**Section 1: Diet and health**

**Plenary lecture**

**P1.1**

**The challenges of molecular nutrition research**

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Due to its complexity, nutrition has traditionally been an observational science. Matching physiology with molecular thinking was next to impossible. As a result, animal models were discarded and human studies were limited to available biomarkers. With these blunt tools, human studies proceeded in heterogeneous patient populations. However, the nature of nutrition is nuanced. Unlike pharmacological interventions, food contains multiple bioactives, usually with low receptor affinities. Consequently, weak or even conflicting biological effects were observed. In seeking the statistical power of large cohorts, subtle differences become blurred. Several times, epidemiology and meta-analysis have killed solid evidence in properly performed human intervention trials and mechanistic research in animal models. Our health status is the result of the combination of our genomic heritage and the cumulative environmental exposure, from conception till now. As our genome is stable (not considering mutations and epigenetics), we just need to carefully quantify this and this is a technology challenge where rapid progress is being made. Thus, the major challenges in the nutrition and health equation are quantification of the other two parts of the equation, i.e. the exposure part (in our case and the major part of exposure, i.e. the diet), and the health status. With the advent of the omics tools and thinking, nutrition science undergoes a dust-off and the transition (started by the functional food wave) from “optimizing our diet” (i.e. optimal amount of macro-and micronutrients in our diet) to “optimizing our health” (i.e. the right diet to optimize health and prevent disease) can be completed. We can now nominate the key areas and major next steps in nutrition research. Of course, one can add or disagree on specific items. However, it is essential to realize and embrace these key drivers:

- Nutrition science is part of the big biology revolution, and a massive integration of fundamental disciplines with nutritional science is essential.
- This integration involves not just technology implementation, but also a coordinated action on standardisation, data warehousing and bioinformatics.
- A conceptual shift from “more” to “better”, where very accurate quantification of food intake, health status and health effects is essential.

In following this path, we may indeed realize that the nutrition and health relationship is not targeted at disease pathologies, but much more towards the “overarching drivers” from which disease states originate, like metabolic stress, inflammatory stress and oxidative stress. These highly balanced processes need a systems approach, where the both the complexity and the individuality need to be taken into account.

If this is properly achieved, we very likely will see a new wave of nutritional sciences, where indeed health optimisation and disease prevention, maybe even at a personal level, can be achieved.

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**Panel I: Nutrigenomics and Health**

**Oral presentations**

**O1.1.1**

**Health and Food - How much can biotechnology learn from local European knowledge?**

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New biotechnological leads are likely to come from plants which already have a use, for example, as food or medicine [1]. Food plants used locally in the Mediterranean or other European regions are one such area. In recent years our understanding of the effects of the various diets has increased substantially. Numerous studies indicate the beneficial effects of certain elements of Mediterranean diets (esp. of selected major elements like olive oil) and of these diets as a whole. As part of an integrated ethnomedical and pharmacological-nutritional project more than three hundred botanical species used locally as food have been identified in communities in Italy, Spain and Greece [2,3], highlighting the importance of these resources, which may be used in very restricted regions or may be pan-Mediterranean. For example in the Italian Greek community of Gallicianò (Reggio Calabria) about 40 wild food taxa like Reichardia picrodes (used raw as a snack or cooked with other wild greens) have been identified [4].

About 140 of these species have been investigated for antioxidant activity using a variety of in vitro assays (incl. guaiaol oxidation, xanthine oxidase, HOCl scavenging, eNOS activity), for effects against a variety of targets of relevance in chronic and acute inflammation, in the comet assay to detect protection from oxidative DNA damage and for angiogenic activity [2]. All extracts are profiled using HPLC-MS and the extract’s polyphenol content was determined. An example is Cynara cardunculus ssp. Cardunculus which shows one of the highest anti-oxidants...
effects in the guaiacol assay (about 95% at 0.2 µg/ml), it stimulates eNOS activity to a level about five folds of the controls (at a concentration of 10^{-6} M gallic equivalents 0.2 mg/µl) and acts as a scavenger of HOCl (28%).

