. K.¹, and Min A. Y.¹

¹Department of Food & Nutrition, Chungnam National University, Korea

²College of Pharmacy, Chungnam National University, Korea, E-mail: mrkim@cnu.ac.kr

Some endocannabinoids have been known to express anti-inflammatory and antioxidant actions independent of cannabinoid receptors. In this respect, we investigated whether N-acyl serotonin might prevent against glutamate-induced oxidative cytotoxicity in hippocampal cells (HT-22 cells), and attempted to elucidate the mechanism for their neuroprotective action. N-acyl serotonin (5-HT) with acetyl, palmitoyl, stearoyl, arachidonoyl or docosahexaenoyl chain expressed a remarkable protective effect on glutamate-induced cytotoxicity. Additionally, glutamate-induced oxidative stress, represented by the increase of ROS and the reduction of glutathione (GSH) level, was prevented markedly by N-acyl 5-HTs at submicromolar levels. Further, N-palmitoyl 5-HT, the most cytoprotective, enhanced antioxidant defense by up-regulating the expression of antioxidant enzymes such as HO-1, GCLC and NQO-1. Consistent with this, N-palmitoyl 5-HT stimulated nuclear translocation of Nrf2, and this effect was remarkably suppressed by inhibitors of PI3K, PDK-1, Akt or p-38 MAPK. In support of this, glutamate-induced cytotoxicity was prevented remarkably by an inhibitor (U0126) of MEK/ERK1/2 pathway. Collectively, it is suggested that N-acyl 5-HTs may attenuate glutamate-induced cytotoxicity via the activation of PI3K/PDK/Akt and p-38 MAPK-dependent Nrf2.

P77. Recent developments of the Czech food composition database

Machačková M.¹, Holasova M.², and Maskova E.²

¹Antonin Svehla Library, Institute of Agricultural Economics and Information, Prague, Czech Republic

²Food Research Institute Prague, Czech Republic, E-mail: machackova.marie@uzei.cz

The main objective of the Czech Food Composition Database (FCD) Project is to develop and maintain a National FCD with a structure compatible with other European FCDs and to disseminate data using an online application (www.NutriDatabase.cz). Sources of data comprise data generated by analysis of foods, data from literature (e.g. food composition tables), calculated and estimated values. In the recent period, significant development of the Czech FCD has been achieved. The database has been enlarged by a set of data for 113 foods generated by chemical analysis of raw materials that are used in the food industry (poultry, pork and beef production meat, semi-products of milk processing, fruits, vegetables, cereals, pasta, rice, oils, legumes, fish). Data have been documented by the EuroFIR (European Food Information Resource www.eurofir.eu) harmonized procedure. Traceability of data sources has been assured by documenting references for each value included into the database. New functionalities have been introduced in to the on-line version of the Czech FCDB: A new additional display for simplified presentation of nutritional data has been introduced into the food record. The set of nutrients in this basic record comprises mandatory nutrients according the Regulation 1169/2011/EC (energy value, content of fat, saturated fatty acids, saccharides, total sugars, proteins, salt). Newly introduced registration of users allows them to choose foods into a clipboard and to create their own output from the database within the range of the mandatory nutrients. The advanced search module has been extended by a new search on front-of-package logo. The system has been tested successfully with selected foods bearing the logo of the Choices Programme. The newly created mobile version of the Czech FCDB web has been introduced to enable users to browse the database wherever they are. Collaboration with the EuroFIR makes possible dissemination of Czech data within the EuroFIR FoodEXplorer – a unique interface for simultaneous searches in 28 national food composition datasets. Cooperation with food producers and extending the set of covered Czech foods is the challenge for the future.

P78. Apple pomace extract improves muscle strength and mitochondrial function in skeletal muscle

Jeong J. W.*, Choi I. D., Park S. D., Lee J. H., Ahn Y. T., and Sim J. H.

Korea Yakult R&BD Center, E-mail: woongshow@re.yakult.co.kr

Ursolic acid is a lipophilic pentacyclic triterpenoid that contributes to the waxy coats on apples, other fruits, and many herbs, including some folkloric herbal medicines for diabetes. Recent studies showed that ursolic acid was proposed as a therapeutic compound in various conditions such as Alzheimer's disease, cancer, diabetes and muscle atrophy. In this study, we evaluated the hypothesis that apple pomace extract (APE, ursolic acid=27.7 mg/g) might increase skeletal muscle strength and mass in a mouse model. Muscle atrophy is one of the common symptoms in aging, several illnesses like cancer and diabetes. To develop a potential therapy for muscle atrophy, the effect of APE on the expression of biomarkers associated with muscle atrophy was investigated. As a result, APE reduced the expression of muscle atrophy

related with mRNA while it activated Akt and AMPK pathway that can enhance muscle hypertrophy and mitochondrial function in C2C12 myotubes. We also examined the effect of APE, fed a diet in mice, for muscle strength enhancement. As a result, APE increased skeletal muscle mass, fast and slow muscle fiber size, grip strength and treadmill exercise capacity. These results suggest that APE might be used for the development of functional foods in muscle atrophy through dietary intake.

P79. Eating behavior in obese 6 to 12 years children, attending the Hospital Civil de Guadalajara “Dr. Juan I. Menchaca”

De la Fuente A.*, Romero E., Hunot C., Vásquez E., and Mariscal A.

Institute of Human Nutrition, Health Sciences University Centre, University of Guadalajara, Mexico, E-mail: amparodelafuente@hotmail.com

Introduction: In recent years studies have evaluated the possible association between eating behavior traits in children and the presence of obesity. There are different tools for assessing appetitive traits (AT); one of the most used is the Children Eating Behavior Questionnaire (CEBQ). The objective was to identify the relationship between AT and the presence of obesity in children aged 6-12 years attending the Hospital Civil de Guadalajara “Dr. Juan I. Menchaca” Mexico.

Methods: A case-control study included 92 normal weight children and 61 obese children (exogenous obesity) (BMI>2.0 SD). With parental consent height and weight were measured and the CEBQ questionnaire was administered to mothers to assess appetitive traits. The CEBQ consists of 8 constructs that assess different dimensions of appetite. Constructs mean scores were calculated and compared between the two groups with the Student t test for two independent samples. The project was authorized by the Ethics and Research Committees of the Hospital.

Results: We recruited 153 children, 61 cases and 92 controls. With mean age of 8.6 ± 1.6 , showed no mean differences between groups. Mean scores for 7/8 appetitive traits were different between cases and controls ($p < 0.01$).

Conclusion: There are differences in eating behavior traits between obese and normal weight children. These traits could contribute to the development or persistence of obesity in children.

P80. DPPH scavenging activities of selected flavonoids and their mixtures

Brachos D.*

Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University in Bratislava, Slovakia, E-mail: sbistras12@hotmail.com

Flavonoids are secondary plant metabolites, the most common phenolics compounds and are widely distributed in plants. Dietary

sources of flavonoids are parsley, blueberries, black tea, wine and many other plants or fruits. Flavonoids possess antioxidative, anti-inflammatory, anti-cancer, antibacterial and many other activities. The aim of this study was to investigate the DPPH scavenging activities of selected flavonoids (quercetin, galangin and luteolin) and their mixtures. These ones consisted of two or three substances in equimolar ratio. We tried to determine possible synergistic or antagonistic effects. According to the results of the present study, quercetin has the highest scavenging activity compared to the two other flavonoids. Moreover, the combination of luteolin and quercetin showed to have the highest activity after quercetin, but having only nearly additive effect. From the point of view of combination index the triple combination has the best value but still appearing to have nearly additive effect. No synergy was detected between any of the flavonoid combinations. DPPH scavenging activities of mentioned three flavonoids equimolar mixtures are described for the first time to our best knowledge.

Acknowledgements: Taking this opportunity, I would like to express my gratitude to my prof. Ing. Milan Nagy, CSc for his guidance, advices and time to help me prepare my thesis assignment and the department of pharmacognosy and botany for the university facilities given. Not to forget, I would like to thank my parents for their strong support and motivation all this time.

P81. Dietary protein for athletes

Vesely A.¹, Hadrova S.¹, and Gazarkova E.²

¹Agroresearch Rapotin Ltd., Výzkumníku 267, Rapotin, Czech Republic

²Masaryk University Brno, Czech Republic, E-mail: ales.vesely@vuchs.cz

Introduction: Protein recommendations are based on nitrogen balance assessment. In athletes, protein and amino acid ingestion is considered essential to sport performance. RDA for protein in healthy adults is 0.8 g/kg body weight per day. This amount of protein intake may be appropriate for non-exercising individuals, but it is likely not sufficient to offset oxidation of protein/amino acids during exercise or for repair of exercise induced muscle damage (Tarnopolsky, 2004). There are many factors need to be considered when we want to find an optimal amount of dietary protein for exercising individuals. The intake of protein and amino acid supplements depends on a few factors such as a type of exercise training, protein quality, carbohydrate and energy intake, a type of dietary supplements and also timing of the protein intake (Lemon, 2000). The main goal of this study was to verify amino acids amounts declared by the manufacturer and to determine the profile of essential amino acids in selected sport products. For analysis, three sport products were selected such as Extreme whey protein (Vanille), Extreme muscle gainer (Strawberry) and Active soja plus (Chocolate).

Methods: After acid and oxidative-acid hydrolysis, all hydrolysates were separated in the Automatic aminoanalyser AAA 400 (Ingos,

Ltd., Czech Republic) using Na citrate buffer system and quantified by reaction with ninhydrin.

Results: Preliminary results of this study are presented in the Table 1. Table 1: Amino acids and crude protein amounts in the sport products (in g/100 g of product)

Sport product	Declared content of protein	Determined crude protein*	Determined sum of total AA**	Determined sum of essential AA**
Extreme whey protein, Vanille	80	80.2	77.9	38.4
Extreme muscle gainer, Strawberry	85	85.9	82.3	38.3
Active soja plus, Chocolate	80	82.6	68.2	31.2

From the assesment of results, the content of determined crude protein is slightly higher in comparison to declared content of protein, whereas variability is in range from 0.25 to 3.25%. There is difference between declared protein content and sum of total amino acids in analysed sport product Active soja plus with flavour Chocolate. In comparison to this one sport product, differences between amounts of amino acids declared by the manufacturer and analysed amino acids amount in Extreme whey protein and Extreme muscle gainer, are only minor.

P82. Change of body composition by branched-chain amino acid ingestion immediately after the training in basketball player

Watanabe T.¹, Sato T.², Takani H.³, and Igawa S.⁴

¹Hachinohe Gakuin University, Japan

²Jissen Women's University, Japan

³Graduate School of Health and Sport Science Nippon Sport Science University, Japan

⁴Nippon Sport Science University, Japan, E-mail: nabetaka@hachinohe-u.ac.jp

It was known that branched-chain amino acid (BCAA) was related to promoting protein synthesis in skeletal muscle, and to reduced delayed onset muscle soreness after exercise. The purpose of this study was investigated to the influence that BCAA intake just after the training gives to the body composition of the basketball player at early stage of the season. Four basketball players who belongs to Tohoku collegiate basketball federation in japan (mean \pm SD; age: 20.1 \pm 0.9; height 171.0 \pm 7.4 cm; weight 62.3 \pm 7.5 kg; percent body fat 13.3 \pm 2.8%; body mass index 20.9 \pm 1.6 kg/m²) participated in this study. Subject was performed resistance training at three times a week, for 6 weeks. BCAA (Leucin: Isoleucine: Valin=2: 1: 1) was taken immediately after exercise.

Body composition was measured by multi frequency segmental body composition monitor (MC-190 TANITA, Japan) on before and after training for 6 week to determine the amount of change, and compared with the 12 weeks of same training which performed one year ago. Statistical analysis was used one way repeated analysis of variance ($p < 0.05$). Result of this study, there was no significant difference of body weight, fat mass, percent body fat and body mass index. Muscle mass and basal metabolic rate were significantly increased by intake of BCAA. Therefore, it was suggested that effect of BCAA ingestion immediately after the training to increase the muscle mass and basal metabolism.

P83. New dietary fibre content results of cereal, vegetable and fruit samples

Pastell H*, and Putkonen T.

Finnish Food Safety Authority Evira, Mustialankatu 3, FI-00790 Helsinki, Finland, E-mail: helena.pastell@evira.fi

The definition of dietary fibre has recently been revised. According to Codex Alimentarius dietary fibre consists of carbohydrate polymers with ≥ 10 monomeric units, which are not hydrolyzed in the small intestine of humans. The polysaccharides, that are classified as dietary fibre occur naturally in food, or can be obtained from food raw material, or are synthetic and have shown health benefits. Also oligosaccharides with 3 to 9 monomeric units are included as dietary fibre. After establishing the new definition, there was a need to update the analysis methods of dietary fibre. In 2013, AOAC published a new analytical method, AOAC 2011.25 for the determination of total dietary fibre. AOAC 2011.25 is a multistage process, where water-insoluble and water-soluble dietary fibre is determined separately. In AOAC 2011.25 method, the starch and protein are enzymatically removed. Water-insoluble dietary fibre is separated from water-soluble portion by filtration. Water-soluble polysaccharides are precipitated by ethanol and filtrated. The filtrate contains oligosaccharides which are analyzed by liquid chromatography (HPLC). The residues from soluble and insoluble fractions are analyzed for ash and protein. Total dietary fibre is calculated by reducing ash and protein content from the sum of the fractions. The method AOAC 2011.25 is equivalent with the method AOAC 2009.01 apart from analyzing the insoluble and soluble polysaccharides separately. These methods are at the moment the only ones capable of analyzing all the components defined as dietary fibre at the same time. Fineli database contains information about Finnish food composition. Dietary fibre results in Fineli are from 1980's and -90's and they were obtained using earlier methods. The dietary fibre results have to be updated using the new method to produce precise, comparable and logical information for the consumers, science, education, risk assessment, health care, food industry, and other users of the database. The sampling plan was designed to represent Finnish consumer intake. All the samples were purchased from the Finnish retail sales with their marketing shares taken into account. For each food, 12 subsamples were collected and pooled together, from which the analyses were performed as three replicates. Vegetables and

fruits were freeze-dried, followed by the grinding of the samples in a grinding mill to pass a 0.5 mm sieve. The cereal samples were also grinded. Different milling fractions of wheat flour, rye flour, oatmeal (rolled oats), wheat bran, rye bran, oat bran, tomato, apple and carrot were analyzed using the method AOAC 2011.25. The method used, produced the results for the water-insoluble and water-soluble high molecular weight polysaccharides, and oligosaccharides separately. These three fractions together form the total dietary fibre content. The results are mainly in line with the results obtained earlier with the older methods, such as AOAC 985.29 and 991.43. However, some differences were also observed. For example, rye flour contains significant amount of oligosaccharides, which has an impact on total dietary content. Many of the now studied samples are analyzed for the first time for the food composition database purposes, since many of the values presented in Fineli has not been obtained by analyzing the samples but by recipe calculation.

P84. Interactions of quercetin with radicals derived from catechin and dinitrosocatechin under the conditions simulating the gastric lumen

Takahama U.¹, Morina F.², Veljovic-Jovanovic S.², Yamauchi R.³, and Hirota S.⁴

¹Kyushu Dental University, Kitakyushu 803-8580, Japan

²University of Belgrade, Belgrade 11030, Republic of Serbia

³Gifu University, Gifu 501-1193, Japan

⁴University of East Asia, Shimonoseki 751-8503, Japan, E-mail: takahama@kyu-dent.ac.jp

Quercetin glycoside and catechins are contained in foods derived from plants such as apple, adzuki bean, and buckwheat. When foods prepared from these plants are ingested, quercetin glycosides are mixed with catechins and salivary nitrite, and are hydrolyzed to quercetin in the oral cavity. The mixture is swallowed in the stomach to be mixed with gastric juice (pH 2). Since the pKa of nitrous acid is about 3.3, almost all of salivary nitrite is transformed into reactive nitrous acid in the gastric lumen. Here we report about the interactions between quercetin and (+)-catechin during their oxidation by nitrous acid. Quercetin was oxidized to 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone and (+)-catechin was oxidized to 6,8-dinitrosocatechin by nitrous acid. The formation of the dinitrosocatechin was due to the addition of nitric oxide (NO) to semiquinone radicals of (+)-catechin produced by (+)-catechin/nitrous acid system. The formation of 6,8-dinitrosocatechin was partly suppressed by quercetin, and the suppression accompanied the enhancement of the oxidation of quercetin. 6,8-Dinitrosocatechin was oxidized by nitrous acid and its oxidation product was the quinone form. The formation of quinone was supported by the results that ascorbic acid reduced the oxidation product to 6,8-dinitrosocatechin and that the oxidation product reacted with thiocyanate under acidic conditions producing 6'-thiocyanate-6,8-dinitrosocatechin. Quercetin effectively suppressed the formation of the quinone form of 6,8-dinitrosocatechin. Interactions of

flavonols other than quercetin with (+)-catechin were studied, and the degree of the interaction increased in the order rutin<quercetin 4'-O-glucoside<kaempferol<quercetin 7-O-glucoside<quercetin. Since quinones are toxic, importance of scavenging of the quinones by quercetin and its glycosides for human health is discussed.

P85. The relationship between MNA, anthropometric and biochemical parameters used in the nutritional status assessment of lung cancer patients

Zabłocka-Słowińska K.¹, Gołdecki M.², Porębska I.², Kosacka M.², Prescha A.¹, Grajeta H.¹, Jankowska R.², and Biernat J.³

¹Department of Food Science and Dietetics, Wrocław Medical University; Borowska 211, 50-556 Wrocław, Poland

²Department and Clinic of Pulmonology and Lung Cancers, Wrocław Medical University; Grabiszyńska 105, 53-439 Wrocław

³Department of Human Nutrition, Wrocław University of Environmental and Life Science; Chelmońskiego 37/41, 51-630 Wrocław, Poland, E-mail: katarzynazablocka0112@gmail.com

Introduction: Lung cancer is a common fatal cancer with a poor prognosis. Assessment of nutritional status before treatment enables medical staff to provide more individual, precise and complex care to patients, taking into consideration their incorrect nutritional condition. Biochemical parameters are the best tools to evaluate nutritional status, but they are invasive and very often costly. Anthropometric parameters and MNA tests are non-invasive and not expensive, but do not evaluate nutritional status in detail. The multitude of nutrition assessment tools available necessitates a search for the most suitable to reflect the actual nutritional status of cancer patients. The aim of this study was, therefore, to assess the nutritional status of lung cancer patients using biochemical and anthropometric parameters, as well as MNA, and to evaluate the relationships between invasive and non-invasive tools.

Methods: Assessment of nutritional status, based on the biochemical parameters: albumin, cholesterol, LDL, HDL, glucose, hemoglobin, total iron binding capacity and iron concentration, and the anthropometric parameters: BMI, body fat percentage, arm circumference (AC), waist circumference (WC), hip circumference (HC) and waist-hip ratio (WHR), as well as MNA, was performed on 92 lung cancer patients. Associations between biochemical parameters and anthropometric measurements, as well as MNA, were calculated using the Spearman rank correlation coefficient.

Results: The majority of the lung cancer group had altered nutritional status, as shown by elevated BMI, high body fat percentage, low AC, high waist circumference, and low MNA scores. Most patients also had hypoalbuminemia, and about one-third of them – decreased cholesterol, Fe and Hgb levels. MNA was found to significantly positively correlate with the largest number of measured biochemical parameters in male patients: albumin, cholesterol, HDL cholesterol, glucose and Hgb, while in female patients MNA significantly negatively correlated only with glucose. BMI positively correlated with total cholesterol, TG, Hgb and TIBC in the male group. Body fat percentage

correlated with: HDL cholesterol, TG, glucose and TIBC in the male group. AC was shown to significantly correlate only with Hgb level.

Conclusion: Lung cancer patients at the time of diagnosis had altered nutritional status, based on anthropometric and biochemical parameters. MNA correlated with the highest number of biochemical parameters, and therefore can be a useful screening tool for routinely monitoring lung cancer patients' nutritional status at the time of diagnosis.

P86. Is vitamin-mineral supplementation during second trimester of pregnancy necessary? – Preliminary study

Pieczynska J.¹, Klósek A.², Sozański R.³, Zimmer M.², and Grajeta H.¹

¹Department of Food Science and Dietetics, Wrocław Medical University, Borowska 211, 50-556 Wrocław, Poland

²Department and Clinic of Gynecology, Obstetrics and Neonatology, Wrocław Medical University, Borowska 213, 50-556 Wrocław, Poland

³Department and Clinic of Gynaecology and Obstetrics, Wrocław Medical University, Chałbińskiego 3, 50-368 Wrocław, Poland, E-mail: joanna.pieczynska@umed.wroc.pl

Pregnant women are particularly vulnerable to nutritional deficiencies because of the increased metabolic demands imposed by pregnancy involving a growing placenta, fetus, and maternal tissues. The health behaviors of pregnant woman, including adequate dietary habits and using of supplements, affects the health condition of woman and development of the fetus. The aim of the study was to assess the dietary vitamins and minerals intake with and without vitamin-mineral supplements among Polish pregnant woman in second trimester. In the study, dietary assessment of pregnant woman was performed using the 24 h dietary recall from the last 7 days. The computer program Diet 5.0 developed by the Institute of Food and Nutrition in Warsaw was used for nutrient intake assessment. The data was collected in 2012-2013. The studied group consisted of 20 pregnant woman in their 15th-27th week of pregnancy. In pregnant women participated in the study were found insufficient intake of vitamin D, calcium and iodine, despite of vitamin-mineral supplementation. Intake of vitamins A, C, B group and copper, phosphorus, and sodium was above recommendations, independently of supplements using. The intake of folate in studied group was consistent with the recommendations, but only if women applied vitamin supplements. It was also found inadequate intake of iron from the diet, but after supplements application its supply exceeded the recommended amounts. 95% of studied pregnant women in second trimester of pregnancy applied vitamin-mineral supplements. However, vitamin-mineral supplementation of the diet should be matched individually for each patient depending on her diet. This fact indicate need of dietary counseling for pregnant women during pregnancy.