Integrated approaches incorporating both the study of local knowledge and biotechnological and pharmacological-phytochemical investigation are used, offer unique opportunities for developing novel health foods or nutraceuticals and my group is currently conducting such studies in Spain, Greece and on Cyprus. One particularly interesting and hitherto neglected area are the numerous fermented foods found in the diets of countries like Poland.

The support and the input of all members of the consortium “Local Food-Nutraceuticals” and R. Alarcon, A. Lardos, T. Hooper and A. Patsoura is gratefully acknowledged. A contract with the EU (“Food, Nutrition and Health” - FP5, QLRT-2001-00173), funding through a BBSRC-CASE award, by the Luxemburg government (stipend) and a contract with Fa. Natra, Valencia makes this research possible.


O1.1.2

Dietary polyphenols and oestrogen-metabolizing genes affect hormone-dependent cancer risk among women

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Changes in lifestyle and dietary habits in the post-industrial era can influence metabolism and the level of sex-steroid hormones [1]. It has been suggested that prolonged exposure to oestrogens during a woman’s life span due to early menarche, late menopause, lower parity and environmental estrogenic toxins may be responsible for the increased rate of breast cancer [2]. Specifically, the rates of production and detoxification of oestrogens may determine the risk of cancer [3]. The mechanism through which oestrogens contribute to the development of human cancer, however, is still unknown. Two hypotheses exist. The first posits that oestrogen-induced tumor development is mediated by the oestrogen–receptor-based proliferation of cells, which increases the probability of mutation and the induction of DNA [4]. The second suggests that the accumulation of genotoxic metabolites, like catechol estrogens (CE) formed by hydroxylation of estradiol, directly induces DNA damage [5]. The first sequence can be prevented from occurring by lowering oestrogen production, for example, by high tea catechols intake [5]. To prevent the second posited mechanisms from functioning requires the efficient detoxification of CE, for example, by their methylation catalyzed by catechol-O-methyl transferase (COMT). The enzyme also detoxifies catechol-containing flavonoids such as catechins or quercetin. Moreover, these flavonoids can efficiently inhibit the COMT-mediated O-methylation of CE. Since the level of COMT enzyme activity is genetically polymorphic in the human population (with a trimodal distribution of low, intermediate and high COMT activity) individuals with low COMT activity and high oestrogens exposure plus chronic administration of dietary flavonoids may be at higher risk for developing hormonal-dependent cancer [6]. The potentially harmful effect of large amounts of ingested dietary flavonoids can be balanced by positive effect resulted from simultaneously occurring enhancement of the SAH level which may effectively prevents the endogenous SAM pool (the main donor of methyl group involves in the methylation of DNA, RNA, protein) from being readily depleted. This folate-mediated one-carbon metabolism is strictly related to the adequate supply of methionine, folate, vitamin B6 and vitamin B12, and is also catalyzed by polymorphic enzymes, the methylenetetrahydrofolate reductase (MTHFR). Therefore, the benefits and safety of a diet high in flavonoid and of flavonoid supplement intake depend on the quality of diet, composition and concentration of dietary supplements and the timing of their exposure; the inherent genetic susceptibility of individuals; and individual variations in the capacity to metabolize particular flavonoids.


O1.1.3

Nutrient sensor, their polymorphism as the metabolic control and risk for cardiovascular disorders