P87. Effect of dietary nickel intake on its content in

whole blood during pregnancy – Preliminary study

Pieczynska J.^{1*}, Klósek A.², Sozański R.³, Zimmer M.², and Grajeta H.¹

¹Department of Food Science and Dietetics, Wrocław Medical University, Borowska 211, 50-556 Wrocław, Poland

²2nd Department and Clinic of Gynecology, Obstetrics and Neonatology, Wrocław Medical University, Borowska 213, 50-556 Wrocław, Poland

³1st Department and Clinic of Gynaecology and Obstetrics, Wrocław Medical University, Chałbińskiego 3, 50-368 Wrocław, Poland, E-mail: joanna.pieczynska@umed.wroc.pl

There is no determine recommended daily intake for nickel, yet. The role of nickel in organism is unclear, and it is not accept as an essential element for human. Ni deficiency, in laboratory conditions, effects in animals increase of perinatal mortality, inhibition of growth, decrease of reproductive performance and metabolism disturbance. This element exhibits embryotoxic, genotoxic and teratogenic actions, too. The aim of the study was to assess the correlations between Ni daily intake and its content in whole blood of pregnant women. 10 pregnant women from Lower Silesia region participated in the study. Nickel content in daily meals and whole blood was assessed using GF-AAS method.

Mean daily Ni intake in pregnancy was 109 µg/d and mean whole blood Ni content 10,2 µg/L. It has been shown statistically significant differences in Ni intake depending on the stage of pregnancy. There was no correlation between the intake of Ni with daily meals and its content in the blood in any of the trimesters of pregnancy. The advancement of gestational age affects the intake of nickel, whereas the content in the blood of pregnant women is independent of the trimester of pregnancy. There is no correlation between the intake of nickel with daily meals, and its concentration in whole blood.

P88. In vivo genotoxic effects of benzoic acid

Aksoy H.^{1*}, Yilmaz S.², Unal F.³, Yuzbasioglu D.³, and Celik M.⁴

¹Sakarya University, Faculty of Arts and Sciences, Department of Biology, Sakarya, Turkey

²Ankara University, Faculty of Health Sciences, Ankara, Turkey

³Gazi University, Faculty of Sciences, Department of Biology, Ankara, Turkey

⁴Kahramanmaraş Sutcu Imam university, Faculty of Medicine, Department of Basic Science, Kahramanmaraş, Turkey, E-mail: haksoy@sakarya.edu.tr

Benzoic acid is a food additive that is especially used in acidification. Benzoic acid was found in flavored drinks, alcohol drinks, different sauces and other foods. The aim of this study was to assess the effect of benzoic acid on bone marrow cells of Swiss Albino mice using chromosomal aberration test. Animals treated with four

benzoic acid doses for 24h. 62.5, 125, 250 and 500 mg/kg doses of benzoic acid were injected to animals by i.p. Our results indicated that benzoic acid significantly increased the abnormal cell frequency and cells with chromosomal aberrations compared with control. Six types of aberrations, structural and numerical, were observed. These are chromatid and chromosome breaks, sister chromatid union, dicentric chromosome, fragment and polyploidy. The results showed that the benzoic acid used in this study have clastogenic, aneugenic and mutagenic effects in in vivo mouse bone marrow cells.

P89. Phytosterols content in the nuts

Pieczynska J.*., Drzewicka M., Fijałkowska M., and Grajeta H.

Department of Food Science and Dietetics, Wrocław Medical University, Borowska 211, 50-556 Wrocław, Poland, E-mail: joanna.pieczynska@umed.wroc.pl

The aim of this study was to evaluate the content of total phytosterols and theirs the most abundant type - β - sitosterol, stigmasterol, campesterol and Δ 5-avenasterolin the edible nuts. Material for the analysis comprised of 10 following kinds of nuts: walnuts, hazelnuts, peanuts, almonds, cashew, macadamia, pecan, pistachio, pine and Brazil nuts. The phytosterols content was determined using the gas chromatography technique previously transferring them into trimethylsilyl derivatives. 5- α -cholestan was used as an internal standard. The fat content was also assessed. On the basis of the results it was shown that the total phytosterols capacity in nuts ranged from 76,9 mg to 252,9 mg/100g. The highest amounts of phytosterols were found in pistachio (252,9 mg/100g) and pine nuts (205,2 mg/100g). The β -sitosterol was the main compound among the analyzed phytosterols in all studied nuts. Its quantity ranged from 64% to 86% of the total sterols content, followed by Δ 5-avenasterol(8,6-18,7%). In the examined products the fat content carried out from 40,3% (pistachio nuts) to 62,4% (pecan nuts).

P90. Analysis of fatty acids composition in HDL lipoproteins of type 2 diabetic patients

Laštovička P.*., Libalova M., Cermak T., Muzakova V., and Cegan A.

University of Pardubice, Faculty of Chemical Technology, Department of Biological and Biochemical Sciences, Pardubice, Czech Republic, E-mail: Monarcha13@seznam.cz

Diabetes is a long-term problem in developed countries. Numbers of patients with this disease have risen because of poor lifestyle (which remains a major problem). Many studies have focused on LDL. However, on the cholesterol deposition and the resulting complications in diabetics participates disruption of lipid metabolism and fatty acid composition of HDL. The ability to reverse cholesterol-transport by HDL decreases with increasing number of VLCFA and SFA in these particles. 21 anonymized plasma samples randomly selected type 2 diabetic patients and 10 healthy blood donors plasma samples as a control group were processed. The group of diabetic patients were categorized into two groups according to the level

of glycated haemoglobin (HbA1c). This indicator appears to be the most reliable because it reflects compensation of diabetes and highlights the development of blood glucose in the last 4 to 6 weeks prior to collection. HDL was obtained by ultracentrifugation of EDTA plasma samples. HDL lipids were divided into five fractions by thin layer chromatography - phospholipids (PL), diglycerides (DG), free fatty acids (FFA), triglycerides (TG) and cholesterol esters (CE) and analysed by gas chromatography. The 18:1n9/18:0 and 16:1n7/16:0 ratios were calculated as indices of SCD1 activity, while the ratio of 18:0 to 16:0 was calculated as an elongase index. FA concentrations were expressed as percentages of total FAs analysed. The data were processed in STATISTICA 12. Differences in variables between the groups were evaluated using t-test. P value less than 0.05 was considered statistically significant. For correlation analyses were used Pearson's correlations. Negative correlation of elongase index with HbA1c in diabetics in PL ($r=-0.47$; $p=0.032$) and FFA ($r=-0.48$; $p=0.029$) was found. Negative relationship with HbA1c was found in case of arachidic acid ($r=-0.47$; $p=0.029$) in PL, lower concentration of arachidic acid occurred in decompensated diabetics compared to healthy donors (0.28% vs. 0.20%; $p=0.019$). In CE fraction we found lower level of nervonic acid in compensated diabetics in comparison to healthy donors (0.58% vs. 0.30%; $p=0.023$). Low elongase activity causes disruption in docosahexaenoic and arachidonic acid metabolism which has an influence on the fluidity of membranes and thus on the activity of transport proteins thereunder. This affects the transfer of cholesterol by HDL lipoproteins as well.

P91. Determination of genistein derivatives in rat urine by HPLC combined with MS/MS

Papaj K.¹, Rusin A.², and Szeja W.¹

¹Bioorganic Chemistry and Biotechnology, Silesian University of Technology, Gliwice, Poland

²Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Memorial Center and Institute of Oncology, Gliwice Branch, Poland, E-mail: katarzyna.papaj@polsl.pl

Semi-synthetic derivatives of genistein synthesized in our laboratory are compounds that show very promising therapeutic potential. Some of these compounds inhibit cancer cell proliferation at the concentration several fold lower than genistein. We found that the most active compounds block the cell cycle in different cancer cell lines. Moreover, some of the derivatives interact with mitotic spindle due to direct influence on microtubules assembly, which eventually leads to apoptotic cell death. A very important feature of these novel genistein derivatives is their lipophilic character, which may improve significantly their bioavailability. In order to appraise the potential therapeutic use of these semi-synthetic genistein derivatives we decided to determine their bioavailability in vitro using Caco-2 cell line and then to confirm the results in animal study. The experiments with use of Caco-2 model allowed us to define the total amount of transported derivatives in time and their intracellular accumulation. The in vitro permeability assay enabled selection of the compounds used in the

animal bioavailability study. This work presents preliminary data on bioavailability and metabolism of selected genistein derivatives in rats after oral administration of the drugs. The animals divided at random into six groups were administered by oral gavage with genistein derivatives and genistein, which served as a reference compound. Next, the animals were placed in metabolic cages for urine collection. Urine was collected during 0-12 hours and 12-24 hours after drug administration. The urine samples were filtrated and analyzed using HPLC-ESI-MS/MS.

P92. Artemisia capillaris extract suppresses bone resorption activity through controlling acidification pathway in RANKL-induced osteoclast differentiation

Lee S. H.*, Kim J. K., Park J. E., Lee J. Y., Choi H. Y., Kwon Y. I., and Jang H. D.

Department of Food and Nutrition, Hannam University, Daejeon 305-811, Korea, E-mail: ggamannom03@nate.com

The regulation of formation and function of bone-resorbing osteoclasts is a key to understanding the pathogenesis of skeletal disorders. Active bone resorption involves acidification with production of protons by binding to V-ATPase with TRAF6. In this study, the suppressive effect of Artemisia capillaris extract (ACE) on acidification by TRAF-6 and V-ATPase in RANKL-induced osteoclast differentiation was investigated using immuno-precipitation and western blotting analysis. The pH reduced by RANKL treatment was significantly recovered by ACE at 1-20 µg/mL. The suppressive effect of ACE on acidification and protein expression of TRAF6 and V-ATPase dose-dependently increased between 1 and 20 µg/mL in RANKL-induced osteoclast differentiation for 5 days. In addition, ACE inhibited a bone resorption activity in a dose-dependent manner. These results indicate that ACE plays a role as an osteoclastogenesis inhibitor through controlling acidification by regulation of TRAF6/V-ATPase signaling pathway. Therefore, ACE may be used as a potential therapeutic agent targeting osteoclast differentiation and bone resorption in skeletal diseases such as osteoporosis.

P93. Cellular antioxidant capacity of 6-shogaol as an bifunctional antioxidant and underlying mechanism in HEPG2 cells

Kim J. K.*, Wang F. F., Park J. E., Kang Y. R., Lee S. W., Kwon Y. I., and Jang H. D.

Department of Food and Nutrition, Hannam University, Daejeon 305-811, Korea, E-mail: kikkne@naver.com

Ginger is the rhizome of the plant Zingiber officinale, used as a medicine or spice. Ginger contains 6-shogaol, gingerol, and gingerone. In dried ginger powder, shogaol, a dehydrated product of gingerol, is a predominant pungent constituent. A recently published research described that in ginger 6-shogaol shows more biological activity than any other component. In previous study, we founded that 6-shogaol increases antioxidant response element (ARE) activity and induces the

mRNA expression of NQO-1 and GSTa2. Therefore, the present study was designed to investigate the indirect antioxidant effects of 6-shogaol through MAPK pathway in HepG2 cells. The transcriptional activity of phase II detoxifying and antioxidant enzymes was measured by ARE-luciferase assay. The transcription and translation level of each phase II detoxifying and antioxidant enzymes was analysed using RT-PCR and western blotting respectively. The expression levels of activated MAPK, Nrf2, and GST were analysed by western blotting assay. The obtained results showed that 6-shogaol induced the mRNA expression and the protein expression of GCS and GST. ERK1/2 and JNK known as a reactive oxygen species signal protein were notably activated and Nrf2 accumulation in the nucleus was enhanced by 6-shogaol treatment. Both ERK1/2 (U126) and JNK inhibitor (SP600125) completely suppressed ERK1/2 and JNK activation, Nrf2 accumulation in the nucleus, and GCS and GST expression. These results propose that 6-shogaol could exert indirect antioxidant capacity by up-regulating phase II detoxifying and antioxidant enzymes as GCS and GST through ERK1/2/JNK-Nrf2 signaling pathway. Therefore, ginger containing 6-shogaol could be used as a potential natural source for the treatment of oxidative stress and related diseases.

P94. Development of a scale to measure the cariogenic potential of the diet and its application in an Australian dental birth Cohort study (VicGen)

Amezdroz E.^{*}, Carpenter L., Johnson S., and Waters E.

The Jack Brockhoff Child Health and Wellbeing Program, The Melbourne School of Population and Global Health, The University of Melbourne, Australia, E-mail: emily.amezdroz@unimelb.edu.au

Introduction: The cariogenic potential of a food or beverage is determined by physical properties, including type and content of carbohydrates, presence of protective nutrients and frequency of consumption. Numerous studies to measure cariogenic potential in food and drinks have been completed however until now a scale to assess the cariogenic potential of a full diet, an essential element in the development of dental decay, has not been created. The aim of this research was to develop an evidence-informed scale and utilise this to track the cariogenic potential of young children's diets. **Methodology:** The VicGen study has been following children from Victoria, Australia from birth to 5 years of age. Dietary data was collected from approximately 350 children at age 6, 12 and 18 months using a 46-item food-frequency-questionnaire (FFQ) completed by mothers. A comprehensive literature review was conducted to classify foods and beverages into 6 categories of cariogenic potential: cariostatic (0), anti-cariogenic (0), low (1), moderate (2), high (3) and severe (4). Four cariogenic diet types were generated (low, moderate, high and severe). Each diet had an identical core diet (based on the Australian Dietary Guidelines) and variation in frequency of consumption of "extra" food and beverages (those not recommended for consumption). A score for each combination of cariogenic potential and consumption frequency was generated for each food and beverage. These scores were applied to FFQ responses and summed to produce an overall

cariogenicity score (0-280) for each child across the three time points. Results: Preliminary results produced mean cariogenicity score of diets of children aged 6 months to be (mean:16.1, sd: ± 13.2, range 0-81), 12 months (mean:39.6, sd: ± 19.6, range 6-117) and 18 months (mean:56.3, sd ± 19.45, range 12-128). The cariogenic score was positively skewed at the 6 month time-point, however became normally distributed at 12 and 18 month time-points. Young children's diets are increasingly cariogenic with age and "extra" foods are being incorporated into children's diets much younger than recommended. Conclusions: The potential cariogenicity of "extra" foods and beverages can be measured utilising this scale. This provides an extremely useful methodological advance for epidemiological studies aiming to compare across age and to efficiently incorporate into causal modelling.

P95. Differences in breast milk fatty acid composition between obese, overweight and normal weight mother

Chisaguano A.¹, Martín-Dinares L.¹, Castellote A. I.¹, Montes R.¹, De la Garza A.¹, Brandi P.², Segura M.², Moreno-Torres R.², Campoy C.², and López-Sabater M. C.¹

¹Department of Nutrition and Food Science, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain

²EURISTIKOS Excellence Centre for Pediatric Research, University of Granada. Granada, Spain, E-mail: amchisaguanot@ub.edu

The main sources of breast milk fatty acids (FA) are the maternal diet, the mobilization of endogenous stores of FA and the de novo synthesis of FA by breast tissue or liver. The study of FA composition of breast milk in obese or overweight populations is still limited. It has been hypothesized that the content of the long-chain polyunsaturated fatty acids (LC-PUFA) in breast milk is related with obesity. We studied the differences of the FA composition of colostrums and mature milk between obese and normoweight mothers from a Spanish population. Maternal nutritional status (normoweight, overweight or obesity) was estimated on the Body Mass Index (BMI). Thirteen obese (BMI ≥ 30 kg/m²), 17 overweight (25 ≤ BMI < 30 kg/m²) and 16 normoweight (18 ≤ BMI < 25 kg/m²) mothers were selected for this study. Participants provided samples of colostrums (2–4 days after delivery) and mature milk (28–32 days after delivery). Breast milk fatty acid methyl esters (FAMES) were separated and quantified by fast-gas chromatography. Differences between groups were assessed. The FA composition of breast milk showed a considerable variation with regards to factors such as the stage of lactation. Thus, we found that colostrums of the three groups have higher levels of n-6 and n-3 LC-PUFA, especially AA and DHA, in relation to mature milk. On the other hand, we observed higher concentrations of total n-6 LC-PUFA and the ratio n-6/n-3 FA in colostrums of obese compared to normoweight mothers. There were no significant differences between the levels of the main saturated and monounsaturated fatty acids in both, colostrums and mature milk.

P96. Plasma vitamin C in normal weight, overweight and obese pregnant women and offspring at birth

De la Garza A.¹, Marysael C.¹, Guerendiain M. E.¹, Castellote-

Bargalló A. I.¹, Montes R.¹, Chisaguano M.¹, Moreno-Torres R.², García-Valdés L.², Campoy C.², and López Sabater M. C.¹

¹Department of Nutrition and Food Science, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain

²EURISTIKOS Excellence Centre for Pediatric Research, University of Granada. Granada, Spain, E-mail: adelagpu9@alumnes.ub.edu

Background and aim: Vitamin C is an essential water-soluble vitamin of great interest due to its role in the prevention of oxidative stress, which is a key feature in the development of complications during pregnancy. The objective of our study was to evaluate plasma ascorbic acid (AA) concentration in pregnant women belonging to different body mass index (BMI) groups and to search for an association between plasma AA levels in both mother and child. **Methods:** Plasma samples were obtained from 302 pregnant women divided in 3 different groups according to their BMI; normal weight, overweight and obesity. Samples were collected at 24, 34 and 40 weeks of gestation and a cord plasma sample from the fetus was collected at offspring along with anthropometric measures such as weight, length and head circumference. Plasma AA concentration was determined by a validated Ultra High Performance Liquid Chromatography (UHPLC) method. **Results:** Plasma AA levels were significantly different between overweight and obese women at 24 weeks of gestation. No differences were observed for other periods even when compared with control group. Overweight women presented significant differences in plasma AA levels at all weeks of sample collection, and there was a tendency of AA values to decrease towards delivery. A positive correlation was found between the newborns and the AA plasma level of the mothers in all groups at 40 weeks of gestation. AA concentration of the newborns was always significantly higher than the mothers'. **Conclusions:** AA plasma concentration of the newborns is related the mothers' at 40 weeks of gestation, independently of the maternal BMI.

P97. Antioxidant protein hydrolysates obtained from by-products from the slaughter industry

Damgaard T.*, and Lametsch R.

Department of Food Science, University of Copenhagen, Denmark, E-mail: tdd@life.ku.dk

The meat industry produces large quantities of low-value by-products which are rich in protein and essential amino acids, and compared to lean meat tissue, rich in a variety of vitamins and minerals. By-products therefore represent an interesting protein source for enzymatic hydrolysis, due to the nutritional value and diverse functionality. By enzymatic processing, meat industry by-products can be converted into a broad assortment of valuable food ingredients or industrial products with a wide range of applications. The aim of the project is to characterize different protein hydrolysates, e.g. regarding peptide size distribution, dry matter yield and bioactivity in order to create a comprehensive knowledge about the usability of protein hydrolysates obtained from low value products from the meat industry. Protein hydrolysates from five different bovine tissues were

generated with the use of 0.1% Alcalase at different incubation times (0, 15, 60 and 600 min.). The antioxidant capacity was investigated with two in vitro methods, namely iron chelation and ABTS radical scavenging capacity. Furthermore, the soluble yield and peptide size distribution of each of the hydrolysed tissues at the different time points was studied by means of dry matter determination and size exclusion chromatography, respectively. The results show that the dry matter yield increased continuously with incubation time, representing the increasing amounts of Alcalase-liberated and solubilized peptides. The peptide size distribution profiles revealed that the peptide size changed with incubation time resulting in progressively smaller peptides. All the hydrolysed tissues yielded antioxidant capacities through all time points. The ABTS radical scavenging capacity showed a small tendency to increase with incubation time until 60 min where after it reversed to lower values. The iron chelation capacity decreased dramatically with increasing incubation times, indicating a correlation between the capacity of peptides to chelate iron to the peptide size. In summary, these results show that meat industry by-products can be transformed into antioxidant hydrolysates, potentially creating added value.

P98. Linoleic acid, dihomog-γ-linolenic acid and total content of MUFA and VLCFA in erythrocyte membranes predict increase of inflammatory response to coronary stent implantation

Čermák T.¹, Mužáková V.¹, Matějka J.², Skalický J.³, Laštovička P.¹, Líbalová M.¹, and Čegan A.¹

¹University of Pardubice, Faculty of Chemical Technology, Dept. of Biological and Biochemical Sciences, Pardubice, Czech Republic

²Regional Hospital of Pardubice, Dept. of Cardiology, Pardubice, Czech Republic

³Regional Hospital of Pardubice, Dept. of Clinical Biochemistry and Diagnostics, Pardubice, Czech Republic, E-mail: tomas.cermak86@seznam.cz

Percutaneous transluminal coronary angioplasty (PTCA) induces an inflammatory reaction that is crucial in the pathogenesis of neointimal proliferation, which is the main cause of stent restenosis. Many inflammatory cytokines, as mediators of inflammation, are closely associated with the fatty acid profile. We study biochemical aspects of complications associated with PTCA and the role of fatty acids in the development of inflammatory reaction. Hs-C-reactive protein (CRP), interleukin-6 (IL-6), serum amyloid A (SAA), malondialdehyde (MDA) and the fatty acid composition of fasting plasma lipids fractions have been analysed in patients with coronary atherosclerosis before and 24 and 48 h after PTCA, aged 39 – 85 years (n=16). Erythrocyte fatty acid profile and fatty acid profile of plasma fractions separated by thin layer chromatography were analysed by GC/FID. Statistical analyses were done with software STATISTICA v. 12.0. It was found significant increase of inflammatory reaction characterised by hs-CRP and SAA with maximum after 48 h post PTCA as well as oxidative stress (MDA). In case of IL-6 maximum was at 24 hours. The initial levels of

total amounts of groups of fatty acids (SFA, trans-MUFA, cis-MUFA, n-3 PUFA and n-6 PUFA) and individual fatty acids of these groups in erythrocyte membranes and plasma lipid fractions (PL, DG, FFA, TG and CE) were correlated with increase of CRP, IL-6 and SAA concentration as well as individually fatty acids of these groups. In case of erythrocyte membrane there were statistically significant positive Spearman correlations between increase of IL-6 and C18:2 n-6 ($r=0.59$) and C20:3 ($r=0.70$) from group of n-6 PUFA and with very long chain saturated fatty acids (VLC SFA) ($r=0.51$), especially with C22:0 ($r=0.56$) and C24:0 ($r=0.54$). Negative correlation with IL-6 was found in case of MUFA – palmitoleic acid ($r=-0.47$) and total trans MUFA ($r=-0.54$). It was found negative correlation between SAA and total n-3 PUFA. Significant negative correlation between increase of MDA and values of MUFA – oleic ($r=-0.45$) acid, vaccenic acid ($r=-0.46$) and total SFA ($r=-0.46$). The total content of VLC SFA (especially, behenic and lignoceric), a high content of linoleic acid and dihomo- γ -linolenic acid (but not the overall proportion of n-6 PUFA) and a reduced content of MUFA in erythrocyte membranes significantly predict increase of inflammatory response after PTCA. Our results confirm close relationship between fatty acid profile (especially in erythrocyte membranes) and the extent of inflammation reaction after stent application. Our data indicate that risk patients could be identified based on the fatty acid profile. Then this profile could be modified by diet or medicaments before the treatment, which would diminish the risk of restenosis.

P99. Assessment of genotoxic and antigenotoxic effects of *Prunus avium* extract

Berber A. A.¹, Sönmez F.², Berber N.³, Güzel N.¹, Aygün B.¹, and Aksoy H.¹

¹Sakarya University, Faculty of Arts and Sciences, Department of Biology, Sakarya, Turkey

²Sakarya University, Pamukova Vocational High School, Sakarya, Turkey

³Sakarya University, Faculty of Arts and Sciences, Department of Chemistry, Sakarya, Turkey, E-mail: aberber@sakarya.edu.tr

In this investigation, genotoxic effects of *Prunus avium* cultivar Lambert extract (PE) and antigenotoxic effects of this extract against Mitomycin C (MMC) induced micronuclei have been evaluated in human peripheral blood lymphocytes in vitro. Two series of experiments were conducted. In the first, only 50, 100, 200 and 400 $\mu\text{g}/\text{mL}$ concentrations of PE were used for 48 h to detect potential genotoxicity. In the second, MMC (0.20 $\mu\text{g}/\text{mL}$) plus 50, 100, 200 and 400 $\mu\text{g}/\text{mL}$ concentrations of PE were used for 48 h to determine antigenotoxic effects. PE significantly increased MN frequency at 100 and 400 $\mu\text{g}/\text{mL}$ compared with negative and solvent controls. However, it decreased the frequency of MMC induced MN. Decreasing was significant at all concentrations.

P100. Characterization of a population concerning vitamins and anemia biomarkers

Mota C.^{1,2}, Tavares N.³, Torres D.^{2,4}, Santos M.¹, Castanheira I.¹, and Matos A.³

¹Food and Nutrition Department, National Institute of Health Doctor Ricardo Jorge, Lisbon, Portugal²FCNAUP, Faculty of Nutrition and Food Sciences of the University of Porto, Portugal

³UNIDEMI, Departamento de Engenharia Mecânica e Industrial, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

⁴Departamento de Bioquímica, Faculdade de Medicina da Universidade do Porto (U-38, FCT), Al. Prof. Hernâni Monteiro, 4200-319 Porto, Portugal, E-mail: carla.mota@insa.min-saude.pt

Folate, vitamin B12, iron and hemoglobin are essential for metabolic functions. Deficiency of these can cause several known pathologies and, untreated, severe morbidity and death. The objective of this study was to characterize a population, concerning serum levels of folate, B12, iron and hemoglobin, as well as finding evidence of correlations between these parameters and illnesses, mainly cardiovascular, gastrointestinal, neurological and anemia. The laboratorial data collected consists of blood analysis in patients, required by the patients' doctors, and were retrieved in 2012. The total population consisted of 728 individuals, residing in Lisbon metropolitan area in Portugal. When coming in for examination, the patients were asked routine questions about their age, gender, county of residence, reason for examination and what medication or supplementation they were taking. The population consisted of 140 male patients and 588 female patients, aged from 2 to 95 years of age. There are 329 persons over 60 years old, these are considered elderly patients.

Clinical data was submitted to multivariate analysis. The data was screened with Spearman correlation and Kruskal-Wallis analysis of variance to study correlations and variability between groups. To characterize the population, we used cluster analysis with Ward's linkage method. A positive correlation between iron with, ferritin, transferrin and hemoglobin was observed with the Spearman correlation. Kruskal-Wallis analysis of variance showed significant differences between these biomarkers in persons aged 0-29, 30-59 and over 60 years old. Cluster analysis, evidence low folate levels for population in general, and hemoglobin levels below the reference values for 42.8% of study population. Iron and vitamin B12 were within the reference range for most of the population. Low levels of the parameters were registered mainly in cardiovascular, gastrointestinal, neurological diseases and anemia patients.

P101. Inhibition of carbonic anhydrase I and II with total anthocyanins extracted from sweet cherry cultivars

Sonmez F.¹, Gunesli Z.¹, Demir T.¹, Bilen C.², Yavuz E.², and Gencer N.²

¹Sakarya University, Turkey

²Balikesir University, Turkey, E-mail: fsonmez@sakarya.edu.tr

Carbonic anhydrase (CA, EC 4.2.1.1) is a ubiquitous zinc enzyme. Basically, there are several cytosolic forms (CA-I, CA-II, CA-III, and CA-VII), four membrane-bound forms (CA-IV, CA-IX, CA-XII, and CA-XIV), one mitochondrial form (CA-V), as well as a secreted CA form (CA-VI). All of them catalyze a very simple physiological reaction, the interconversion between carbon dioxide and the bicarbonate ion, and are thus involved in crucial physiological processes connected with respiration and transport of CO₂/bicarbonate between metabolizing tissues and the lungs, pH and CO₂ homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (such as the gluconeogenesis, lipogenesis and ureagenesis), bone resorption, calcification, tumorigenicity, and many other physiologic or pathologic processes. Sweet cherry (*Prunus avium*) belongs to Rosales order, Rosaceae family, Prunoidea subfamily and Prunus genus. This fruit, which can conveniently be grown in mild climate zones, is widely used in a variety of areas such as fruit juice industry, table consumption, cake and jam production. Hence, it has a high market value and demand potential. Fruits are considered a natural source of antioxidants including polyphenols and anthocyanins. These compounds can reduce the risk of degenerative diseases caused by oxidative stress, such as cancer, cardiovascular disease and stroke. Red fruits, including sweet cherries, are rich in these types of compounds. Sweet cherry cultivars, known throughout the world, are used for both table consumption and processed products. In this study, total anthocyanins were extracted from six sweet cherry cultivars ('Z0900', 'Hasan Kazak', 'Lambert', 'Karakiraz', 'Napoleon', and 'Churchill') grown in Sakarya, Turkey and their effects on carbonic anhydrase I and II were evaluated.

P102. Characterization of honeys from the great Hungarian plain: Determination of the mineral content by inductively coupled plasma mass spectrometry (ICP-MS)

Poór P.*, Ördög A., Körmöczy T., Barhács E., and Tari I.

University of Szeged, Hungary, E-mail: poorpeti@bio.u-szeged.hu

Honey is a key product of traditional agriculture in the southern region of the Great Hungarian Plain and it is also considered an important export item in Hungarian commerce. The geographical origin and the special plant sources determine the characteristics and the quality of honey. Many types of unifloral (robinia, sunflower, rapeseed, milkweed) and multifloral honeys are produced in the southern region of the Hungarian Great Plain. Our aim in this study was to determine the macro- and microelement content in honey samples and compare them in terms of variety and origin within the region. The measurements were carried out using inductively coupled plasma mass spectrometry (ICP-MS). Our results show that the element found in highest amount in samples collected in the region was potassium and sodium. As for microelements; boron, zinc, iron and copper were present in the largest amounts.

P103. Sequences suitable for DNA barcoding analysis of cryptic diversity of a *Botrytis cinerea* species complex

Szojka A.*, Sándor E., Karaffa L., and Asadollahi M.

University of Debrecen, Hungary, E-mail: aniko.szojka@gmail.com

The necrotrophic fungus *Botrytis cinerea* Pers. Fr. is the causal agent of grey mould, a disease affecting many economically important crops. At the present, *B. cinerea* is considered a species complex consistent of two phylogenetic or cryptic species, *B. pseudocinerea* (also called group I) and *B. cinerea* sensu stricto (group II). It is extremely difficult to be distinguished by classical taxonomic criteria. For identification of two cryptic species of *B. cinerea*, vegetative compatibility analyses, genealogical concordance of the phylogenetic species recognition using multiple gene sequences and selected several molecular markers are commonly required which takes a lot of effort and is time-consuming. Screening an appropriate DNA barcode for identification of the two cryptic species is essential. DNA barcoding aims to provide an efficient method for species-level identifications. Our purpose was to select useful molecular markers as candidates, to investigate the possible DNA barcode for *Botrytis cinerea* species complex. Although ITS is the most widely used marker for species identifications in mycology, its sequence has earlier stated not suitable for differentiation among members of the genus *Botrytis* as providing limited phylogenetically informative characters. Two other sequences have been tested for differentiation of *Botrytis cinerea* species complex (i) b-tubulin sequence, and (ii) cyanide resistant alternative oxidase (AOX). The b-tubulin sequences has been used earlier for differentiate *B. cinerea* from *B. pseudocinerea*. The parsimony analysis of the b-tubulin sequences clearly separated the two *B. cinerea* cryptic species. A short sequence fragment has been chosen for DNA barcoding with nucleotide differences in eight positions. The AOX sequences nicely correlated with the phylogenetic connection of the studied Ascomycetous fungi. It also proved to be suitable for differentiate *B. cinerea* from *B. pseudocinerea*.

P104. The interplay between nutritional status and anemia among lung cancer patients

Zabłocka-Słowińska K.¹, Porębska I.², Golecki M.², Kosacka M.², Grajeta H.¹, Jankowska R.², and Biernat J.³

¹Department of Food Science and Dietetics, Wrocław Medical University, Poland

²Department and Clinic of Pulmonology and Lung Cancers, Wrocław Medical University, Poland

³Department of Human Nutrition, Wrocław Medical University, Poland, E-mail: katarzynazablocka0112@gmail.com

In the course of cancer, accompanying symptoms are often more important clinical problems than underlying disease and may influence treatment schedule. Malnutrition and altered blood counts appear particularly very often. Because hematological and nutritional disturbances may coexist in lung cancer, it is interesting to evaluate the association between symptoms of them. Knowledge of interplay between nutritional status and blood parameters related to anemia could help clinicians to better evaluate lung cancer patient condition and therefore to better adjust the oncological treatment. The aim of

this study was to evaluate the associations between nutritional status and hematological parameters indicating anemia in lung cancer patients. Material and method: Among 116 patients, nutritional status was evaluated using anthropometric parameters and Mini Nutritional Assessment (MNA) and the risk of anemia was estimated with routinely performed blood counts: Hgb, Htc, RBC, MCV, MCH, MCHC. Associations between nutritional status and blood parameters were carried out using Pearson and Spearman correlation coefficients. The sensitivity, specificity, positive and negative predictive values (PPV and NPV) of anthropometric parameters and MNA were compared to blood counts Results: HC and AC significantly, positively correlated with the largest number of blood parameters: Hgb, hematocrit, RBW and Hgb, hematocrit, RBW respectively. Additionally weight was found to positively correlate with Htc and RBW while MNA – with Hgb and hematocrit. Weight and HC were shown to negatively correlated with MCV. The MNA test was the most sensitive but also the least specific in screening for hematological disturbances. The highest specificity in screening for anemia was observed for AC but this parameter had also the lowest sensitivity. Conclusions: Anthropometric parameters and MNA interplay with blood parameters, however they are not good indicators of prevalence of anemia.

P105. The importance of commercial and self-brewed beers in our nutrition

Szabó E.*, and Sipos P.

University of Debrcen, 138. Böszörményi Street, Debrecen H-4032 Hungary, E-mail: szabo.edina@agr.unideb.hu

Beer is known from the beginning of civilization, and very popular nowadays too (Piendl, 2005; Eßlinger, 2009). However it is the most consumed from the alcoholic beverages, but few people knows, that it has more positive effects to our body (Kalušević et al., 2011; Hofta et al., 2007; Gerhäuser, 2005). Beer is usually alcoholic beverages, which is brewed from malt with water, boiled with hop, fermented with brewer's yeast (Codex Alimentarius Hungaricus, 1997; Imure – Sato 2012; Nogueira et a., 2005). Thanks to these raw materials, beer is rich in nutrients such as in carbohydrates, proteins, minerals and polyphenols (Kalušević et al., 2011; Hofta et al., 2007; Gerhäuser, 2005). We tried to find out during our research weather the commercial light or the self-brewed light beers can contribute better to our daily needs. Our aims were to determine the protein, the carbohydrate, the nutrient content and the mineral composition of commercial, and self-brewed beers, and to compare the results with the GDA (Guideline Daily Amounts). Beer samples were bought in Hungary, and the self-brewed beers were produced by Zip's Micro Brewery Equipment in the laboratory of the Institute of Food Science at the University of Debrecen, using the recipe of the equipment. We could establish that the self-brewed beers can contribute better to our daily needs than the commercial beers.

P106. Grapevine trunk diseases in the Tokaj wine region, Hungary

Kovács C.¹, Szojka A.¹, Bihari Z.², Sándor E.¹, and Peles F.¹

¹University of Debrecen, Institute of Food Science, Hungary

²Research Institute for Viticulture and Oenology, Tokaj, Hungary, E-mail: k.csilla20@gmail.com

Grapevine trunk diseases (GTD) are one of the most important diseases in vineyards worldwide, which can be found in Hungarian vineyards as well. GTD are caused by a variety of pathogen fungi, like: *Phaeoacremonium aleophilum*, *Phaeomoniella chlamydospora*, *Eutypa lata*, *Fomitiporia mediterranae*, *Diplodia seriata*. There is very limited information about the occurrence of different pathogens causing GTD in the Tokaj wine region nowadays. Currently there is no effective fungicide, which could be used against the disease. The objective of the research were estimated the occurrence of grapevine trunk diseases in Tokaj wine region in cooperation with local specialists; furthermore, to isolate and identify fungi associated with symptoms of GTD in vineyards. Tokaj wine region have indigenous grape varieties (Hárslevelű and Furmint). The samples came from the Bakonyi area (101 samples). We cooperated with the staffs of the Research Institute for Viticulture and Oenology (Tokaj) in the collection of the grapevine trunk samples. Pure fungal cultures were used for morphological (colony formation, pigment production and the shape of the spore) and molecular identification of fungal species. The woody tissue samples were cut in a small part, and were disinfected with 10% Neomagnol (tosylchloramide sodium B) solution for 10 minutes, than were washed twice with sterile distilled water. Afterwards they were put on malt extract agar medium and incubated at room temperature (25°C) for 3-8 days. The isolates were maintained on potato-dextrose agar (PDA) at 4°C. Fungal DNA was extracted from three days old fungal mycelia growth on 50 ml potato dextrose broth on a rotary shaker (125 rpm) at room temperature, and was harvested by vacuum filtration. MagNaLyser (Roche) was used for disruption, and isolation was carried with NucleoSpin Plant II (Macherey-Nagel). The ITS4 and ITS5 primers were used for PCR amplification of the internal transcribed spacer regions. Purified amplicons were sequenced by MWG Biotech Company in Germany. The sequences were aligned with deponated reference sequences with Clustal X program, and manually corrected with GeneDoc. Phylogenetic analyses were performed with MEGA 5.05. The foliar symptoms could be observed on 0.46% (Szemere-dűlő), 3.49% (Dorgó-dűlő); 4.27% (Szarvas-dűlő) and 0.34% (Várhegy) of the examined trunks. However, dieback could be detected only in few cases. More than one hundred fungi were isolated in case of Bakonyi area from symptomatic woody tissues. In case of Bakonyi area the majority of species (72.3%) were determined as member of *Botryosphaeriaceae*, sixty-six samples were determined seventy-three samples as *Diplodia seriata*, and six samples as *Botryosphaeria stevensii*. Other fungi, like *Alternaria* (four), *Mucor ramossimus* (nine), *Phoma* sp. (one), *Diaporthe* (two) and *Xylaria* (seven), *Gibberella zea* (one), *Pheniophora* sp. (one), *Xanthomendoza* sp. (one) were also identified from grapevine trunk samples.

P107. Effect of long-term administration of Arginyl-fructose (AF) on hyperglycemia and HbA1c in db/db mice model

Choi H. Y.*, Lee J. Y., Kang Y. R., Lee S. W., Ha K. S., and Kwon Y. I.

Department of Food and Nutrition, Hannam University, Daejeon 306-791, Korea, E-mail: kelolo123@naver.com

To test whether Arginyl-fructose (AF) play a role in the blood glucose lowering in db/db mice model, db/db mice were randomly assigned to high-carbohydrate diets (66.1% corn starch), in AF contents (4% in diet) for 6 weeks. Changes in body weight, blood glucose level, and food intake were measured daily for 42 days. Administration of AF resulted in a significant decrease of blood glucose level and body weight. The level of HbA1c relevant to seriousness of diabetes was also significantly decreased during 6 weeks. Administration of acarbose® (0.04% in diet), positive control, also significantly alleviated the level of blood glucose, HbA1c, and body weight. These results indicate that AF Maillard reaction product may have anti-diabetic effect by suppressing carbohydrate absorption in the gastrointestinal level.

P108. A study of student diet and lifestyle and correlation with cardiovascular disease factors

Alamri N.*, and Morris P.

Heriot-Watt University, United Kingdom, E-mail: Naa46@hw.ac.uk

Most cardiovascular disease (CVD) is caused by risk factors that can be controlled, treated or modified, such as high blood pressure, cholesterol, diet, overweight, smoking, lack of physical activity and diabetes. However, there are also other major CVD risk factors that cannot be controlled, such as age, gender, and family history. The aim of this cohort study was to evaluate potential CVD risk factors associated with diet and lifestyle among a sample of 20 first year University students (70% male, age 18 to 23 years). The nutrients consumed in the diet for 3 days were evaluated using the WinDiet program. The following biometric data were also collected for each student: body mass index (BMI), waist/hip ratio, body fat, blood pressure, oral glucose tolerance test (OGTT), fasting plasma insulin, low density lipoprotein (LDL) and high density lipoprotein (HDL). Lifestyle factors included vegetarianism, consumption of fruit/vegetables every day and taking dietary supplements such as vitamins and minerals. Eleven statistically significant relationships were identified. These included smoking x BMI; vegetarianism or consumption of fruit/vegetables x physical activity; LDL x fasting glucose; LDL x OGTT; blood pressure x OGTT; protein consumption x OGTT; blood pressure x manganese consumption; insulin x biotin consumption; and body fat x cholesterol consumption. The relationships between these factors may be important in predicting CVD in the future. A follow-up study, to evaluate how the observed relationships between the CVD risk factors may change over time, will be conducted in December 2014.

P109. Risk assessment of arsenic contamination

Várallyay Sz.*, Bacskainé Bódi E., Garousie F., and Kovács B.

University of Debrecen, Faculty of Agricultural and Food Science

and Environmental Management, Institute of Food Science, Hungary, E-mail: varallyayszilvia@gmail.com

The agricultural environment is contaminated with heavy metals and other toxic elements, which means more and more threats. One of the most important toxic element is the arsenic. Arsenic damages the whole body, has been shown to cause cancer, but it increases the risk of non-cancer diseases too. Consequences of arsenic toxicity in the plant organism is decreases the weight of the roots, and causes discoloration and necrosis of leaves. Therefore, we analyse the concentration of arsenic in the different layers of the soil and in the plants and also in the each parts of the plants. Furthermore, we would like to analyse, how the arsenic concentration increases in the plants if the soil contains different doses of arsenic. In 1991 Dr. Imre Kádár made a long-term experiments in the Institute for Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research, Hungarian Academy of Sciences which located in Nagyhöröcsök, Hungary. Dr. Imre Kádár used 13 different elements, among others arsenic. The plot size was 21 m². Different doses were applied: 0 kg/ha (control), 30 kg/ha, 90 kg/ha, 270 kg/ha, 810 kg/ha and different plants were grown in each years: eg. maize, sunflower, potato and etc.. This experiment was sampled for plant samples year by year and the sampling of soil samples were made in each 3-6 years (from different layers). Naturally we have these samples from the experiment, so we analyse these using ICP-OES and ICP-MS, that is the concentration of the total arsenic, which contains the each parts of the plants and of the soil. We can gain information about how the arsenic migrates into the deeper layers of the soil, and how it accumulates in the different parts of the plants. Two different plant was chosen from this experiment to present the result in poster presentation. After the analysis we are able to make food-safety risk assessment for human health which based on our analysis results.

P110. Effect of molybdenum treatment on the element uptake of spinach and garden sorrel in a long-term field experiment

Bodi E.*, Varallyay Sz., Garousi F., and Kovacs B.