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Adipose tissue is viewed as a dynamic fuel reserve mobilized during food deprivation with the release of fatty acids (FFA) for oxidation. From the other site the accumulation of FFA in circulation produces the endothelial dysfunction, oversecretion of insulin and lipotoxicit. Adi-
pose cells are derived from pluripotent stem cells, and the process of adipogenesis involves a complex communication network between various transcription factors, some of which are sensors for nutrients (carbohydrates, fatty acids, proteins) its metabolites and hormones. These nuclear receptors bind to specific target sequences in the promoter regions, and control the transcription of specific genes in response to nutrient signals. Integrators of homeostatic control of nutrient intake and energy production are: for energy and glucose metabolism the peroxisome proliferator-activated receptors gamma (PPARgamma), carbohydrate-response-element-binding protein (ChREBP) and Forkhead transcription factors (FOXO); for fatty acid, triglyceride and lipoprotein metabolism: PPARalpha, beta, and gamma; hepatocyte nuclear factor 4 alpha (HNF4), sterol regulatory element binding proteins (SREBP's); for reverse cholesterol transport and cholesterol absorption and xenobiotic metabolism: the liver X receptors (LXRs) and liver receptor homolog-1 (LRH-1), farnesol X receptor (FXR), pregnane X receptor (PXR), steroid and xenobiotic receptor (SRX); and for aminoacids and protein: the mammalian target for rapamycin (mTOR). Most nuclear receptors are active as monomers, dimers or heterodimers with retinoid X receptor/retnoic acid receptors (RXR/RAR), vitamin D receptor (VDR) and the others. The induction by the exogenous stimuli the CCAAT/enhancer binding proteins (C/EBP-beta,-delta,-alpha) allows the cross-talk among these factors. The phosphorylation of corepressors (NcoR; SMRT, RIP)/ coactivators (PGC-1,-2; p300/CBP, p/CIP and others) is an integral part of regulation of gene expression by regulation of NAD-dependent histone deacetylase recruitment and chromatin remodeling. Thus the AMP/ATP ratio (the energy status) as well as the nutrient (amino acids, glucose) supply regulate the the mTOR-dependent mitochondrial morphogenesis thus regulate the adipose tissue differentiation and insulin sensing leptin production. Caloric restriction as well as resveratrol induce sirtuins, the longevity genes – the NAD-dependent histone deacetylases. This results in inhibition of adipose tissue mass (activation of lipolysis), inhibition of inflammatory cytokine production and augmentation of adiponectin release. Thus nutrient and energy supply (AMPK-dependent phosphorylation) as well as epigenetic modification of DNA) supply - induced changes in the elegant cooperation of transcriptional factor activity results in the imbalance in metabolism. Polymorphism of HNF-4 in the induction of diabetes type MODY, or FOXO gene polymorphism leading to the carbohydrate-diet dependent development of obesity. It is the one of the genetically based mechanisms resulting in metabolic changes followed by appearance of metabolic disorders generating risk of cardiovascular disease.

Supported by the Framework 6EU IP project NuGO contract nr FP6-2004-506360

### O1.1.4 Evaluation of genetic predisposition to insulin resistance by nutrient-induced insulin output ratio (NIOR)

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Background: Metabolic regulation, from genes to metabolites, dictates biochemical functions as well as the nutritional and dietary demand. Therefore, genetic disposition and metabolic needs are important in determining the optimal diet for an individual in prevention of metabolic syndrome. There is the need for the implementation of new tools to identify the genotype-phenotype interactions. The aim of this study was to find the correlation between risk originating from gene variation and diet-dependent development of insulin resistance.

Methods: The insulin output as an area under curve for insulin after standard glucose tolerance (AUCIns OGTT) and lipid tolerance tests (AUCIns OLTT) were measured in 167 overweight/obese patients. Estimation of the 18 common “obesity risk-genes” polymorphisms and standard phenotyping were performed.

Results: Insulin output during oral glucose tolerance test (AUCInsOGTT) correlated strongly with insulin output after standard high fat meal (AUCIns OLTT) in the whole group. However, within the genotype sub-groups the correlation was lower or did not exist. Using a nutrient-induced insulin output ratio (NIOR), calculated as AUCIns OLTT/AUCIns OGTT, values ranged from 0.42 to 5.83, and correlated significantly with body mass index (BMI) and leptin but not with age, gender, waist/hip ratio (WHR) and insulin resistance index (HOMA) or plasma adiponectin. High NIOR was found in a subgroup of carriers of rare allelic variants of genes characteristic for the worse tolerance to lipids in the diet. Low NIOR values were found within a sub-group with rare genetic variants regulating carbohydrate metabolism.

Thus, the new insulin index NIOR may distinguish gene variant carriers into groups, members of which are glucose- or lipid-content sensitive phenotypes.

Conclusion: We suggest that the OLTT/OGTT insulin output ratio (NIOR) may be predictive for identifying individuals (genotypes) who are phenotypically susceptible to insulin resistance in response to high fat or carbohydrate in their habitual diet.
O1.1.5

Beta-carotene and arachidonic acid-induced changes in human endothelial cells and its progenitors.

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DNA methylation is a mechanism regulating gene expression. Beta-carotene (BC), potent pro-vitamin A/retinoic acid source in human, was shown to have pro-chemo-tactic activity and stimulate expression of pro-angiogenic genes in endothelium. Angiogenesis is an important mechanism in tumour malignancy. Fatty acids stimulate BC uptake. The arachidonic acid (AA) metabolites were shown the procancerogenic activity. This study was undertaken to define the possible changes in DNA methylation in endothelial cells and its progenitors after incubation with BC and AA.

Human umbilical vein endothelial cells (HUVEC) and isolated from cord blood endothelial progenitors (EPC) were incubated with BC (1-10mcM) and 3mcM arachidonic acid (AA) for 24 hours. The CpG island methylation was quantified using the Combined Bisulphite Restriction Analysis (COBRA) method (HotStarTaq Master Mix Kit, Qiagen) and the PCR products were digested by restriction enzymes (NewEngland BioLabs). Global DNA methylation was analysed with cytosine extension assay using methyl-sensitive restriction enzyme HpaII (New England Biolabs), which allows [3H]dCTP to be incorporated into the DNA strands.

The global DNA methylation analysis pointed to the tendency to down-regulation of DNA methylation in HUVEC and EPC, after incubation with AA (p=0.919) or BC (p=0.227). Of the 18 investigated genes connected with the endothelial cell proangiogenic activity, DNA methylation was regulated in the promoter regions of: integrin beta3, connexin 43, CXCR4, KDR, MMP-2, laminin, Notch4 and VCAM1 genes.

Conclusion: The CpG island methylation might be an important mechanism of changes in the expression of pro-angiogenic genes after stimulation with beta-carotene and arachidonic acid. The presented results are from a pilot study and need further observation.

Project supported by Polish Committee of Science Grant No: 0143/F01/2006/31 and The European Nutrigenomics Organisation NUGO Exchange Grant.

O1.1.6

Antioxidant properties of several Polish nectar-honey types.

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Polish honey is known for its high quality as it is produced in natural, ecologically clean areas, unaffected by industrial activity, excess fertilizers or chemical plant protection chemicals. A multitude of available honey species results from both the efforts of experienced bee-keepers and from the access to sites rich in biodiverse floral sources.

Most of advantageous nutritional and therapeutic values of honey are due to the presence of various substances revealing antioxidant activities. Such activities are crucial for preventing destructive oxidation reactions caused by free radicals. Bio-active compounds appear in honey either as nectar constituents or as a consequence of metabolite enrichment during preparation of the final product.

Antioxidant properties of several nectar honey types were evaluated. The comparative study was performed using acacia, lime, buckwheat, heather, rape and multiflorous honeys. Honey extracts were made using both polar and non-polar solvents. The obtained extracts were then further fractionated with liquid column chromatographic techniques: HPLC and FPLC. Active compounds were identified and their concentrations determined using spectroscopic methods (UV/VIS), electrophoretic techniques, and gas chromatography linked to a mass-spectrometer (GC/MS). In order to measure antioxidative activities in extracts and fractions, a unique electron paramagnetic resonance (EPR) method was employed, using a laboratory-made spectrometer working at microwave frequency of 1.2 GHz (L-band EPR). In this method, the capabilities to reduce stable free radicals (TEMPO, DPH) by honey antioxidant constituents were evaluated and measured in kinetic experiments.