University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Food Science 4032 Debrecen, Böszörményi Street 138. Hungary, E-mail: bodieva@agr.unideb.hu

Molybdenum (Mo) is an essential microelement equally for plants, animals and people. Molybdenum has an important role in functioning of several enzymes as metal constituent of enzymes, among which nitrogenase, nitrate reductase, xanthine oxidase and sulfite oxidase are significance for plant physiology. Our research is based on the long-term field experiments of Nagyhöröcsök, where different levels of soil contamination conditions are represented. Molybdenum was supplemented to the soil as ammonium paramolybdate [(NH₄)₆Mo₇O₂₄.] in four different concentrations as follow: 0 (control), 30, 90, 270 mg kg⁻¹. In our work, molybdenum content of spinach (*Spinacia oleracea*) and garden sorrel (*Rumex rugosus*) samples were measured; they were collected from the above-mentioned

experiment station. Wet digestion with nitric acid and hydrogen peroxide were applied during sample preparation of these plants. Total element concentration were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). As a result of our experiments, we can draw the conclusion that both leaf vegetables took up only a small amount of molybdenum from the control soil. Molybdenum treatments that were applied in the experiment, raised molybdenum concentration in plants, however difference was observed in the intensity of growth concentration in the spinach and garden sorrel. We increased more intense of concentration in spinach. In addition, the results of our research also showed that treatments with increasing concentrations of molybdenum have significant Mo concentration differences in leaf and stem of spinach.

P111. New methodology for the safety control of lupin seeds

Marková L.¹, Vaněrková D.¹, Portychová L.¹, Horna A.¹, Jelínek M.², Fuchs M.³

¹Institute of Nutrition and Diagnostics, RADANAL Ltd., Okružní 613, 530 03 Pardubice, Czech Republic ²University of Hradec Kralove, Faculty of Science, Czech Republic, and ³EXTRUDO Becice Ltd., Czech Republic, E-mail: markova@radanal.cz

In recent years there has been growing interest in replacing (genetically modified) soya by lupin, mainly because of lupin great nutritional value. Lupin proteins are more and more used to replace animal proteins and other plants ingredients in foods such as bakery products, imitation dairy and meat products, and beverages. Due to increased use of lupin seeds, flours and lupin containing food, there is an increasing risk of an undesired exposition to quinolizidine alkaloids, bitter compounds produced by lupin plants as a defence against predators that have shown to possess acute oral toxicity mainly related to neurological effects. Lupin flours have been analysed in order to assess the relevance of a potential health hazard given by naturally occurring alkaloids. Our research was aimed at developing method for quantification of quinolizidine alkaloids - lupanine, lupinine, sparteine in lupin seeds by high performance liquid chromatography coupled to a triple quadrupole tandem mass spectrometry UHPLC/MS/MS. A simple extraction technique with 70% (v/v) methanol in water was applied for the isolation of the analytes from the lupin flour. The separation on a C18 reverse-phase column was achieved using a gradient elution with the mobile phase composed of a mixture of 0.05% (v/v) formic acid in water and methanol. The mass spectrometer was operated in Multiple Reaction Monitoring (MRM) mode with the positive electrospray ionization (ESI) interface. In Czech Republic the maximum limit of quinolizidine alkaloids in food product have not been established yet. Our methodology is based on data fixed by the Health Authorities of Australia, New Zealand, Great Britain, and France. The amount of 200 mg/kg (0.02%, w/w) is considered as the maximum concentration of quinolizidine alkaloids in lupin flours used in food industry. Main purpose of this methodology is to provide

the appropriate conditions for the safety testing of lupin used for the preparation of common foods and dietary and nutritional products. This methodology will provide conditions for the safety control of imported lupin, where is the potential danger of imports not precisely identifiable lupin varieties with greater risk of toxicity.

P112. Postprandial blood glucose level reducing effect of low molecular weight chitosan oligosaccharide, GO2KA1, in health individuals

Lee S. W.*, Lee J. Y., Yu-Ri Kang, Hwang-Yong Choi, Sung-Hoon Jo, and Young-In Kwon

Department of Food and Nutrition, Hannam University, Daejeon 305-811, Korea, E-mail: leesewoon257@naver.com

We investigated the effects of chitosan oligosaccharide (GO2KA1) on the level of postprandial blood glucose in adult subjects with normal blood glucose level. Postprandial blood glucose levels were tested for 2 hr after sucrose administration with or without 500 mg of GO2KA1. GO2KA1 significantly lowered the mean, maximum and minimum level of postprandial blood glucose at 30 and 90 min after meal. Additionally, GO2KA1 treatment reduced the AUC and Cmax while Tmax was significantly increased. Our findings suggest that GO2KA1 reduces postprandial blood glucose rise due to slow absorption of glucose in the small intestine, via carbohydrate hydrolyzing enzymes inhibition.

P113. Diet quality of children suffering from juvenile idiopathic arthritis

Prescha A.¹, Gorczyca D.², Postępski J.³, Olesińska E.³, Lachór-Motyka I.⁴, Nytko B.¹, and Grajeta H.¹

¹Department of Food Science and Dietetics, Wrocław Medical University, Borowska 211, 50-556 Wrocław, Poland

²Department and Clinic of Paediatrics, Immunology and Rheumatology of Developmental Age, Wrocław Medical University, Koszarowa 5, 51-149 Wrocław, Poland

³Clinic of Paediatrics Pulmunology and Rheumatology, Medical University of Lublin, Poland

⁴Department of Paediatric Rheumatology, John Paul II Paediatric Centre, Gabrieli Zapolskiej 3, 41-218 Sosnowiec, Poland, E-mail: anna.prescha@umed.wroc.pl

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic childhood disease. JIA is a heterogeneous group of disorders characterized by chronic inflammation of one or more joints. The etiology of JIA includes genetic, hormonal, and environmental factors, and their interactions lead to pronounced activation of the patient's innate immune system. Diet is one of the most important environmental factors, which may not only contribute to the development but also modify the course of the disease. Scientific data on the role of nutrition in JIA are scarce. The aim of this study was to assess dietary habits and nutrient intake in children with JIA. 116 children (72 girls and

44 boys) admitted to one of three Paediatric Rheumatology Centers in Poland (Wrocław, Lublin, and Sosnowiec) were divided into two equal groups: children with JIA and healthy children (control group). Dietary assessment was performed using a questionnaire developed by the Department of Nutrition and Dietetics WMU and the 7-day dietary record. The computer program Diet 5.0 developed by the Institute of Food and Nutrition in Warsaw was used for nutrient intake assessment. Statistical analyses were carried out using Statistica 10.0. StatSoft. A significantly lower intake of vitamin E was observed in children with JIA compared to the control group. Moreover, an insufficient intake of energy, water, polyunsaturated fatty acids, vitamin D, folate, calcium, potassium and iron was found in both groups. Children with JIA consumed less good dietary sources of antioxidants than healthy children, and this could affect their antioxidant status. An excessive consumption of saturated fatty acids, sucrose, and sodium was found in both groups of children. Observed dietary mistakes in children with JIA indicate a need for nutritional education and monitoring, as impaired nutritional status may affect their development as well as the progress of their disease.

P114. Effect of long-term supplementation of low molecular weight chitosan oligosaccharide (GO2KA1) on fasting blood glucose and HbA1c in db/db mice model and elucidation of mechanism of action

Kang Y. R.*, Lee J. Y., Lee S. W., Cho C. Y., Ha K. S., Jo S. H., and Kwon Y. I.

Department of Food and Nutrition, Hannam University, Daejeon 305-811, South Korea, E-mail: kang0606@hotmail.com

Type 2 diabetes is a serious problem for developed countries. Prevention of prediabetes progression to type 2 diabetes with the use of natural products appears to be a cost-effective solution. Previously we showed that enzymatically digested low molecular weight chitosan-oligosaccharide with molecular weight (MW) below 1,000 Da (GO2KA1) has potential for hyperglycemia management. In this study we evaluated the effect of long-term supplementation of GO2KA1 on hyperglycemia using a db/db mice model. Additionally, we evaluated the effect of GO2KA1 on sucrase and glucoamylase activities and expression, using the same db/db mice model. After 42 days we observed that GO2KA1 supplementation reduced both the blood glucose level and HbA1c in a similar manner with a known anti-diabetic drug, acarbose. Long-term GO2KA1 supplementation in db/db mice results to significant blood glucose and HbA1c reduction, to levels similar with those of acarbose. When the sucrase and glucoamylase activities of GO2KA1 and control mice were evaluated using enzymatic assay, we observed that GO2KA1 significantly inhibited sucrase in all 3 parts of the intestine, while glucoamylase activity was significantly reduced only in the middle and lower part. When the sucrase-isomaltase (SI) complex expression on mRNA level was evaluated, we observed that GO2KA1 had minimal inhibitory effect on the upper part, more pronounced inhibitory effect on the middle part, while the highest inhibition was observed on the lower part. Results from this study

provide a strong rationale for the use of GO2KA1 for type 2 diabetes prevention, via inhibition of carbohydrate hydrolysis enzymes.

P115. Anti-hyperglycemic and antioxidants activity of 6 selected medicinal plants extracts in traditional digestive medicine

Lee J. Y.¹, Lee S. W.¹, Choi H. Y.¹, Kim E. J.², and Kwon Y. I.¹

¹Department of Food and Nutrition, Hannam University, Daejeon .306-791, Korea

²Institute of Genofocus, GENOFOCUS Inc., Daejeon .305-500, Korea, E-mail: seembeeks@hanmail.net

Inhibition of α -amylase and α -glucosidase involved in the digestion and absorption of carbohydrates can significantly decrease the postprandial increase of blood glucose level after a mixed carbohydrate diet. In the course of screening useful these enzyme inhibitors, we selected 6 kinds of medicinal plants from Korean traditional digestive medicine, Hwalmyoung-Soo[®] using in-vitro enzyme inhibition assay. Furthermore, total phenolic, total flavonoids, and oxygen radical absorbance capacity (ORAC) of these selected 6 extracts were evaluated. Generally all ethyl alcohol extracts tested had a high α -glucosidase inhibitory activity and a low α -amylase inhibitory activity. Among tested plants *Myristica fragrans* Houtt (70.1%), had the highest α -glucosidase inhibitory activity, followed by *Fraxinus excelsior* Linne (61.8%), and *Uncaria gambir* Roxburgh (41.7%). The α -glucosidase inhibitory activity of extracts correlated to those of phenolic content and antioxidant activity. These results indicate that ethyl alcohol extracts of medicinal plant which has antioxidant and α -glucosidase inhibitory activity has the potential to contribute as a useful dietary strategy for controlling postprandial hyperglycemia.

P116. Effect of long-term supplementation of selected medicinal herbs on activity of intestinal α -glucosidase and expression of SI-complex in rat model

Kang Y. R.¹, Choi H. Y.¹, Lee J. Y.¹, Park S. H.², and Kwon Y. I.¹

¹Department of Food and Nutrition, Hannam University, Daejeon 305-811, South Korea

²Institute of Genofocus, GENOFOCUS Inc., Daejeon .305-500, Korea, E-mail: kang0606@hotmail.com

In this study, we investigate the effect of long-term supplementation and suggested mechanism of action (via inhibition of carbohydrate hydrolysis enzymes) of selected medicinal herbs such as *Cinnamomum loureirii* Nees, *Magnolia officinale* Rehder et Wils, *Brassica oleracea* var. *capitata* L. powders, and extract of *Ficus carica* L. Briefly, the effect of long-term supplementation of selected medicinal herbs in Sprague-Dawley (SD) rat model on intestinal α -glucosidase activities and the expression of SI (sucrose-isomaltase)-complex was evaluated. To confirm the mechanism of action via carbohydrate hydrolyzing enzymes inhibition, after the completion of the animal trial the small intestines of the tested animal were recovered and the sucrase, glucoamylase

and sucrase-isomaltase complex activities were determined. Using 4 groups of 11 male Sprague-Dawley rats, rats of each group were treated with Cabagin-S® (positive control) and herbal mixture at low or high concentration given daily for 2 weeks. After postprandial blood glucose lowering test with starch and sucrose feeding for evaluating a digestibility of starch and sucrose, small intestine was collected from each rat and the expression levels of sucrase-isomaltase in each group was evaluated by western blotting method.

P117. Results of nutritional status screening among patients who moved to hospitals from primary care

Kiss A.¹, and Lelovics Z.²

¹Semmelweis University, Faculty of Health Sciences, Budapest, Hungary

²Kaposvár University, Kaposvár, Hungary, E-mail: kiss.anna891@gmail.com

Aims: to assess the nutritional status of hospitalised people, especially to determine the amount of people with different risks and to describe a tendency compared to earlier results. **Patients and Methods:** We screened in 2013 the nutritional status of 1290 voluntary people elder than 18 years (48% male, 52% female) who moved to hospitals from the primary care with the validated Malnutrition Universal Screening Tool (MUST). We defined the means and standard deviations and 95% Confidential Intervals of the groups and we performed Student's T-test to analyse the data. We examined the parameters both of different groups and of the full population. **Results:** According to our results the risk of malnutrition for these people is high (36.5%), but the prevalence of the malnutrition depends on the assessed and number of those parameters. The proportion of malnutrition among people according to their Body Mass Index is 19%, and according to their weight loss is also 19.9%. We found that the effect of acute illnesses on the malnutrition risk is 14.4%. Comparing the age of the people, the men between 31–40 and the women between 61–70 have the lowest risk of malnutrition. The patients with gastroenterological, oncological and pulmonary illnesses had the highest risk of malnutrition. The nutrition team have measurable, proven impact on reducing the incidence of malnutrition. In institutions where nutrition teams operates, nutritional status of the patients improved. **Conclusions:** We received only partially the same results as in the nutritional status studies performed earlier. The prevalence of malnutrition was reduced compared to the last several years. Our country almost reached the prevalence recorded in the United Kingdom. To reduce the prevalence of malnutrition, it is important to screen the nutritional status.

P118. Nutrition status of Parkinson's disease patients

Kiss A.¹, Hartmann E.², and Lelovics Z.³

¹Semmelweis University, Faculty of Health Sciences, Budapest, Hungary

²Szent Lukács Hospital, Dombóvár, Hungary

³Kaposvár University, Kaposvár, Hungary, E-mail: kiss.anna891@

gmail.com

Introduction: risk of malnutrition with Parkinson's disease patients is high(er), however, this prevalence is yet to be well-defined. **Goal:** The screening of nutrition status of Parkinson's disease patients and ascertainment of their prevalence. **Method and study subjects:** the authors of this study screened the nutrition status of 245 volunteer Parkinson's disease patients older than 18 years (28,2% men and 71,8% women, average age 71,7 ± 16,9 years) partly with nDay, but valid nutrition status screening tools (MUST)[1] were used at each and every case. The youngest patient was 18,6, the oldest was 97,5 years old. Data analysis was conducted with univariate linear regression analysis and two-sample t-tests. **Results:** average Body Mass Index (BMI) of men with Parkinson's disease was 24,8 ± 4,2, while women's was 25,5 ± 4,8 [kg/m²]. 44,1% of men and 46,5% of women belonged to the optimal Body Mass Index category (18,5<BMI<25,0). 39,7% of men, and 29,7% of women belonged to the first-degree obesity category (preobes, 25,0 ≤ BMI<30,0), and 10,3% of men and 19,8% of women belonged to the second-degree obesity category (obes, 30,0 ≤ BMI<40,0). 5,9% of men and 4,1% of women belonged to the malnourished category from the BMI's perspective (BMI<18,5), which means that their protein-energy status was most probably disagreeable, and only one man belonged to the severely malnourished category (BMI<16,0). In the three-six months prior to the screening, large-scale decrease (> 10%) in body mass was not characteristic: only 5,9% of men and 4,1% of women were affected. According to MUST, the risk of malnutrition is low among the 60,9% of male and 63,1% of female Parkinson's disease patients, and mediocre among the 10,1% of men and 9,7% of women patients. Men are at higher risk of malnutrition (29,0% vs. 27,3%). **Conclusions:** prevalence of malnutrition risk among Parkinson's disease patients is 29,0% with men, and 27,3% with women; it appears eminently among men below the age of seventy, and women above the age of sixty. Knowing the nutrition status is informative and serves as guidance in the planning of personalized treatment. Through optimal nutrition, personal rehabilitation of Parkinson's disease patients will come to realization.

P119. Extreme long endurance exercise and performance based nutrition

Lelovics Z.¹, and Kiss A.²

¹Kaposvár University, Kaposvár, Hungary

²Semmelweis University, Faculty of Health Sciences, Budapest, Hungary, E-mail: lelovics@yahoo.com

Introduction: Some races (for example, the Vienna-Budapest Supermarathon, Ultrabalaton, Supermarathon around The Lake Balaton, Ironman, Spartathlon, Marathon des Sables) mean extreme endurance test for the human body. The successful delivery/finish is unimaginable without an adequate diet, on the go "charging" and upgrading. Our aim was to explore the available and adequate products on these distances. **Methods:** Our method was qualitative and quantitative analysis of the available assortment in the food store of the food for sport and energy drinks (n=60) based on manufacturing data. The data were characterized by descriptive statistical analysis. The

competition was modelled energetically and logistic regression (in case of different types of motion with multiple logistic regression analysis) was used to calculate values from items available. Results: The energy- (0-261 kJ, 0-62 kcal) and nutrient content (protein 0-62.0g, fat 0-15.0g, carbohydrate 0-14.3g) of the items (100 ml) is widely distributed, but its ion content (Na <45mg, K <26mg, Ca <33mg) does not fulfil the needs during endurance training (confidence interval 95%). The average content of stimulating agents in 100 g: 38 mg caffeine (range: 0-200 mg) and 382 mg taurine (range: 0-1000 mg). We could not find any food industry products or combination of them ($p < 0.075$) for a daily 1800 kcal energy intake that fit our models (Goodness of fit, Hosmer and Lemeshow Test, χ^2 : 7.9; df: 8; $p = 0.44$). We faced osmolarity problems in 70% of the products. Conclusion: Optimal nutrition during exercise means, that the ratio of the components, quality and energy content of the food is tailored to absorption possibilities during exercise, and takes into account the needs of the specific sport, the individual tolerance and fitness status. However, the found products do not match these.

P120. Flocculation as the critical parameter in the stabilization of egg-free mayonnaise

Emadzadeh B.^{1*}, Kadkhodae R.¹, and Naji S.²

¹Department of Food Nanotechnology, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran

²Department of Food Science and Technology, Ferdowsi University of Mashhad, Iran, E-mail: b.emadzadeh@rifst.ac.ir

By increasing the consumers' knowledge about the effect of diet on the prevention of certain diseases, production of egg yolk-free food products have been improved seriously. Mayonnaise is one of the most popular dressings around the world, but many people who are allergic to the egg yolk are not able to enjoy its consumption. Moreover, its emulsion based nature has made its pasteurization process impossible. Concerning these limiting conditions, in this study the production of egg yolk free mayonnaise has been investigated. The effect of modified starch and xanthan gum on the flocculation process has been evaluated. The particle size, stabilization, zeta potential and the rheological characteristics have been studied. The application modified starch was not able to produce a stabilized emulsion system. However, the addition of xanthan gum made the system more stabilized probably due to the intensive effect of depletion flocculation produced by the added gum.

P121. Ethanol: a confounding factor in diagnostics based on levels of particular anabolic-androgenic steroids?

Jarek A.^{1*}, Kowalczyk K.^{1,2}, Szczepańska Z.¹, Wójtowicz M.¹, Pokrywka A.¹, Bulska E.², and Kwiatkowska D.¹

¹Department of Anti-doping Research, Institute of Sport, Warsaw, Poland

²Faculty of Chemistry, Biological and Chemical Research Center, University of Warsaw, Poland, E-mail: anna.jarek@insp.waw.pl

The effects of alcohol ingestion have already been the subject of numerous researches covering all branches of medicine. Although its influence on metabolism and development of particular diseases is well recognized, both for animals and humans, the misinterpretation of diagnostic tests for non-alcohol related diseases caused by ethanol ingestion is not fully recognized. The aim of the project was to evaluate the influence of alcohol consumption on the levels of selected endogenous anabolic-androgenic steroids (namely: testosterone, epitestosterone, dehydroepiandrosterone, androsterone, etiocholanolone, 5- α -androstadiol, and 5- β -androstadiol). The group of above 40 healthy volunteers, both men and female (18-40 years old) were involved in this study. Prior to the experiment, all volunteers had been tested for the presence of UGT2B17 gene to ensure that their urine would qualify for analysis of testosterone based on determination of its glucuronide. Each individual was asked to ingest 2 mg of alcohol per kilogram of body mass in less than half an hour. Then, all participants were asked to collect urine samples according to the schedule, moreover they were asked to avoid substances and activities that might disturb alcohol metabolism. In order to observe the correlation between the levels of steroids and remaining alcohol, the content of ethyl glucuronide was also measured. Finally, the results were analyzed in order to determine whether single ethanol ingestion can disturb the levels of selected steroids enough to result in misinterpretation of diagnostic analysis.

P122. Comparison of antioxidant activity and phenolic content of garlic, leek and onion vegetables in different cooking methods

Afshoun E.^{1*}, Mirzaei A.¹, and Afshoun A.²

¹Medicinal Plant Research Center, Yasuj University of Medical Sciences, Yasuj, Iran, and ²Dameghan University, Dameghan, Iran, E-mail: esfandafshoon@gmail.com

Epidemiological studies have shown consumption of vegetables and Fruits were related with reduced risks of several age-related and degenerative human disease, cardiovascular and cancers. Among the widely consumed vegetables garlic (*Allium sativum* L.), leek (*Allium porrum*) and onions (*Allium cepa* L) are important medicines to the ancient Iranian. Material and Methods: The vegetable samples (2.5 kg) were collected and five portions (500 g each) were prepared. One portion was kept raw as control stored at 4°C in the fridge and others were subjected for four thermally treatmentssuch asboiling, Pressure-cooking, Microwave cooking and frying.Raw and cooked vegetables were analyzed by different antioxidant activity diphenyl-2-picrylhydrazyl (DPPH) and trolox equivalent antioxidant activity (TEAC methods and total phenol contents. All data were expressed as means \pm standard deviation .For statistics analysis samples one-way analysis of variance (ANOVA) was applied. Results: After cooking procedures, the total phenolics content of onion samples was insignificantly reduced. However significant reductions in leek and garlic were reported. In total phenol procedure the highest losses were reported by leek in boiling method .Leek showed highest scavenging

activity (32.7 $\mu\text{M TE/g}$) whereas onion had lowest activity with 21 $\mu\text{M TE/g}$ by diphenyl-2-picrylhydrazyl (DPPH), assay. According to result of the trolox equivalent antioxidant activity (TEAC) radical scavenging activity the highest losses were reported in garlic in boiling method. The boiling cooking method in all assays was reported with maximum of % losses. Conclusion: Among spice vegetables tested, garlic had the highest phenolic contents and antioxidant activity. All antioxidant activities decrease in the vegetables except leek in thermal treatments by different methods.