It was shown that the maximum total antioxidative activity was revealed by buckwheat and heather honeys. Phenolic antioxidants contributed much to the overall activity. HPLC technique enabled precise determination of flavonoids (quercetin, kaempferol, apigenin) and phenylpropenic acids (caffeic, chlorogenic and ferulic). Again, buckwheat and heather, together with lime honey, proved to be particularly rich in these compounds.

Attempts were made to identify other antioxidants since significant antioxidative activities were detected in several fractions of different polarity, solubility and chemical nature. In particular, the aqueous fraction of the buckwheat honey revealed radical-reducing activity that was dramatically elevated relative to other honey types. As this fraction contained proteins, SDS-PAGE electrophoresis was performed, and the obtained protein profiles of
each honey were compared. In the buckwheat honey pattern the presence of low-molecular proteins (18–32 kDa) was exclusively manifested. It is now of interest to study these unique proteins in detail and to find out whether their activity contributes to the particularly high antioxidative action of this honey.

**O1.1.7**

**Relationship between the hormonal status and folate and vitamin B12 concentration in maternal serum during pregnancy**

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**Introduction:** The maintenance of pregnancy, fetal development and delivery at term require proper maternal steroid hormonal milieu. The maternal progesterone to 17beta-estradiol ratio is significantly lower among women who delivered prematurely than among those who delivered at term. In turn, the deficiency in folate increases risk of neural tube defects, fetus heart problems and other complications. Serum folate concentration depends on its dietary intake and on the activity of the methylenetetrahydrofolate reductase (MTHFR). This enzyme reduces the 5,10-methylene-THF, the substrate for purine and thymidilate synthesis, necessary for supplying rapidly dividing cells in fetus, 5-methyl-THF, the principal circulating form of folate in serum, used for remethylation of homocysteine. The gene for MTHFR is polymorphic at nucleotides: 677 (C/T) and 1298 (A/C). The wild type 677C gene product is more active form, associated with higher plasma folate concentration.

The wildtype 1298A allel has a protective effect on the pregnancy outcome. The importance of this polymorphism in determining MTHFR activity depends on its inhibitor, 5-adenosylhomocysteine, a final product of homocysteine remethylation in B12-dependent methionine synthesis. Thus, the vitamin B12 and folate concentration in serum, in addition to hormonal status, may be crucial for fetal development.

**Objective:** to examine association of MTHFR polymorphisms and concentrations of 17beta-estradiol, progesterone, folate and vitamin B12 in serum during 8 months of pregnancy.

**Design:** the study included 65 pregnant women from 6 to 39 week of gestation. Blood samples were collected one to four times during each trimester. MTHFR polymorphism was determined by RFLP.

Results: During pregnancy serum folate concentration was relatively stable. The concentration of vitamin B12 in serum showed a significant decline during gestation (p = 0.000046). The folate concentration increased with folate dietary intake (p = 0.051) and was significantly higher in the group of women with concentration of B12 above median. In the group with low vitamin B12 concentration, the folate concentration was not affected by the MTHFR1298C allele. Concentration of estradiol and progesterone increased during pregnancy (p = 0.0000) and estradiol/progesterone ratio was significantly lower in group with low (below median) vitamin B12/folate ratio.

**Conclusion:** The two most important factors in maternal regulation of fetal development, the hormonal status and vitamin concentration in serum involved in methyl cycle, seems to be interrelated.

**O1.1.8**

**Angiogenic response in animal models of human metabolic syndrome.**

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**Introduction:** Obesity, insulin intolerance, hypertension, dyslipidaemia, hypoadiponectinaemia and hyperleptinaemia are the main clinical symptoms of metabolic syndrome leading to vascular injury, atherosclerosis, diabetes and pathological angiogenesis. Aim of the study was to define the possible link between biochemical parameters of metabolic syndrome and angiogenesis.