P123. Hepatoprotective effect and antioxidant activity of aqueous cherry extract on rats

Borzabad A.¹, Ali M.¹, and Alireza A.²

¹Medicinal Plant Research Center, Yasuj University of Medical Sciences, Yasuj, Iran

²Dameghan University, Dameghan, Iran, E-mail: afshoon.es@yums.ac.ir

Abstract: Fruits and vegetables rich in antioxidant compounds which combat against free radicals. Many of food products contain synthetic antioxidants, with undesirable effects so, many of scientist have interest to the natural antioxidants with plant sources, especially medicinal plants. In this research, the hepatoprotective effects of cherry on the hepatotoxic rats' liver which induced by carbon tetrachloride was studied. Materials and methods: In this study, it was used five tests such as diphenyl picryl Hydrazyl(DPPH), trolox equivalent antioxidants activity(TEAC), Ferric reducing antioxidant power (FRAP), total phenol and flavonoid contents. For in vivo study used 28 wistar male rats which divided into four groups of seven rats each (olive oil control, plant control, carbon tetrachloride control(toxin) and treat groups). At the end of seventh day the for evaluation of hepatoprotective effects of cherry seed Alkaline phosphatase ALP, Alanine transferase ALT, aspartate transferase AST enzymes and bilirubin was measured in rat serums. Results: There was a significant increase in hepatic enzymes and bilirubin levels in carbon tetrachloride group compare to negative control. The extraction of cherry in 250 mg dose was able to alleviate the induced damages compare to toxic group ($p < 0.05$). Conclusion: it can be suggested that cherry extract show hepato-protective potential in selected dose.

P124. Validation of rapid microbiological methods

Esteve-Romero J.¹, Peris-Vicente J.¹, Ochoa-Aranda E.², Raviolo M. A.³, Kassuha D.⁴, and Carda-Broch S.¹

¹Dep. de Química Física i Analítica, E.S.T.C.E., Universitat Jaume I, Campus Riu Sec, 12071 Castelló, Spain

²Biopatología Molecular, Hospital Provincial, Castelló, Spain

³Dep. Farmacia. Universidad Nacional Córdoba, Argentina

⁴Química Biológica y Farmacología, Facultad de Ciencias de la Alimentación, Bioquímicas y Farmacéuticas, Universidad Católica de Cuyo, San Juan, Argentina, E-mail: josep.esteve@uji.es

Classical microbiological methods have nowadays unacceptably long cycle times. Rapid methods, available on the market for decades, are already applied within the clinical and food industry, but the implementation in pharmaceutical industry is hampered by for instance stringent regulations on validation and comparison with classical methods. In order to encourage the implementation of these methodologies, they must be validated to assess that the results are straightforward. The comparison with traditional methods should be also performed. In this manuscript, several information about the validation of Rapid Microbiological Method are provided, explaining the difficulty of validation of microbiological methods. This would be useful for industries and laboratories which would implement these methods.

P125. Quantification of antibiotics in milk and egg samples by micellar liquid chromatography

Esteve-Romero J.*, Rambla-Alegre M., Carda-Broch S., Peris-Vicente J., Villareal-Traver M., and Tayeb-Cherif K.

Dep. de Química Física i Analítica, E.S.T.C.E., Universitat Jaume I, Campus Riu Sec, 12071 Castelló, Spain, E-mail: josep.esteve@uji.es

Antibiotics, or antimicrobial agents, are defined as drugs of natural, semisynthetic, or synthetic origin with antibacterial, antifungal, or antiparasitic activity. Antibiotics are used in human and veterinary medicine because they are equally effective against bacterial diseases in plants or aquaculture. The widespread use of antibiotics may leave residues in foodstuffs of animal origin, and can also produce allergic reactions in humans, especially when drugs are used wrongly or abusively (e.g., withdrawal periods are too short, incorrect doses, self-medication, etc.). Antibiotic use is a major contributor to the selection and propagation of resistant bacterial strains that pose a serious health risk to humans and animals. Thus, in the European Union and the United States, many efforts have been made to protect consumers' health from exposure to residue levels. The U.S. Food and Drug Administration (FDA) and the European Commission have established lists of tolerance levels, also known as maximum residue limits (MRLs), for different food contaminants for several raw foods on the basis of toxicological data, acceptable daily intake values, and the performance of present-day analytical technology. Food surveillance programs used to enforce legislation on drug residues in animal products rely heavily on the availability of fast and accurate analytical screening methods. Micellar liquid chromatography (MLC) is an attractive alternative to conventional RP-HPLC that uses a surfactant solution above the critical micellar concentration instead of aqueous-organic solvents as mobile phases for the determination of compounds in a variety of matrixes with direct injection. This work includes the methods developed in MLC for the determination of antibiotics in egg and milk samples.

P126. Determination of antibiotics in fish grown in fisheries using micellar liquid chromatography

Esteve-Romero J.*, Peris-Vicente J., Rambla-Alegre M., Carda-Broch S., Tayeb-Cherif K., Garrido-Cano I., and Álvarez-Rodríguez L.

Dep. de Química Física i Analítica, E.S.T.C.E., Universitat Jaume I, Campus Riu Sec, 12071 Castelló, Spain, E-mail: josep.esteve@uji.es

A simple and sensitive method was optimized and validated for the simultaneous analysis of five antibiotics (oxolinic acid, flumequine, enrofloxacin, difloxacin and sarafloxacin) in seven different fish muscles (gilthead, salmon, trout, sea bass, mussel, prawn and turbot). Quinolones are used mainly in the treatment of human and animal diseases. However, their extensive use in fish grown in fisheries implies drug residues persisting in the edible tissue, which can reach human and provoke drug resistance. For this reason, the European Union has set maximum residue limits (MRL) for most of them. Therefore, reliable analytical methods are required to analyze fish samples before they are available in the market, in order to assess their safety. In the sample preparation, the analytes are extracted by mixing the minced solid sample in a micellar solution and shaking. An aliquot was filtered and injected. No organic solvent was used. Under optimized extraction conditions, high recovery was found for the five quinolones. The chromatographic resolution was achieved using a C18 as stationary phase and a micellar solution made of sodium dodecyl sulfate, propanol and triethylamine at pH 3, without interferences. A fluorescence program was used at the following times and excitation/emission wavelengths: 0-10 min, 260/366 nm and 10-200 min, 280/450 nm. The analytical method was validated following the requirements of European Commission Decision 2002/657/EC. Selectivity, linearity, linear range, sensitivity, decision limit, detection capability, accuracy, precision, robustness and recovery were calculated with excellent results. Finally, the micellar chromatographic method was applied to quantify the five studied quinolones in fish samples purchased at a local supermarket.

P127. Use of micellar liquid chromatography to analyze hydroxytyrosol in olive extracts

Peris-Vicente J.¹, Esteve-Romero J.¹, Beltrán-Martinavarró B.¹, Marco-Peiró S.¹, Kassuha D.², Raviolo M. A.³, and Carda-Broch S.³

¹Dep. de Química Física i Analítica, E.S.T.C.E., Universitat Jaume I, Campus Riu Sec, 12071 Castelló, Spain

²Química Biológica y Farmacología, Facultad de Ciencias de la Alimentación, Bioquímicas y Farmacéuticas, Universidad Católica de Cuyo, San Juan, Argentina

³Dep. Farmacia. Universidad Nacional Córdoba, Argentina, E-mail: juan.peris@guest.uji.es

Olive is a fundamental ingredient of the Mediterranean diet. Over the past few years, its diffusion and consumption have spread remarkably outside the Mediterranean basin. The growing interest in olive lies in its unique taste and nutritional properties. Recent findings demonstrate that hydroxytyrosol, olive phenolic compounds, is a powerful in vitro and in vivo antioxidant, and display other biological activities that could partially account for the observed healthy. Micellar liquid chromatography (MLC) is a liquid chromatographic technique in which aqueous solutions of surfactant over the critical micellar concentration are used as the mobile phase. While the surfactant

modifies the nature of the stationary phase, micelles interact with analytes, as natural compounds of olive oil, and direct injection of the samples could be performed. Using a chemometric strategy, we develop a method for the determination of these compounds in olive extracts, using a Kromasil C18 column and a micellar mobile phase of 0.05 M of SDS-4% methanol-pH 7, flow rate of 1 mL/min, volume injection of 0.020 mL and detection at 210 nm, was performed in the UV region. Samples were directly injected into the chromatographic system after dilution with 0.05 M SDS. Method validation was performed following the U.S. Food and Drug Administration guideline.

P128. Detection of melamine in milk by micellar liquid chromatography

Peris-Vicente J.¹, Esteve-Romero J.¹, Beltrán-Martinavarró B.¹, Marco-Peiró S.¹, Carda-Broch S.¹, Raviolo M. A.², and Monferrer-Pons L.¹

¹Dep. de Química Física i Analítica, E.S.T.C.E., Universitat Jaume I, Campus Riu Sec, 12071 Castelló, Spain,

²Dep. Farmacia, Universidad Nacional Córdoba, Argentina, E-mail: juan.peris@guest.uji.es

Melamine is a nitrogen-rich non-expensive industrial chemical. Because of these properties, it has been used to apparently increase the nitrogen content, and then the protein content and consequently the price, in milk samples. In September 2008, melamine-tainted milk resulted in nephrolithiasis and renal failure in infants in China. Due to the proved toxicity of melamine, safety limit of melamine ingestion has been officially set by the US FDA at 2.5 ppm for adult's food, and at 1 ppm for infant formula. Therefore, a reliable method is needed to determine melamine residues in milk, particularly in products for children, in order to eliminate the potential threat to human health. A micellar liquid chromatography method was developed to quantify melamine in liquid and powdered milk. Samples were solved in a sodium dodecyl sulphate solution, filtered and directly injected, thus avoiding long, time consuming and contaminant extraction steps. The chromatographic separation was performed using a C18 column as stationary phase and a micellar solution of 0.05 M sodium dodecyl sulphate - 7.5% propanol buffered at pH 3. Detection was set at 210 nm. Melamine was eluted in nearly 9.3 min without overlapping with matrix compounds. The validation was performed following the requirements of the European Decision 2002/657/EC, in terms of: selectivity, linearity ($r^2 > 0.9990$), calibration range (0.02-100 ppm), limit of detection (5 ppb), Decision limit, detection capability, accuracy (-9.8 to +6.0%) precision (< 9.7%), and robustness (< 7.4%). Sensitivity was enough to detect melamine under the safety limits proposed by FDA. The method was applied to powdered and liquid milk samples, for adult and infant consumption, purchased at a local supermarket.

P129. Analysis of melamine in swine kidney by micellar liquid chromatography

Peris-Vicente J.*, Esteve-Romero J., Beltrán-Martinavarró B., Marco-Peiró S., Carda-Broch S., and Fabregat-Safont D.

Dep. de Química Física i Analítica, E.S.T.C.E., Universitat Jaume I, Campus Riu Sec, 12071 Castelló, Spain, E-mail: juan.peris@guest.uji.es

Melamine is a toxic compound illegally used for the adulteration of animal feed to boost protein content. It increases the nitrogen concentration, then provoking false measuring in the protein concentration if determined with Kjeldahl-related method. Therefore, the spiked feed product seems of high quality and can be sold at a higher price. The ingestion of products contaminated with melamine has been related to renal diseases, urolithiasis and bladder cancer in both animals and humans. Thus, the level of melamine intoxication of animal should be determined if the use of contaminated feed is suspected. This can be performed the measuring of melamine concentration in the kidney, as this organ accumulates melamine previous to its filtration. An easy and sensitive method was reported for the routine identification and quantification of melamine by micellar liquid chromatography in swine kidney samples. The analyte was extracted from the samples by simply shaking in methanol, solved in sodium dodecyl sulphate aqueous solution and filtered previous injection. Melamine was determined in a C18 column using a micellar mobile phase of 0.11 M sodium dodecyl sulphate and 7.5% propanol at pH 3. Absorbance detection was set at 210 nm. Under these conditions, melamine was eluted at 6.0 min with no interference by the endogenous compounds. The method was validated using a non-contaminated sample of swine kidney, according to the Food and Drug Administration guidelines in terms of: sensitivity (LOD and LOQ were 100 and 300 ppb, respectively), calibration range (0.3 to 20 ppm), linearity ($r^2 > 0.9997$), intra- and inter-day precision ($< 7.2\%$ and $< 7.6\%$, respectively) and accuracy (91.7-103.6% and 91-109.3%, respectively), and robustness ($< 5.1\%$). The methodology was applied in routine analysis of animal kidney to post-mortem diagnose the melamine intoxication in animals and prevent further human intoxication by ingestion of contaminated food.

P130. Analysis of biogenic amines in Anchovy sauce using micellar liquid chromatography

Roca-Genovés P.^{*}, Esteve-Romero J., Peris-Vicente J., Rambla-Alegre M., Carda-Broch S., Romero-Cano R., and Monferrer-Pons L.

Dep. de Química Física i Analítica, E.S.T.C.E., Universitat Jaume I, Campus Riu Sec, 12071 Castelló, Spain, E-mail: pasqualroca@hotmail.com

Biogenic amines are biological metabolites present in foods either as natural products or after fermentation, decay microbial contamination, decomposition or putrefaction processes. The main biogenic amines are: putrescine, tyramine, cadaverine, 2-phenylethylamine, histamine, spermidine and spermine. The presence of these compounds in food indicates a high level of bacterial contamination and a high degree of spoilage. The consumption of an excess of biogenic amines, known as histamine intoxication, is mainly related to heart, gastrointestinal and skin diseases, as well as headache. Fish products, as fish sauces, can be spoiled in low time by incorrect handling or stocking conditions (freezer at -18°C). Then the determination of biogenic amines is

of the utmost importance to assure that the fish sauce can be eaten without health risk. A practical micellar liquid chromatography-based methodology has been developed for the selective measurement of the levels of each biogenic amine in anchovy sauce, after derivatization with 3,5 dinitrobenzoyl chloride. Because of the strong differences between the hydrophobicity of the biogenic amines, they were studied by three groups: (putrescine and tyramine) (cadaverine, cadaverine, 2-phenylethylamine, histamine, spermidine) and spermine. Each group was resolved using a different mobile phase. The common conditions of the method were: stationary phase, C18 column (125 X 4.6 mm; 5 mm particle size); mobile phase containing SDS/organic modifier buffered at pH 7; detection set at 260 nm; flow-rate 1 mL/min running under isocratic mode at room temperature. The difference was the composition of the mobile phase (SDS amount, organic modifier nature and concentration) and the optimization performed using an interpretative model by testing only five mobile phases. The analytical method was successfully validated according to FDA guidelines in terms of: linearity ($r^2 > 0.999$), detection and quantification limits, precision ($< 4.2\%$), accuracy (88.6–103.7%) and robustness ($< 4.8\%$). These results are in agreement with the requirements of FDA guidelines. The proposed method was successfully applied to the monitorization of biogenic amines in unsalted and salted fish sauce samples. According to the results, salting was a useful technique to avoid anchovy sauce spoilage. The suggested methodology was found useful in routine analysis of biogenic amines in fish sauce samples.

P131. Simple chromatographic method for determination of thiram, cabendazim and ziram in agricultural samples

Roca-Genovés P.¹, Esteve-Romero J.¹, Peris-Vicente J.¹, Bose D.², Durgbanshi A.², Carda-Broch S.¹, Garrido-Cano I.¹, and Álvarez-Rodríguez L.¹

¹Dep. de Química Física i Analítica, E.S.T.C.E., Universitat Jaume I, Campus Riu Sec, 12071 Castelló, Spain

²Department of Criminology and Forensic Sciences, Dr. H. S. Gour University, Sagar, India, E-mail: pasqualroca@hotmail.com

Fungicides are a very important group of pesticides and the most commonly used are carbendazim, thiram and ziram. Carbendazim is a broad spectrum benzimidazole carbamate systemic fungicide while thiram and ziram are protective fungicides belongs to dithiocarbamate family. The most common uses of these compounds in agriculture are as foliar spray, seed and soil treatments agent, seed treating agents or as rubber accelerators. After chemometric optimization, best conditions for the determination of the three compounds in the minimum analysis time, were: a Princeton SPHER-100 C18 100A (250 mm x 4.6 mm, 5 μm particle size) column, coupled to a DAD set at 275 nm, flow rate at 1 mL/min and injection volume of 20 μL , using a mobile phase composed for 0.125 M SDS-2.5% (v/v) pentanol-pH 7. Under these conditions, the three compounds could be resolved in less than 10 min. Validation studies were performed using ICH guideline obtaining: good linearity ($r^2 > 0.999$), adequate LODs (3s criteria) of 25, 25 and

30 ng/mL for thiram, carbendazim and ziram respectively, and intra- and inter-day precision (R.S.D,%) was less than 2.5. The developed method was applied to the determination of selected fungicides in seeds, commercial formulations, plant material, soil and water with any prior pretreatment step (apart from washing, mincing or filtering) and injecting directly onto the chromatographic system, with good recoveries.

P132. Green method to determine melamine in dietetic supplements

Roca-Genovés P.*, Esteve-Romero J., Peris-Vicente J., Beltrán-Martinavarró B., Carda-Broch S., and Marco-Peiró S.

Dep. de Química Física i Analítica, E.S.T.C.E., Universitat Jaume I, Campus Riu Sec, 12071 Castelló, Spain, E-mail: pasqualroca@hotmail.com

Micellar liquid chromatography has proven a fast, sensitive, and selective technique for the determination of melamine in a wide variety of dietetic supplements for infants and adults. The developed method allows the rapid determination of melamine by the direct injection of the samples into the chromatographic system, after filtration, thus avoiding long, tedious extractions, the analysis time was under 10 min. Conditions were: 0.15 M SDS- NaH₂PO₄ pH 3 as mobile phase, detection at 210 nm, flow rate of 1 mL/min in a Kromasil C18 column (5 micro-m particle size, 150 mm×4.6 mm i.d). Validation was done following the European Commission (Decision 2002/657/EC) validation guideline. The parameters evaluated were: linearity, LOD and LOQ, precision, accuracy, selectivity, decision limit, detection capability, robustness and recovery, with satisfactory results. This method meets the requirements of the “green chemistry” concept since no organic solvent has been used. Besides, it is relatively inexpensive compared to other methods, thus making it a more appealing method. The proposed technique could be recommended as a routine method for the analysis of melamine in dietetic supplements for both infants and adults. Finally, samples from 12 dietetic supplements for adults and 15 for infants) were analyzed and in all cases results were in agreement with the declared content by the manufacturer.

P133. Simultaneous multi-determination and transfer of eight pesticide residues from green tea leaves to infusion using gas chromatography

Kim S. W.¹, Cho S. K.², Abd El-Aty A. M.^{1,3}, Rahman M. M.¹, Choi J. H.¹, and Shim J. H.¹

¹Biotechnology Research Institute, College of Agriculture and Life Sciences, Chonnam National University, 300 Yongbong-dong, Buk-gu, Gwangju 500-757, Republic of Korea

²National Agricultural Products Quality Management Service, Products Safety Inspection Laboratory, Gwangsan-Gu, Gwangju, Republic of Korea

³Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt, E-mail: jhshim@jnu.ac.kr

A method for determining eight pesticide (cyhalothrin, flufenoxuron, fenitrothion, EPN, bifenthrin, difenoconazole, triflumizole, and azoxystrobin) residues in made green tea as well as a tea infusion (under various brewing water temperatures; 60, 80, and 100°C) using gas chromatography (GC) micro-electron capture detector (μ ECD) was developed and validated. The extraction method adopted the relatively commonly used approach of solid sample hydration, with the green tea hydrated before being extracted through salting out with acetonitrile followed by a cleanup procedure. The analytes were confirmed using GC-coupled to tandem mass spectrometry (GC/MS/MS) with a triple quadrupole. The linearity of the calibration curves yielded determination coefficients (R^2)>0.995. Recoveries were carried out using blank samples spiked with all analytes at two levels. The results demonstrated that all pesticides were recovered within the range of 77–116% with a relative standard deviation (RSD) \leq 14%. The quantification limits of 0.015 to 0.03 mg/kg were lower than the maximum residue limits (MRLs) set by the Korea Food and Drug Administration (KFDA) for all analytes (0.05–10 mg/kg). The infusion study indicated that cyhalothrin, flufenoxuron, and bifenthrin did not infuse into the tea brew from the made tea.

Increases in brewing time resulted in increased transfer of azoxystrobin, fenitrothion, and difenoconazole from the made tea to the brew; however, this was not the case with triflumizole or EPN. We conclude that transfer of pesticides appeared to be dependent on their water solubilities and drinking a cup of tea is recommended to be at a water temperature of 60°C.

P134. Evaluation of nutritional status and relationship between intelligence and academic achievement elementary students

Afshoun E., Kamkar A.*, Akbartabar M., and Afshoun T.

Social Determinants of Health Research Center, Yasuj University of Medical Sciences, Yasuj, Iran, E-mail: esfandafshoon@gmail.com

Introduction: A competitive country in the field of science and children's health today is founded. According to the teaching and learning of school children should be based on a scientific process. The purpose of this study was survey of relationship between nutritional status, intelligence and academic achievement of students. Material and methods: In this descriptive analytical study 397 boys and girls, fifth grade elementary students were examined. Evaluation of nutritional status, measured height (cm) and weight (kg) by the nutrition expert on intelligence tests and educational status was measured by an expert psychologist. Data analysis and testing of software EPI & SPSS Chi-square test and regression analysis was performed. Results: The findings showed that malnutrition of school students related to the present, past and present and past malnutrition 21.3, 44.2 and 31.2 percent respectively in the mild and moderate and severe malnutrition rates was 3.4, 13.9 and 21.2 percent ($P<0.046$). Between nutritional status (of present and past) with the IQ of the students showed a statistically significant dependence. And between IQ and academic achievement of students also showed a significant correlation ($P<0.0001$).

P135. Antioxidant properties evaluation in some vegetables

Afshoun E.¹, Afshoun A. R.², and Mirzaei A.¹

¹Medicinal Herbal Research Center, Yasuj University of Medical Sciences, Yasuj, Iran

²Dameghan University, Dameghan, Iran, E-mail: esfandafshoon@gmail.com

Introduction: Antioxidant potential is one of the most important sources of food and vegetables. The aim of this study was evaluate the antioxidant properties of 7 vegetables include: red radishes, white radishes, beets, broccoli, turnip, radish and red cabbage leaves. **Methods:** Antioxidant activity, (DPPH) Diphenyl picryl hydrazyl, (TEAC) Trolox equality Antioxidant capacity, (FRAP) Ferric Reducing Antioxidant potential, was performed on methanol extracts. Total phenol and vitamin C were analyses in all vegetable samples. **Results:** The lowest average total phenol of red radishes was (2.5 ± 20.7) and the highest of the leaves of radish (4 ± 109) and the lowest anti-radical activity of DPPH in the radish leaves (0.4 ± 203) and highest in white radishes (1.9 ± 12.4) mmol/kg of fresh vegetables. maximum and minimum of anti-radical activity of ABTs were (0.8 ± 5.3) and (turnip (0.2 ± 0.8) respectively. the mean FRAP antioxidant activity in radish leaves was (5.9 ± 93.3), cauliflower (2.1 ± 13.7) mmol /kg. VITC content in turnip was (8.6 ± 102), beets (2.3 ± 16.3) mmol/ kg. **Conclusion:** The highest antioxidant activity of the leaves of cabbage and radish leaf, minimum to red radishes, white radishes, beets and cauliflower, respectively have been.

P136. Washing and blanching substantially reduced the residue levels of Pyridaben in cumulative manner in hot pepper fruits and leaves

Kim S. W.¹, Abd El-Aty A. M.^{1,2}, Choi J. H.¹, Rahman M. M.¹, and Shim J. H.¹

¹Biotechnology Research Institute, College of Agriculture and Life Science, Chonnam National University, Yongbong-ro 77, Buk-gu, Gwangju 500-757, Republic of Korea

²Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211-Giza, Egypt, E-mail: jhshim@jnu.ac.kr

Analysis of residual quantities of insecticides in raw agricultural crops and in processed food is of particular importance from the public health point of view. In the present study, hot pepper fruits and leaves were grown under greenhouse conditions and pyridaben was applied either in single or double the recommended doses. Following QuEChERS and tandem mass spectrometry analysis, the residual levels were determined in unprocessed and processed fruits and leaves. The effect of various household processes, including washing, blanching, frying, and drying under different conditions (water amount, blanching time, and temperatures) on the residual concentrations were also evaluated. The recoveries at various concentrations were 79.9 – 105.1% with relative standard deviations $\leq 15\%$. The limits of quantitation

of 0.003 – 0.012 mg/mg were comparatively very low compared to maximum residue limit (MRL) set by the Ministry of Food and Drug Safety. Both washing and blanching (in combination with high water amount and time factor) are significantly reduced the residue levels in hot pepper fruits and leaves compared to other processes. It is therefore recommended to blanch food commodities/leaves after washing before being consumed, to protect consumers from the negative health effects of detected pesticide residues.

P137. Matrix enhancement effect: A blessing or curse for gas chromatography?

Shim J. H.¹, Rahman M. M.¹, and Abd El-Aty A. M.^{1,2}

¹Biotechnology Research Institute, College of Agriculture and Life Sciences, Chonnam National University, Gwangju, 500-757, Republic of Korea

²Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211-Giza, Egypt, E-mail: jhshim@jnu.ac.kr

The matrix enhancement effect in gas chromatography (GC) has been a problem for the last decade and results in unexpected high recovery. Most efforts, including the use of different types of injectors/matrix simplification procedures, and further clean-up associated with removing this effect has focused on equalizing the response of the standard in the solvent and matrix. However, after eliminating the matrix enhancement effect, the sensitivity of GC remained unchanged. But, GC sensitivity can be increased by utilizing this matrix effect originating from a matrix matched standard. Very few studies have highlighted utilizing the matrix effect but have rather advocated eliminating it. Analyte protectants (3-ethoxy-1, 2-propanediol, gulonolactone and sorbitol) have been introduced as an alternative for GC-mass spectroscopy (GC-MS) (not examined for other GC detectors), as they equalize the response without removing the matrix effect, and, hence, increase sensitivity. Versatile applications of analyte protectants are not observed in practice. The European guidelines recommend the use of matrix-matched standard calibration for residue measurements. As a result, numerous applications are available for matrix-matched standards that compensate for the matrix effect. Moreover, the matrices (among them pepper leaf matrix) act as a protectant for thermolabile analytes in some cases. A lower detection limit should be achieved to comply with the maximum residue limits. Therefore, the matrix enhancement effect, which is considered a problem, can play an important role in lowering the detection limit by increasing the transfer of analyte from the injection port to the detector.

P138. Hydrocolloids as fat replacers: A review on novel Iranian sources

Emadzadeh B.*

Department of Food Nanotechnology, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran, E-mail: b.emadzadeh@rifst.ac.ir; bahareh_e2020@yahoo.com

The fact of terrifying statistics of the growth of obesity through

the world has led the pathway of food researches to the point of calorie control esp. the fat reduction in food products. In this way, the application of hydrocolloids as fat replacers is a common and important issue. The water holding capacity, texturizing effect, stability and acceptable mouth feel are just a few effects of hydrocolloids application as fat replacers. The Iranian natural hydrocolloids as potential novel sources have been the subject of many recent studies. Their rheological and sensory properties as novel sources of fat replacer have been studied in the model and real food systems. From this point of view, they have shown brilliant comparable results. In this study, I have reviewed the recent studies on the applicability of Iranian natural hydrocolloids as novel sources of fat replacers.

P139. Detection of dicinnamates in plants and foods

Grúz J.*, Pospíšil J., Kozubíková H., Pospíšil T., Doležal K., and Strnad M.

Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR & Palacky University, Šlechtitelů 11, 783 71 Olomouc, Czech Republic, E-mail: jiri.gruz@upol.cz

Cinnamates are common phenolic compounds present in plants and plant foods in substantial quantities. Their high in vitro antioxidant activity means that they can be easily oxidized under oxidative conditions. In this study, we found that in vitro oxidation of coumaric, ferulic and sinapic acids resulted mainly in dimeric compounds. By using both, enzymatic and non-enzymatic in vitro approaches, we found that the most common oxo-products are various dimers, such as diferulic, disinapic and dicoumaric acids, which were relatively stable. These compounds were then monitored by specific UPLC-MS/MS methods in several plants and plant foods. As a result, we were able to confirm that these substances are naturally present in plants/foods as free compounds. The presence of oxidation products also means that simple phenolic acids act as antioxidants in plants. All identified structures were confirmed by comparing their retention times, MS fragmentation patterns and UV-VIS spectra with those of synthesized chemical standards.

P140. Analysis of etoxazole in red pepper after major modification of QuEChERS for gas chromatography-nitrogen phosphorus detection

Kim S. W.¹, Jang J.¹, Rahman M. M.¹, Abd El-Aty A. M.^{1, 2}, Ko A. Y.¹, Choi J. H.¹, and Shim J. H.¹

¹Biotechnology Research Institute, Chonnam National University, Gwangju, 500-757, Republic of Korea, and ²Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211-Giza, Egypt, E-mail: jhshim@jnu.ac.kr

A major modification of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method was developed to analyze etoxazole in red pepper using gas chromatography coupled with nitrogen-phosphorus detector (GC-NPD). Etoxazole was extracted

with acetonitrile, partitioned with magnesium sulfate, and purified with a solid phase extraction (SPE) cartridge. The method showed good linearity with a determination coefficient (R²) of 0.998 for the 0.02–2.0 mg/L concentration range. The method was validated using blank red pepper spiked at 0.2 and 1.0 mg/kg, and the average recovery rate was 74.4–79.1% with relative standard deviations <5% for intra- and inter-day precision. The limits of detection (LOD) and quantification (LOQ) were 0.007 and 0.02 mg/kg, respectively. The developed method was successfully applied to field incurred samples, and etoxazole residues were confirmed using gas chromatography/mass spectrometry (GC/MS).

P141. Electrospraying: A novel route toward synbiotic production

Ghorani B.¹, Sarabi Jamab M.², and Zaeim D.²

¹Department of Food Nanotechnology, Research Institute of Food Science & Technology (RIFST), Mashhad, Iran

²Department of Food Biotechnology, Research Institute of Food Science & Technology (RIFST), Mashhad, Iran, E-mail: behrooz.ghorani@gmail.com

Encapsulation of probiotic bacteria amidst prebiotic substances to protect or even enhance their survival whilst passing upper GI tract seems an enchanting topic for academia and industries. Protection shell can be fermented by colon microbiota and probiotic bacteria will be released in the colon. Different methods were suggested, examined and applied to encapsulate and dry probiotics which mostly reduce the viability of bacteria considerably. Electrohydrodynamic atomization or electrospraying is introduced as a state of the art technique for the drying and encapsulation of bacteria among a polymer substance that can produce capsules in micro and sub-micro range. In this versatile method, a probiotic culture and a prebiotic solution are atomized simultaneously using electrical forces. Charged droplets are dispersed in the space as a result of electrical repulsion that prevent agglomeration and coagulation of fine droplets. Solvent evaporation and drying are occurred at low temperature thank to the large surface area to volume ratio of small droplets and solidified particles are deposited on the collector. By optimizing different parameters such as voltage, feed rate, surface tension and viscosity of the solutions and exploiting a co-axial nozzle, micro-sized uniform spherical capsules which contain probiotic bacteria will attain. Moreover, molecular structure and chain length of prebiotic substance plays a substantial role in electrospraying process also probiotic protection during storage and passing upper GI tract.

P142. Electrospinning of cyclodextrin of functionalised cellulose acetate nanofibres

Ghorani B.*, and Kadkhodae R.

Department of Food Nanotechnology, Research Institute of Food Science & Technology (RIFST), Mashhad, Iran, E-mail: behrooz.ghorani@gmail.com

Electrospinning is introduces as a novel delivery vehicle for

supplementary compounds in food science technology and in the last couple of years, significant progress has been made in understanding the mechanisms of electrospinning and the development of electrospun fibres that in addition to the base polymer contain supplementary substances that transform the bulk properties and performance of the fibre web. Fibres can be produced containing insoluble nanoparticles and nanotubes as well as an array of soluble additives. Depending on the intended application these substances may be permanently immobilised within the polymer matrix or may be expected to be removed later, either for the purpose of delivery, e.g. drug delivery or to facilitate templating of the fibre. The incorporation of biomolecules as supplementary components within a polymeric electrospun fibre by first solution blending prior to electrospinning is an emerging technique for increasing the performance of materials, particularly in active food packing and Nanoencapsulation. Cyclodextrins are cyclic oligosaccharides consisting of 1,4-linked glucopyranoside units having either six, seven, or eight glucose units arranged in a cyclic structure, named as alpha-, Beta- and gamma-cyclodextrins, respectively. The hydrophobic cavity of CDs allows them to form noncovalent host-guest inclusion complexes (CD-ICs) with various molecules. The molecular encapsulation of food ingredients with cyclodextrin improves the stability of flavours, vitamins, colorants and unsaturated fats, and other lipophilic molecules, both in physical and chemical sense leading to extended product shelf-life. The purpose of this research was to study the production and properties of electrospun webs made from cellulose acetate (CA) and finding a new solvent system to establish means of incorporating the Beta- Cyclodextrin (CD) which is known to have a toroid like structure with a hydrophobic cavity, having the ability to accommodate, trap or include other molecules and can be described as a nanoencapsulation at molecular level.

P143. Fundamentals of molecular imprinting technique and their utilisation in selected target recognition in food sample analysis

Ghorani B.*

Department of Food Nanotechnology, Research Institute of Food Science & Technology (RIFST), Mashhad, Iran, E-mail: behrooz.ghorani@gmail.com

Since the introduction of the molecularly imprinting technique in 1970s, it becomes an emerging technology in the recent years with the potential for wide-ranging applications in food manufacturing, processing, analysis and quality control. The design and synthesis of materials possessing specific binding and catalytic behaviour has attracted considerable attention as a means of producing polymers exhibiting molecular recognition or template properties. Molecular recognition plays an indispensable role in nature for the recognition of antibodies, enzymes and nucleic acids. Biomimetic fibrous nonwoven materials are being developed that are capable of acting as highly sensitive and selective artificial receptors based on molecular recognition sites in the constituent fibres. It has been successfully applied in food microbiology, removal of undesirable components

from food matrices, detection of hazardous residues or pollutants and sensors. Conventionally, the production of molecularly imprinted polymers involves introducing binding sites wherein highly cross-linked co-polymers are formed around analyte molecules that act as cavity-creating templates. Subsequent removal of the template molecule provides recognition sites in the polymer that ideally resemble the template in terms of shape, size and functionality. Rebinding of the target molecule within these pre-formed sites can occur when the polymer is incubated in the presence of the template molecule.

The paper presents the fundamentals of molecular imprinting technique as applied to fibrous materials and electrospun nanofilters suitable for selected target recognition in food sample analysis.

P144. Evaluation instrument of nutrition literacy on adults (EINLA) the study of validity and reliability

Cesur B.¹, Koçoğlu G.², and Sümer H.²

¹Cumhuriyet University, Faculty of Health Sciences, Midwifery Department, Sivas, Turkey

²Cumhuriyet University, School of Medicine, Public Health Department, Sivas, Turkey, E-mail: bbusracak@gmail.com

Introduction: Literacy is said to be a key role in differences in eating habits. With the integration of nutrition science and literacy models in the community to create a vision by providing nutrition literacy of the people to increase control over their eating behaviors with more healthy and sustainable quality of life. Objective: The aim of this study is to evaluate the nutrition literacy is to develop a reliable and valid evaluation instrument. Materials and Methods: The Evaluation Instrument of Nutrition Literacy on Adults which had been developed with the purpose of evaluating the nutrition literacy had been implemented to 266 individuals who live in Sivas city Centrum in Turkey, who are at least primary school graduate and who are between the ages of 18-64. On evaluation of the data, content validity, construct validity, the average item severity and distinctiveness indexes had been implemented. The expert opinions regarding the evaluation instrument had been observed as compatible. In the result of the factor analysis made towards the construct validity, among the items that take place in the evaluation instrument, the factor load point that belong to all of them except the 1st item had been determined as above .30 and as they are gathered in 5 factors. After three weeks the individuals surveyed to investigate the reliability of responses to questions selected by systematic sampling method was applied to 60 people re-evaluation instrument and test-retest correlation coefficient of the scale was 0.85. For the evaluation instrument, the Cronbach Alfa reliability coefficient is 0.75, the average item severity and distinctiveness indexes are 0.552 and 0.730, respectively. In the result of validity and reliability analysis, 3 questions had been removed from the instrument that constituted of 38 questions and the Evaluation Instrument of Nutrition Literacy on Adults had been developed with totally 35 questions. Result: Consequently "The Evaluation Instrument of Nutrition Literacy on Adults" can be used as a valid and reliable instrument for the evaluation of nutrition literacy.

P145. The effectiveness of lifestyle modification intervention in obese children

Esfarjani F.¹, Mohammadi F.², Khalafi M.², Roustae R.², and Etebarian A.³

¹National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

²Food and Nutrition Policy and Planning Research Department National Nutrition & Food Technology Research Institute (NNFTRI), Shahid Beheshti University of Medical Sciences, Tehran, Iran

³School of Dentistry at Tehran University of Medical Sciences Tehran, Iran, E-mail: fesfarjani@hotmail.com

Background: Obesity has increased all over the world and becomes a major health problem. The purpose of study was to assess the effect of lifestyle modification intervention in elementary school obese children. **Materials and methods:** This study is a field trial and was accomplished in a random sample of 7 year- old obese children (n=156) who were selected from 3 area of health clinic in Tehran. Randomly divided to case (n=70) and control (n=86). Anthropometric indices were measure according to standard procedure. Biochemical tests were done on children blood sample. Questionnaires on demographic characteristics, lifestyle and food frequency (FFQ) were completed. Data were analyzed by SPSS. **Results:** Evaluation of anthropometric indices in obese children showed that although weight and height increased significantly in both groups, Weight increased more slowly in intervention group. The largest declined in waist and hip. Among biochemical indices, only blood triglyceride and cholesterol decreased significantly. Evaluation of food group consumption showed that not only, utilization of milk, dairy and nuts group increased significantly in intervention group but also consumption of braes and cereals, butter, cream, mayonnaise, sugar and confectionery ingredients significantly decreased. Use of oil and fat group decreased dramatically in case group. **Conclusion:** Overall, if obesity interventions were done in longer periods including all family and school members, weight control and lifestyle modification would be more efficient. It is obvious that Government policy and organization support are also effective ways to control children obesity in order to have a healthy society in the future.

P146. Functional foods can decrease burden of non communicable diseases and raise food security

Esfarjani F.^{*}, Mohammadi F., and Alikhanian H.

National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran, E-mail: fesfarjani@hotmail.com

Introduction: urbanization and decrease in rural population leads to fundamental changes in food consumption patterns, its processes and hazards. These complications establish challenges & resulted in more prevalence of non communicable diseases. Cardiovascular diseases

(CVD) are the main cause of death in patients with diabetic type 2. Risk of CVD and its risk factors in diabetics is 2-4 times more than healthy people. High blood pressure, increased level of triglyceride and LDL & low level of HDL which is features of dislipidemi, known as risk factors of CVD in diabetics. **Results:** probiotics are none- pathogenic micro organisms that if they are administered in adequate amount and live in special food items can have beneficial effects, by developing microbial balance in intestine. Probiotics are used as part of fermented functional foods. WHO introduce probiotics as none pathogenic non toxic, with appropriate adhesion strength to epithelial cells and proliferation capability, which is primary condition for probiotics establishment in different parts of body and significant factor for competing with harmful bacteria. Since these bacteria have effects on components of diet in intestine like digesting lactose and lipid metabolism such as cholesterol, causes an increase in host's health and supply nutrients like vitamin B which leads to decrease in nutrient's deficiency in society and food security. Nowadays yogurt is the most consumed probiotic product which has been made this product as the most popular and an important dairy product. It is probable to make some changes in processing steps or adding specific additives to basic media in order to increase biological capability and improvement in sensory properties in final product. In addition a particular type is designed aiming to treat or prevent specific diseases, their complications type of probiotic organizes in nutrient culture and their numbers in ml or g of product are two significant determinant of probiotic property of the product. **Conclusion:** Many researches show effectiveness of probiotics in diabetes type 2, so that probiotics yogurt decreases fast blood sugar, total and LDL cholesterol, improve antioxidants status and leads to reduction in cardiovascular risk factors. Therefore consuming probiotic yogurt as complementary treatment can be recommended to these patients. As a consequence, when all cells are fed with adequate and safe nutrients health and active life is gained and food security will be achieved.

P147. Studying the therapeutic effects of dietary iron on human gut microbiota activities between ulcerative colitis patient using an in vitro batch culture models

Khalil N. A.¹, Andrews S. C.², Gibson G. R.³, and Tuohy K. M.⁴

¹Faculty of Home Economics, Menoufia University, Egypt

²School of Biological Sciences, University of Reading, UK

³School of Chemistry, Food Biosciences and Pharmacy, University of Reading, UK

⁴Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Trento, Italy, E-mail: nazahkhalil@yahoo.co.uk

There is much current interest in the relationship between diet, human health and disorders such as inflammatory bowel diseases, particularly Ulcerative Colitis (UC). The most prominent colonic bacteria linked with UC are Sulphate reducing bacteria (SRB) because of their toxic fermentation end product, hydrogen sulphide (H₂S). A

possible approach to reducing the harmful impact of dietary sulphate could be trapping such gas before it is able to exert its harmful effects on the epithelium. Iron, zinc and bismuth have been indicated effectively for this purpose when used in in vitro batch culture (healthy faecal inocula). However, such experiments from UC patients have not been reported. So, triplicate 48-batch cultures were carried out (0.5 mM iron citrate; 0.5 mM sodium sulphate; 0.5 mM sodium citrate; mix of 0.5 mM iron citrate and sodium sulphate; and no additions). All cultures were sampled (0, 5, 10, 24, 36 and 48 h) for determination of bacterial densities (FISH), SCFA detection, H₂S levels and cytotoxicity effects. Results show that SRB and Clostridial growth are favoured by sulphate, whereas Bifidobacterial and Eubacterial groups were disfavoured. Sulphate shifted SCFA production away from beneficial butyrate towards acetate – inclusion of iron with the sulphate partly reversed this shift in the SCFA profile. H₂S increased by sulphate addition, but this increase was not observed when iron was also included. Culture supernatants from the sulphate-supplemented cultures displayed significant cytotoxicity against HT-29 cells reflecting the raised H₂S content. However, the supernatants from cultures containing sulphate plus iron showed no marked cytotoxic effects.

P148. Plasma free metanephrines as diagnostic markers of pheochromocytoma

Portychová L.^{1,2}, Nývltová Z.³, Brabcová Vranková A.⁴, Bartoš M.³, Vermousek I.¹, and Horna A.¹

¹Institute of Nutrition and Diagnostics, RADANAL Ltd., Okružní 613, Pardubice, Czech Republic

²Department of Analytical Chemistry, Faculty of Science, Palacký University, 17.listopadu 12, Olomouc, Czech Republic

³Research Institute for Organic Synthesis Inc., Rybitví, Czech Republic, and 43rd Internal Department, 1st Faculty of Medicine and General Teaching Hospital, Charles University in Prague, Czech Republic, E-mail: Lenka.Portychova@vuos.com

Quantitative determination of catecholamines and their metabolites plays an important role in the diagnosis of pheochromocytoma (PHEO) – adrenal medulla tumour. This kind of tumour synthesizes, stocks, metabolizes and mostly secretes catecholamines. For this reason it is possible to use elevated concentrations of catecholamines and their metabolic products as diagnostic markers of this tumour. The determination of metanephrines, mainly normetanephrine (NMN), metanephrine (MN) and 3-methoxytyramine (3-MT), is preferred against the determination of catecholamines because tumour cells produce free metanephrines continuously and irrespective of the release of catecholamines. Moreover 3-MT is a biomarker for metastatic PHEO. The project aims to develop a new kit for the determination of plasma metanephrines. Solid phase extraction (SPE) with ionexchange columns was used for a pre-treatment of plasma samples. The determination was performed by high performance liquid chromatography (HPLC) with electrochemical detector Coulochem III. A core-shell column was heated to 28°C and a flow rate of a mobile phase consisted of mixed buffer and acetonitrile (total pH 2.9) was set

at 0.7 mL/min. The potential of conditioning cell was set at +400 mV. The working potentials were +100 mV (1st electrode) and -350 mV (2nd electrode).

P149. Resting energy expenditure in chronic kidney disease

Angélico L.^{*}, Mattos A. M., Figueiredo L., Romão E., and Chiarello P.

Internal Medicine, Ribeirao Preto Medical School – University of São Paulo, Ribeirao Preto, Sao Paulo, Brazil, E-mail: lari_net@hotmail.com

Background: Chronic kidney disease (CKD) is associated with several metabolic disturbances that can affect energy metabolism. As resting energy expenditure (REE) is scarcely investigated in patients CKD non-dialysis, we aimed to determine REE in this population and to evaluate whether the Harris & Benedict (HB) equation is reliable for estimating REE. Methods: A total of 45 patients with CKD stages 3-4 were recruited in a High Complexity Hospital. REE was measured by indirect calorimetry using a computerized metabolic system (VO2000) after a 12-h fasting. The Harris & Benedict (HB) equation were chosen for comparison with the REE measured. In overweight patients we used the adjusted body weight in HB equation. Prediction of REE below 90% measured by indirect calorimetry was defined as underestimation and above 110%, overestimation. Body Mass Index (BMI) also was determined. Data are reported as means±SD. Wilcoxon test was used to compare groups, considering $p < 0.05$. Results: In the sample, the mean age of patients was 56.6 ± 8.7 years. Most patients (69%) were in stage 3 CKD and 60% were male. We found an average of 28.9 ± 5.6 kg/m² for the Body Mass Index and 66% were overweight. REE determined by HB equation showed higher REE in comparison with the indirect calorimetry (1449 kcal/day, from 1127 to 1996 kcal/day, versus 1203 kcal/day, from 851 to 2697 kcal/day; $p=0.01$). The ratio estimated/ measured was 1.16 ± 0.3 (ranging from 0.59 to 1.96). The overestimation of REE by the predictive equation was present in 50% of patients and underestimation in 21% of them. Conclusion: This study showed that the main available prediction equation overestimated the REE of CKD stage 3 and 4 (not on dialysis). There is the need to promote further studies to confirm our findings and also to develop CKD population-specific equations for estimating REE.

P150. Effect of ingestion of low dose of ethanol on the senescence in senescence-accelerated mice (SAMP1)

Kimoto A.¹, Osaki A.¹, Izu H.², and Kato N.^{1,2}

¹Graduate School of Biosphere Science, Hiroshima University, Japan

²National Research Institute of Brewing, Japan, E-mail: m136039@hiroshima-u.ac.jp

Accumulating epidemiological evidence suggests light to moderate alcohol intake confers multiple beneficial effects including risk reduction of several diseases such as type 2 diabetes, brain infarction,

liver cirrhosis, cancers, etc. However, there is limited information regarding the effects of low alcohol intake in animal studies. This study was conducted to examine the effect of low dose of ethanol on the senescence of senescence-accelerated mouse (SAMP1). Male SAMP1 mice (11 wk old) had free access to a commercial stock diet with drinking water containing 0, 1 or 2% (v/v) ethanol for 22 wk. Mean of daily ingestion of ethanol during the feeding period was 1.4 g/kg body wt in the 1% ethanol group and 3.0 g/kg body wt in the 2% ethanol group. Drinking ethanol did not affect food and water intakes. The growth did not differ between the three groups. Serum levels of triglyceride, total cholesterol, glucose, ALT and AST were not also affected by ethanol consumption. The mean of total grading score of senescence, indicating the degree of senescence, in the 1% ethanol group was the lowest among the three groups until 28 wk, and the difference depended on the scores of their conditions of eyes, skin and hair, and behavior. The suppressive effect of 1% ethanol on the senescence disappeared at the end of the experiment (32 wk). Thus, the intake of 1% ethanol is likely to be beneficial to retard the development of senescence in SAMP1 mice, but the effect was attenuated by intake of 2% ethanol and for longer term.

P151. Probiotic utilization of the novel glucosylceramide-hydrolyzing bacterium to the alleviation of inflammatory bowel disease

Asanuma N.*

Department of Life Science, College of Agriculture, Meiji University, Japan, E-mail: asanuma@isc.meiji.ac.jp

Ceramides and sphingoids are important bioactive substances for their diverse biological function, such as the modulation of immune system pathways and the induction of apoptosis. Glucosylceramide (GluCer), a precursor of ceramide, is ingested daily from plant food material, and it can be hydrolyzed to ceramide by intestinal enzymes and are taken up by mucosal cells. However, since GluCer hydrolysis activity is very low in the intestinal tract, most of GluCer is excreted in feces. Therefore, enhancement of GluCer hydrolysis by utilizing intestinal bacteria, as a probiotic, might improve the physiological effects of dietary GluCer. We isolated the novel bacterial strain that hydrolyzes GluCer, previously. Thus, the probiotic utilization of this bacterium with the simultaneous administration of GluCer was examined for the alleviation of inflammatory bowel disease (IBD). Four-week-old male Jcl:ICR mice were used. Mice were permitted free access to sphingolipid-free diet and water. After acclimatization, mice were fed sphingolipid-free diet supplemented with 0.1% (wt/wt) GluCer. The experimental enterocolitis was induced by drinking 2% (wt/vol) dextran sodium sulfate (DSS) to mice. Live cells of the newly isolated strain were administered to mice using a stomach tube at a level of 10⁹ cfu/mouse/day. To measure the GluCer digestibility, GluCer was extracted from each diets or feces with chloroform-methanol (2:1, v/v) and separated by thin-layer chromatography. The newly isolated GluCer-hydrolyzing bacterium was examined as a probiotic candidate. This bacterium hydrolyzed Plant GluCer, but did not hydrolyze

sphingomyelin. The severity of IBD as expressed by the disease activity index (DAI) markedly increased with DSS administration. Feeding a diet containing GluCer slightly lowered the DAI value. Oral administration of the novel strain with dietary supplementation of GluCer to DSS-treated mice alleviated the symptoms of colitis including body weight loss, diarrhea, and bloody stool. Probiotic utilization of this strain improved DAI and apparent GluCer digestibility. Myeloperoxidase (MPO) activity levels in colonic tissue were increased greatly in response to DSS, but probiotic utilization of this strain with dietary GluCer lowered MPO activity significantly. Thus, this novel strain might be used as a probiotic with dietary supplementation of GluCer-containing plant food material without the adverse effects.

P152. Antioxidant and antimicrobial activity of extracts obtained from leaves of some varieties of *Malus domestica* L.

Sowa A.¹, Szykuła A.¹, Gamian A.², Franciczek R.³, Krzyżanowska B.³, Sosna I.⁴, and Sroka Z.¹

¹Department of Pharmacognosy Wrocław Medical University Borowska 211a, 50-556 Wrocław, Poland

²Department of Medical Biochemistry Wrocław Medical University, Poland

³Department of Microbiology Wrocław Medical University, Poland

⁴Department of Horticulture Wrocław University of Environmental and Life Sciences, E-mail: alina.sowa@umed.wroc.pl

Introduction: Currently, it can be seen growing interest in natural antioxidants is due to lack of confidence in the synthetic food additives. On the basis of studies it was found that the natural antioxidants in many plant species play a significant role in the prevention and treatment of lifestyle diseases such as diabetes, atherosclerosis, heart disease and cancer. Among the many products containing substances with antioxidant and antimicrobial activity to be a species *Malus domestica*. Aim of study: The aim of this study was to determine the antioxidant and antimicrobial activity leaves five selected varieties of *Malus domestica*: *Malus domestica* "Mutsu", *Malus domestica* "Ligol", *Malus domestica* "Golden Delicious", *Malus domestica* "Elstar", *Malus domestica* "Jonagold" Materials and methods: Methanol extracts were obtained from leaves of five varieties of *Malus domestica*: *M. domestica* "Mutsu", *M. domestica* "Ligol", *M. domestica* "Golden Delicious", *M. domestica* "Elstar", *M. domestica* "Jonagold". Raw material was obtained from experimental orchard of Wrocław University of Environmental and Life Sciences in Samotwór (Lower Silesia, Poland). Material was collected in the late autumn. Voucher specimen is deposited in Wrocław University of Environmental and Life Sciences. There were prepared methanol, ethyl acetate, and aqueous extracts as well as non-soluble precipitate. Extracts were investigated for antiradical and antimicrobial activity and for the amount of phenolic compounds. Antiradical activity was measured with ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical but antimicrobial features were studied with disc-diffusion method. Total amount of phenolic

compounds was measured by the method of Singleton and Rossi using Folin-Ciocalteu's phenol reagent. Results: The strongest antiradical activity expressed as antiradical units (TAU734) was observed for ethyl acetate extracts from leaves of *M. domestica* "Jonagold" ($71,6 \pm 1,17$) and *M. domestica* "Ligol" ($65,6 \pm 0,54$). The highest amount of phenolic compounds expressed as gallic acid equivalents per mg of extract (mgGAE/mg) was measured for ethyl acetate extract from leaves of *M. domestica* "Golden Delicious" ($0,368 \pm 0,025$) and *M. domestica* "Jonagold" ($0,335 \pm 0,003$) but the lowest amount of phenols was noted for precipitates from *M. domestica* "Mutsu" ($0,032 \pm 0,002$). The highest antimicrobial properties were observed for ethyl acetate extracts. It's strongly inhibited the growth of *Staphylococcus aureus*, *Enterococcus faecalis* as well as *Candida glabrata*. Conclusions: In general, ethyl acetate extracts showed the highest antiradical and antimicrobial activity what positively correlated with the amount of phenolic compounds in these extracts.

P153. Antiradical and antimicrobial activity of polyphenols fraction obtained from Polish honeys

Mazol I.¹, Sowa A.¹, Ostrowska A.¹, Dryś A.², Gamian A.³, and Sroka Z.¹

¹Department of Pharmacognosy, Wrocław Medical University, Borowska 211A 50-556 Wrocław, Poland

²Department of Physical Chemistry, Wrocław Medical University, Wrocław, Borowska 211A, 50-556 Wrocław, Poland

³Department of Medical Biochemistry, Wrocław Medical University, Chafubińskiego 1050-368 Wrocław, Poland, E-mail: irena.mazol@umed.wroc.pl

Introduction: Honey is a natural product consisting of multiple components which determine its dietary and medicinal properties. Among others, propolis contain a small amount of phenolic compounds with antiradical and antibacterial activity. The aim of the research was to determine amount of phenolic compounds in bee glue and its antibacterial activity. Materials/Methods: Seven honeys were collected from the region of Lower Silesia. Melissopalynologic studies of honeys were made. Then phenolic fractions were obtained with SPE method. Phenolic compounds amount in these fractions was determined with Folin-Ciocalteu's phenol reagent. Antiradical activity was measured with DPPH and antibacterial activity was tested with microplate Alamar Blue method. Results: Melissopalynologic studies revealed that two of the honeys were polyfloral, and five were classified as monofloral (two buckwheat and three rapes). The amount of phenolic compounds in honeys varied from 0.09 to 0.38 mg per g of honey. Honeys harvested in 2010 were the richest in phenolic compounds and especially rich was buckwheat honey, comparing to 2011-2012. Determination of antioxidant potential with the DPPH radical revealed that the strongest antiradical activity was exhibited by extracts obtained from polyfloral (1.22 TAU515/mg) and buckwheat (1.06 TAU515/mg) honeys, while the highest number of antiradical units was observed for rape honey (3.64 TAU515/g). Polyphenolic fractions exhibited various bactericidal activities against *Klebsiella pneumoniae* and *Staphylococcus aureus* and weak or no activity was observed against *Pseudomonas aeruginosa*.

Discussion: Investigated honeys exhibited weak antiradical and antimicrobial activity what positively correlated with small amount of phenolic compounds.

P154. Home parenteral nutrition in oncology patients

Čermáková D.¹, Hornová J.², Vejmelka J.¹, Kohout P.¹, Büchler T.², and Beneš Z.¹

¹Department of Internal Medicine, Thomayer Hospital, Prague, Czech Republic

²Department of Oncology, Thomayer Hospital and First Faculty of Medicine, Charles University, Prague, Czech Republic, E-mail: jana.hornova@gmail.com

Introduction: Cancer patients represent the largest group of patients referred for home parenteral nutrition (HPN). Nutritional support including parenteral nutrition is an important part of comprehensive cancer treatment. Cancer patients comprise 40- 45% of all patients on HPN, however in our institution oncology patients represent 78%. The most important reason for referral of oncology patients for HPN is gastrointestinal dysfunction, usually due to gastrointestinal obstructive symptoms. Malnutrition is a significant cause of mortality in these patients. For choosing the right nutritional strategy, it is important to consider not only the medical condition of patients (self-care, mobility), the expected treatment benefit, but also patients preferences. Patients, methods and results: In our study we have followed 13 patients on home parenteral nutrition treated in the Thomayer Hospital from 1/2013 to 3/2014. There were three men and ten women. Mean age was 66,5 years. Six of the patients were on palliative care, two of them had generalized carcinoma of urinary bladder, three patients had generalized colorectal cancer and one patient gynecological malignancy. There were also four patients with intestinal obstruction presented at the time of diagnosis, with minimal nutritional intake. Two patients had short bowel syndrome. Venous access devices used for administration included a port catheter primarily implanted for chemotherapy in two patients, a long term tunneled catheter (Broviac) in one patient, and three patients had central venous catheter that was changed regularly. Five patients received preformed parenteral nutrition and hydration, one patient had an all-in-one bag. Conclusion: Improved quality of life and/or increased survival are the main objectives of oncology palliative care. Individualized approach to nutrition and timely institution of HPN is one of the crucial parts of palliative care in patients with advanced cancer and severe gastrointestinal dysfunction.

P155. Effects of chard (*Beta vulgaris L. var cicla*) on skin in valproic acid induced toxicity

Ustundag U. V.¹, Tunalı S.², Alev B.¹, İpekci H.¹, Emekli-Alturfan E.¹, Akbay T. T.¹, Yanardag R.², and Yarat A.¹

¹Department of Biochemistry, Faculty of Dentistry, Marmara University, 34365, Nisantasi, Istanbul, Turkey

²Department of Chemistry, Faculty of Engineering, Istanbul University, 34320, Avcılar, Istanbul, Turkey, E-mail: uvustundag@

gmail.com

Valproic acid (VPA) is an antiepileptic drug used for the treatment of seizures in children and adults. Moreover in recent years VPA has been shown to be effective in various cancers and Alzheimer disease. The side effects of VPA were shown in many studies. Chard (*Beta vulgaris* L. var. cicla) is a herbaceous biennial leafy vegetable cultivated in many parts of the world, low cost plant and widespread use in many traditional dishes. It has been demonstrated that chard has antioxidant, antiacetylcholinesterase, antidiabetic, antitumor and hepatoprotective effects. The aim of this study is to evaluate whether VPA might interfere with oxidative metabolism in skin and whether chard ameliorates these effects. Female rats were divided into four groups as intact control animals, VPA (0.5 g/kg/day, i.p.), chard (100 mg/kg/day, gavage) and VPA+chard (in same dose and time) given groups for seven days. Chard extract (500 mg/kg) were given 1 h prior to the administration of VPA. On the 8th day the animals were sacrificed under anesthesia and skins were homogenized in saline. Oxidant-antioxidant biochemical parameters were determined in homogenized skin samples. Results were evaluated statistically and discussed.

P156. Allyl isothiocyanate ameliorates angiogenesis and inflammation in dextran sulfate sodium-induced acute colitis

Sung J. M.*

Research Division Emerging Innovative Technology, Korea Food Research Institute, Songnam, Keongki, Republic of Korea, E-mail: dulle5@kfri.re.kr

Allyl isothiocyanate (AITC) is a phytochemical found in cruciferous vegetables that has known chemopreventive and chemotherapeutic activities. Thus far, the antiangiogenic activity of AITC has not been reported in in vivo studies. Herein, we investigated the effect of AITC on angiogenesis and inflammation in a mouse model of colitis. Experimental colitis was induced in mice by administering 3% dextran sulfate sodium via drinking water. To monitor the activity of AITC in this model, we measured body weight, disease activity indices, histopathological scores, microvascular density, myeloperoxidase activity, F4/80 staining, inducible nitric oxide synthase (iNOS) expression, cyclooxygenase-2 (COX-2) expression, and vascular endothelial growth factor (VEGF)-A/VEGF receptor 2 (VEGFR2) expression in the mice. We found that AITC-treated mice showed less weight loss, fewer clinical signs of colitis, and longer colons than vehicle-treated mice. AITC treatment also significantly lessened the disruption of colonic architecture that is normally associated with colitis and repressed the microvascularization response. Further, AITC treatment reduced both leukocyte recruitment and macrophage infiltration into the inflamed colon, and the mechanism these activities involved repressing iNOS and COX-2 expression. Finally, AITC attenuated the expression of VEGF-A and VEGFR2. Thus, AITC may have potential application in treating conditions marked by inflammatory-driven angiogenesis and mucosal inflammation.

P157. Lupin utilization in food products

Beran M.*, Urban M., Drahorad J., Adamek L., and Molik P.

Food Research Institute Prague, Czech Republic, E-mail: m.beran@vupp.cz

The lupin seed protein fraction is of great interest as it is rich in some amino acids as arginine and presents a good balance of essential amino acids. Moreover, the seed contains high concentration of lutein and other biologically active compounds. In Europe, lupins are used to replace cereal grains or soy in food products such as baked goods, small goods and noodles and pasta. Because of excellent emulsion properties, the lupin flour can replace eggs in many different food products. We have developed a food product on the basis of hemp and sunflower seeds and lupin flour with well balanced content of proteins, fatty acids, polysaccharides, fiber and biologically active compounds. Stability of unsaturated fatty acids has been tested after baking and during long term storage. The product was evaluated by sensory analysis.

P158. Antiradical and antimicrobial activity of extracts obtained from leaves of three species of the Genus *Pyrus*

Żbikowska B.^{1*}, Sroka Z.¹, Janicki K.¹, Francizek R.², Krzyżanowska B.², Dryś A.³, and Gamian A.⁴

¹Department of Pharmacognosy, Faculty of Pharmacy, Wrocław Medical University, Borowska 211a, 50-556 Wrocław, Poland

²Department of Microbiology, Faculty of Medicine, Wrocław Medical University, Chałubińskiego 4, 50-368 Wrocław, Poland

³Department of Physical Chemistry, Faculty of Pharmacy, Wrocław Medical University, Borowska 211a, 50-556 Wrocław, Poland

⁴Department of Medical Biochemistry, Faculty of Medicine, Wrocław Medical University, Chałubińskiego 10, 50-368 Wrocław, Poland, E-mail: beata.zbikowska@umed.wroc.pl

Introduction: An increasingly important problem is the growing resistance of bacteria to antibiotics. New substances to replace the inactive ones are obtained by synthesis but also by isolation from natural raw materials, for example plants. Aim of study: In this study extracts were obtained from leaves of *Pyrus communis* L., *Pyrus elaeagrifolia* Pall. and *Pyrus pyrifolia* (Burm.) Nak. These extracts were tested for antiradical and antibacterial activity as well as for the amount of total phenolic compounds, hydroquinone and arbutin. Methods: The antioxidant activity was measured using the DPPH radical, and antibacterial activity was determined with the disc-diffusion method. The amount of phenolic compounds was calculated using colorimetric technique with Folin-Ciocalteu's Phenol Reagent but the amount of hydroquinone and arbutin was measured with high performance liquid chromatography (HPLC). Results and conclusions: The strongest antiradical activity was observed for ethyl acetate extract from leaves of *Pyrus communis* L., the lowest for the poorly soluble fraction (precipitate) from leaves of *Pyrus elaeagrifolia* Pall. The highest number of antiradical units per gram of raw materials was noted for leaves of

Pyrus communis L. The strongest antibacterial activity was measured for ethyl acetate extracts. The calculation of Spearman rank correlation coefficients indicated the existence of a positive correlation between contents of hydroquinone in extracts and their antibacterial activity, for almost all investigated bacterial strains. The strains of fungi such as *Candida albicans* and *Saccharomyces cerevisiae* were completely resistant to the action of extracts.

P159. Honey as a source of antibacterial agents

Tan K. L. M.*

College of Naturopathic Medicine, London, United Kingdom, and Antioxidant4life, United Kingdom, E-mail: marktan007@doctors.org.uk

Bacterial infections once again threaten to reach epidemic proportions as the indiscriminate use of antibiotics has led to the emergence of antibiotic resistant strains. Thus there is a pressing need to find new and novel chemicals. For centuries honey has been claimed to be an effective antibacterial agent; in recent decades several publications have confirmed this claim. This study employed a modification of the agar well diffusion technique to compare the activity of 25% (w/v) solutions of Tesco Honey (U.K.) and monofloral Manuka Honey (New Zealand), against three strains of bacteria; *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. In addition a similar solution of artificial honey and saline were included to identify the role of osmotic factors in inhibition of growth, and to confirm any inhibition of growth was not inherent. The aims of the study were to first determine the hydrogen peroxide based and the non-peroxide antibacterial activity of each honey. Furthermore, the study sought to and identifies some characteristics of the non-peroxide activity and determine the contribution of osmotic factors to the antibacterial activity of honey. In the presence of saline alone no inhibition of growth (recorded as area in mm²) was observed with any of the bacterial strains, confirming that any inhibition of growth was due to solely to added honey solutions. Temperature had little effect upon the antibacterial activity of Manuka honey with all three bacterial species, suggesting that neither the hydrogen peroxide nor the non-peroxide activity of Manuka honey is adversely influenced by high temperature (70°C). Inhibition decreased by approximately 40% as temperature increased for *Staphylococcus* when Manuka honey was used without catalase. It is thus possible that hydrogen peroxide limits some non-peroxide antibacterial agent(s) normally active in Manuka honey against *Staphylococcus*. When *E.Coli* were incubated with catalase treated Manuka honey, over 40% less area of inhibition was observed upon application of catalase treated Manuka honey at both 37°C and 70°C. It appears *E.Coli* may be much more sensitive to hydrogen peroxide than the non-peroxide components of Manuka honey. In this study Manuka honey inhibited growth of *Pseudomonas aeruginosa* only in the undiluted state. No antibacterial activity whatsoever was observed with 25% (w/v) Tesco honey. Artificial honey proved the most effective antibacterial substance, being the only agent effective at 25% concentration (w/v) against *Pseudomonas aeruginosa*.

These results demonstrate the immense contribution of osmotic effects to antibacterial activity, particularly against *E.coli*. In contrast, *Staphylococcus* was found to be the most sensitive to the antibacterial activity of hydrogen peroxide. This study highlights the differences in response to any given agent (including honey) by different bacterial species.

P160. Effect of high glucose levels on the respiratory burst using cultured U937 cell line

Tan K. L. M.*

College of Naturopathic medicine, London, United Kingdom, and Antioxidant4life, United Kingdom, E-mail: marktan007@doctors.org.uk

Several studies have reported that high glucose concentrations accelerate cell death and hamper replication of some cultured human cells. However, it appears not all cells are affected. Thus, this study proposes to study the effects of high glucose concentration, osmolality, and dimethyl sulfoxide (DMSO) on respiratory burst using U937 cell line. Leukemic monoblast U937 cell line originated from the pleural fluid of a patient with diffuse histiocytic lymphoma. All U937 lines were grown in suspension in RPMI-1640 medium. The superoxide ion (O₂⁻) production assay was based on the modified method of Pick (1986) and as summarised in a flow diagram. Superoxide dismutase (SOD) was assay by a modification of the method of Jones and Suttle (1981). To study the effects of hyperglycaemia and osmolality on U937 cell line, 5mM glucose, 30mM glucose, 5mM mannitol and 30 mM mannitol were used. The O₂⁻ production increased by 22.2% when glucose concentration was increased from 5 mM to 30 mM. The O₂⁻ production decreased by 74% when DMSO was added to the 30mM glucose cultured cells. When glucose concentration was increased from 5 mM to 30 mM, SOD level was decreased by 20%. This study shows that hyperglycaemia caused damage to cells by subsequently enhancing production of free radical, but is reversed by antioxidant, SOD, and the hydroxyl radical scavenger, DMSO. It is reasonable to speculate that hyperosmolality may contribute at least to cellular damage, in hyperglycaemic conditions.

P161. Fatty acids in beebread: A high Omega-3 fatty acid content

Ceksteryte V.¹, Jansen E.²

¹Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry Akademija, Kedainiai dist., Instituto aleja, LT-58344, Lithuania

²National Institute for Public Health and the Environment, Centre for Health Protection, PO Box 1, 3720 BA Bilthoven, the Netherlands, E-mail: violeta@lzi.lt

Introduction: The most important essential fatty acids (EFAs) EFAs are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), belonging to the omega-3 family and linoleic acid (LA) and arachidonic acid (AA) as a representative

for the omega-6 family. In early life the human population lived on a diet with a ratio of ω -6 to ω -3 of approximately 2:1, whereas presently in Western diets this ratio is about 10:1 and can be even as high as 25:1. The imbalance created by an over-consumption of ω -6 fatty acids can lead to an increased formation of inflammatory prostaglandins and thromboxanes, which can have a detrimental effect on public health. The aim of the present study is to determine the content and composition of different fatty acids in beebread and pollen. Materials and methods: Beebread was collected in the apiary of Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. After removing the combs, the beebread was cleaned and only pieces of desirable length (0.3-1.0 cm) were used for analysis. Samples of beebread were dried at 35°C or 40°C to the moisture level of 8.0–10.0%. The samples with fresh beebread were kept in the refrigerator at the 5–8°C in hermetically sealed dishes. Pollen have been collected by pollen traps both in spring and summer. A mixture of spring polyfloral and monofloral pollen of white and red clover were used for determination of fatty acids. Results and discussion: Beebread contains a high amount of ALA and a lower amount of LA, which results in a favorite omega 3 to 6 ratio. The highest concentration of ALA was found in all samples of beebread collected in spring and summer. The concentration of ALA in beebread which was collected in spring varied between 21.0 and 42.7% of the total fatty acids present in the sample. In the summer the concentration of ALA was between 36.6% and 42.0%. The ratio of ω -3 to ω -6 fatty acids in these samples was close to 5.0 what means a very high content of ω -3 compared to ω -6 fatty acids. Spring pollen mixture has the ratio of fatty acids from 0.47 to 0.57 and monofloral rape, dandelion and chestnut from 1.5 to 5.0. Conclusion: The present findings implicate the consumption of beebread and pollen could restore the ratio between ω -3 and ω -6 fatty acids in nutrition.

P162. NOS - nutraceutical oligosaccharides from sugar cane bagasse and pine sawdust Ligno(Hemi)Celluloses through thermopressurized diluted phosphoric acid for *Lactobacillus* and *Bifidobacterium* probiotic bacteria growth

Fontana J.^{1,2*}, Tiboni M.², Grzybowski A.¹, and Scremin L.¹

¹PPGCTA - UTFPR - Graduate Program on Environmental Science and Technology - Federal Technological University of Paraná; Curitiba-PR-Brazil

²PPGCF - UFPR - Graduate Program on Pharmaceutical Sciences - Federal University of Paraná; Curitiba-PR-Brazil, E-mail: anatnof2012@gmail.com

Since (bio)ethanol is currently addressed through glucose arising from enzymatic cellulolysis, the fate of plant hemicelluloses [25-30% of any L(H)C - Ligno(Hemi)Cellulose model] remains less explored. For instance, this is the case of sugar cane bagasse (SCB) hetero-xylan and pine sawdust (PS) hetero-xylan and galactoglucomannans. The thermopressurized diluted phosphoric acid (oPA) technology (3-12 atm / 144 - 192°C / and pH 1.5 - 2.5) - already used to convert inulin to FOS - FructoOligosaccharides, as well a satisfactory

catalytic tool for the conversion of starch into glucose and/or maltosyrups - if adjusted to the harder reaction severity parameters, may be applicable to SCB (angiosperm) and PS (gymnosperm) L(H)Cs to obtain nutraceutical XOS - XyloOligosaccharides and gMOS - glucoMannoOligosaccharides. The best found kinetic conditions for the oligosaccharidogenic hydrolysis of both sources were pH 2.25 - 2.5 oPA, 7 atm (171°C) and 1-2 min reactor residence time at the peak temperature. The NOs hydrolytic products supported a marked in vitro growth of probiotic intestinal microflora such as *Lactobacillus casei* Shirota and *Bifidobacterium animalis* subsp lactis which produced short chain fatty acids (SCFA), thus reducing the pH, as expected for a health gut.

P163. Comparative enzymatic hydrolyses of seed gum galactomannans with *Achatina fulica* snail gut or *Aspergillus niger*-induced enzymes regarding nutraceutical oligosaccharides

Grzybowski A.^{1*}, Tiboni M.², and Fontana J.¹

¹PPGCTA - UTFPR - Graduate Program on Environmental Science and Technology - Federal Technological University of Paraná; Curitiba-PR-Brazil

²PPGCF - UFPR - Graduate Program on Pharmaceutical Sciences - Federal University of Paraná; Curitiba-PR-Brazil, E-mail: adeliagr@yaho.com.br

Seed gum galactomannans have an already established market as food thickeners and stabilizers given their differential viscosity deriving from the galactose: mannose ratios from 1.0: 3.5 (locust bean) or 1.0 : 1.5 (guar). These different degree of galactose branching was able to induce specific *Aspergillus niger* enzymes production (α -galactosidases and / or β -mannanases), while the snail (*Achatina fulica*) gastric juice is a plenty of several glycohydrolases, including the hetero-xylanase and hetero-mannanase complexes. As substrates, hemicelluloses (4-O-Me-galucuroarabinoxylan and galactoglucomannan) and galactomannan gums, were hydrolyzed during 96 h, 30°C under 160 rpm agitation. The *Aspergillus niger* enzymes showed a different fragmentation profile demonstrating that microorganism enzymes specificity was determined by different inducers. For instance, when locust bean gum was used as inducer, the produced enzymes were not efficient for the removal of the galactosyl residues from the more branched guar galactomannan. The snail gastric juice proved be extremely active, hydrolyzing substrates to complete monomerization due to disaccharidases and hence opposed to the desired purpose, short chain nutraceutical XOS and gMOS oligosaccharides.

P164. Food safety information center

Götzova J.*

Ministry of Agriculture of the Czech Republic, E-mail: karolina.mikanova@mze.cz

The Ministry of Agriculture's Food Safety Information Center ("FSIC") is designed primarily as a communication centre in relation to

the public. Its aim is not only to improve mutual communication of all the interested parties but, in particular, to generate accurate, generally accessible information covering the entire food production chain from the farm to the consumer (including feed) referred to as "F2F" ("Farm to Fork"), and make it available early enough to the general public. The Ministry of Agriculture also provides the comprehensive informational background for non-governmental (other than state-run) organisations involved and co-operation in the sphere of food safety. The main goal of FSIC is to familiarize the general public with the problems of food safety. Consequently FSIC provides websites and applications, lectures for children and laymen, informative material, leaflets and professional brochures. Websites www.bezpecnostpotravin.cz, www.foodsafety.cz include among fundamental information about food and feed safety, press releases from competent authorities, reports from Rapid Alert System for Food and Feed, the current information about the possible risk of food hygiene as well as dietary guidelines, food labelling, additives, genetically modified organisms and novel food. Consumers can find here also comprehensive A-Z dictionary of food safety containing about 900 items. Website www.viscojis.cz is primarily focused on issues of nutrition and possible risk from food consumption, including eating disorders or allergies, it was created in cooperation with the Ministry of Health experts. Website www.viscojis.cz/teens was designed for teachers and youth aged 10 – 15 years, the materials are based on the Five Keys to Safer Food Programme created by World Health Organization explaining the basic principles that each individual should know to prevent foodborne diseases. FSIC also provides information from European Food Safety Authority ("EFSA"). EFSA was set up in January 2002, following a series of food crises in the late 1990s, as an independent source of scientific advice and communication on risks associated with the food chain. Its remit covers food and feed safety, nutrition, animal health and welfare, plant protection and plant health. Stakeholders can follow FSIC on Facebook and Twitter.

P165. Lupine fibre concentrates and protein enriched lupine flour

Jelínek J.¹, Hubalovsky S.¹, Jelínek M.¹, Fuchs M.², and Horna A.³

¹University of Hradec Kralové, Faculty of Science, Czech Republic

²Extrudo s.r.o. Bečice, Týn nad Vltavou, Czech Republic

³Radanal s.r.o. Pardubice, Czech Republic, E-mail: jiri.jelinek@uhk.cz, fuchs@extrudo.cz, horna@radanal.cz

The lupine bean is a unique constitution - high protein, high crude and dietary fibre, no starch, low but good oil and low Glycaemic Index. Lupine flour has evidence its benefits in various food applications e.g. bread, wafer, biscuits, s and battered products. Lupine flour improved freshness and structure, and longer shelf life. We tried yet more to improve those specific functionalities of lupine flour. Lupine have a thick seed coat (25%) which consists mainly of cellulose (insoluble fibre-bran) and its removal is the first step in lupine processing. The kernel (split) of lupine is rich in protein (35-40%), fibre (35-40%) and fat (8%) made up largely of unsaturated fatty acids. We make

dehulling lupine beans as dry protein enrichment method. We had two fractions. 1. Lupine hull (27%) is rich on polysaccharides (cellulose 52%, pectin 29%) low protein (8%) and low oil (2%). 2. Lupine kernel (73%) is rich on protein (39%), oil (11%), polysaccharide (pectin 26%, oligosaccharides 7% and cellulose only 2%). Hull fraction we parboiled on alkali solution and washed. Product is as supplement crude fibre. That fibre concentrate is possible use as supplement with variety of health benefits and is especially in reducing the risk of chronic disease such as diabetes, obesity, cardiovascular disease and diverticulitis. The hull fraction could have numerous functional food properties particularly for increasing fibre content when added to food products. Lupin hull is a natural food ingredient that can add value to food distributors and manufacturers. Kernel fraction - flour is possible use for many food applications (to bread, biscuits, pasta waffles, confectionery etc.). Lupin kernel flour is used in the human consumption market and can be included into many different foods. Addition already 10% these flour improve nutritional valuable food product without influence on tastes. Lupine protein has high lysine than cereals and is major natural source of arginine - precursor for NO & GABA. Kernel flour especially after defeating (by CO₂) is very good material for isolate lupine protein in production lupine protein hydrolyzes. Defeated kernel flour contain near 45% protein. We use rest after that process as concentrate dietary lupine fibre. Those lupine kernel dietary fibres concentrate have health benefits, especially:

- Lowers cholesterol - Reduced fat intake
- Additional mechanisms
- High satiety factor - Suppresses appetite
- Satiety effect
- Bowel health - Lowers colon pH
- Reduces transit time
- Prebiotic properties

That lupin kernel fibre may improve the function of the colon and potential reduce the risk of colon cancer.

P166. Determination of metallothioneins in fish

Bušová M.* and Havlíková G.

Department of Biochemistry and Biophysics, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, E-mail: busovam@vfu.cz

Toxic metals are major contaminants found in aquatic environments and also in aquatic organisms. Their presence in the environment and enter the food chain is associated with many negative health effects in the case of long term exposure. Living organisms are equipped with mechanisms that are partially able to eliminate risks arising from the accumulation and effects of these contaminants, heavy metals, in tissues and organs. Production of metallothioneins (MT) is one of this decontamination way. Heavy metals that can contaminate foods of animal origin, particularly fish, are Cd, Pb, Hg and As. Fish and

fish meat are important in our diet for dietetic and protective effects on the body. They can also be an important source of toxic metals from the environment. Not just marine fish, but also freshwater fish from some sites in the Czech Republic on the basis of monitoring and studies have sometimes exceeded limits for freshwater fish muscles. The content of Cd, Pb and Hg is selected for marine and freshwater fish species by Commission Regulation (EC) No.1881/2006, or by Commission Regulation (EU) No. 420/2011. Fish is very sensitive to all changes in their surroundings environment. Water pollution can cause major health problems in fish. Bioaccumulation of any metals above its limited levels results in stress and can cause irreversible physiological and biochemical changes in fish body. Fish contaminated with heavy metals can cause major health problems in human body or, due to their accumulation ability. In terms of defense of the organism and in terms of safe fish meat eating it seems to be very important detoxification pathway by formation of metallothioneins. Metallothioneins (MT) are a group of low molecular weight intracellular metal binding proteins rich in cysteine. MT are induced in animals after exposure to metals, such as cadmium, lead, mercury and others. In this study, we analyzed liver samples of silver crucian carp (*Carrasius gibelio*) from the site on a tributary of the Morava River. Fish were caught and killed. Liver after opening the abdominal cavity were removed and immediately frozen in box at -80 ° C until analysis. We analyzed 34 individuals of silver crucian carp. For the determination of MT in this study we have verified using the spectrophotometric method. Samples of liver tissue of fish were homogenized in buffer. Metallothioneins after reaction with the reagent to form a colored complex, and were evaluated on the basis of calibration with standard solutions MT. The measured concentrations of metallothioneins ranged from 2.12 mg MT/1 g of liver tissue to 7.43 mg MT/1 g liver tissue. The aim of this study was to verify the possibility of spectrophotometric detection of MT in fish as a marker of environmental contamination. The method is applicable to the detection of MT from fish tissues.

P167. The potential of a portable modular medium pressure liquid chromatography system for in-field food analysis

Dvorak M.^{1,2}, Li Y.¹, Nuchtavorn N.^{1,3}, Nesterenko P.¹, Stanley R.⁴, and Macka M.¹

¹University of Tasmania, Australian Centre for Research on Separation Science (ACROSS) and School of Physical Sciences, Private Bag 75, Hobart 7001, Australia

²Department of Food Chemistry and Biotechnology, Faculty of Chemistry, Brno University of Technology, Brno, Czech Republic,

³Siam University, Faculty of Pharmacy, Bangkok, Thailand

⁴Centre for Food Innovation, University of Tasmania, Locked Bag 1370, Launceston 7250, Australia, E-mail: mirek.macka@utas.edu.au

In a current project we developed an approach based on off-the-shelf components assembled on a microfluidic platform to produce a portable modular medium-pressure liquid chromatography (LC), as

detailed in our ISSS-2014 communication. Here the aim was to test the performance of our portable LC platform as a means for screening or fingerprinting of food samples where chromatography is necessary. The on-column or on-capillary LED-based absorbance photometric and end-column (wall-jet arrangement) electrochemical (amperometric) detection combination used was part commercial and part in-house designed to cover a wide range of analytes. The combination of detections also allowed judgement on which analytes from those detected in UV-photometric detection can be oxidised therefore are antioxidants. A review of selected areas of food analysis has indicted where portable in-field or in-process LC platform could bring major benefits. These areas include profiling/fingerprinting of antioxidants or vitamin composition, the presence of undesirable food additives, evidence of falsification of food, rapid on-site screening of biological and chemical risks and in-process control of food manufacture for consistency and quality. In biotechnology processes such as in food fermentation an on-line portable LC could provide rapid results to adjust process control. Experimental characterisation of the portable LC platform is detailed. The separation performance was tested using food dyes (Tartrazine, Orange G, Brilliant Yellow, etc.) with both isocratic and gradient elution using mobile phases of ammonium acetate in water (A) and in methanol (B). The performance of gradient elution reversed phase analysis showed a dependence of the LED-photometric signal on the % A-% B mobile phase composition through the refractive index controlled Schlieren effect, and experimental approaches to minimise this effect were studied. Selected performance characteristics of the portable LC such as retention time and peak area were reproducible and compared well with bench top LC. Applicability to LC analysis of real samples was explored and results are discussed.

P168. Requirements of veterinary medicinal products with antimicrobial substance for food producing animals

Hera A.¹, Billová V.¹, and Novotná P.²

¹University of Veterinary and Pharmaceutical Science in Brno, Czech Republic

²Institute for State Control of Veterinary Biologicals and Medicines, Czech Republic, E-mail: hera@uskvbl.cz; sedlakova@uskvbl.cz

Increasing bacterial resistance towards antimicrobial substances is one of the most pressing problems in current medicine and it is possible to note that antibiotics are slowly becoming “an endangered species” and it seems probable that we are already standing on a threshold of the post-antibiotic era. However, there is no simple solution to the problem of resistance towards antimicrobial substances because it is a long-term, multi-factorial and worldwide issue. But it is still to be hoped that the international cooperation in that field can bring gradually to a standstill the trend of the rising resistance. At the same time it is necessary that exist measures at national (or even regional) level in a form of national antibiotic programmes, which would otherwise respect international recommendations and guidelines but simultaneously may make provisions for specificity of a given country. It is imperative that a

cooperation exists between legislative and executive authorities (the European Parliament, the European Commission, parliaments and governments of individual countries) and between organizations that are in an advisory capacity and represent the professional part of the problem (OIE, WHO, CDC, EMA, EFSA, ECDC, national professional groups and suchlike bodies). Formulation of fundamental, globally valid ways out and legislative documents for a war against antimicrobial resistance is the basis but to achieve results in practice it is inevitable to stand up for implementation of the recommended measures even in local conditions (at least at level of individual countries). Therefore, it

is necessary to put a great stress on professional public awareness that represents the power, which turns a great part of the legislative measures into reality. Also research of new substances with antibacterial effect should be supported, although it is mostly a tedious process which does not produce results during a few years. The antibiotics represent one of the most effective means for treatment of infections in human beings and animals and it is necessary to realize that they act by other mechanisms than the remaining medicinal products. If we do not respect the rules for their proper use we shall lose cardinal weapon in the war against pathogenic bacteria in the foreseeable future.