**Methods:** Three animal models with different metabolic dysfunction were used in the study: hepatocyte RXRAlpha deficient mice (hyperleptinaemia, hypercholesterolaemia), NZO and backcross NZO/F1(NZOxSJL) mice with obesity, insulin resistance and hyperleptinaemia (less expressed in the backcross population). Mice were fed with standard or the high fat diet for seven weeks. Body weight, serum glucose, triglycerides, cholesterol, insulin, leptin and adiponectin were monitored. Angiogenesis was measured in subcutaneously implanted matrigel with 25 nM bFGF during the last week of study. The angiogenic response was assessed by a number of CD31 positive structures. Gene expression in matrigel plug cells was analysed by microarray (Mouse 430A_2, Affymetrix, 14 000 genes).

**Results:** High fat diet increased leptin and cholesterol concentrations in the liver RXRAlpha deficient mice. The tendency to decrease number of vessels with lumen was observed in these animals fed with high fat diet. The number of CD31 positive cells correlated positively with the blood glucose and insulin. In microarray assay, the
activation of insulin receptor pathway, glucose transporters, glycolysis enzymes, fatty acid synthetase; transcription factors related to adipogenesis was observed. High fat diet elevated body weight, serum concentration of glucose, cholesterol, insulin and leptin in NZO and NZO/F1 mice. The high-fat diet raised number of CD31 positive capillaries, what positively correlated with palma glucose level. Analysis of gene expression in the matrigel plaque harvested cells in NZO model revealed the upregulation of genes related to cytoskeleton remodeling, endothelial cell migration as well as induction of proangiogenic growth factors.

Conclusions: High fat diet caused different effect in animal models of metabolic syndrome, leading to decrease of angiogenesis in the hepatocyte RXRalpha deficient mice and increase of angiogenesis in obese and insulin resistant (NZO, NZO/F1) ones. The blood glucose/insulin, but not proangiogenic leptin/adiponectin concentrations correlated with angiogenic responses.

Project supported by Polish Committee of Science Grant No: PBZ-MIN-005/P04/2002/5.

O1.1.9

Proangiogenic activity of progenitor cells isolated from human adipose tissue

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Introduction: Angiogenesis, a physiological process involving the formation of new blood vessels, is essential for many physiological processes, but is also fundamental in the certain pathological steps such as inflammation. Recently, it has been reported that human adipose tissue, active endocrine organ, contains a population of non-characterized cells, called stromal vascular fraction (SVF), which are able to differentiate to several lineages with the possible therapeutic potency. These cells may however take part in adipose tissue mass extension also due to participation in angiogenesis. Aim of the study was to confirm the proangiogenic properties of the SVF cells isolated from human subcutaneous adipose tissue. Methods: SVF cells were isolated according to modified Hauner's method and, after adaptation, cultured in proangiogenic, or proadipogenic medium. Cells were characterized by the expression of surface antigens (flow cytometry) and by the expression of genes characteristic for endothelial progenitor cells as well as for adipocytes performed using quantitative real-time PCR. The cell proliferation assay was performed using BrdU incorporation. Migration assay was assessed using the cell culture inserts. The capillary network formation of GFP labeled SVF cells with not transfected HUVEC was prepared using the 3D matrigel model of angiogenesis. The differentiation to adipocytes was confirmed by the accumulation of lipid droplets stained with Oil-red-O.

Results: SVF cells are a heterogeneous population. Depending to the used culture medium they may differentiate to adipocytes as well to endothelial-like cells respectively. This was documented by the pattern of gene expression. The proangiogenic SVF cells proliferate, migrate and form the capillary-like structure in the in vitro 3D model of angiogenesis. They also incorporate into tubules formed by HUVEC in this model. The longest tube formation was observed during co-culturing HUVEC with the GFP labeled SVF cells.

Conclusions: The differentiation of human SVF cells towards endothelium is stimulated by the presence of human serum in the absence of adipogenic hormone mixture. It argues for the deep influence of the environment on the differentiation of adipose derived progenitors towards adipose versus angiogenesis promoting cells.

This work was supported by SC&CR (LSHB-CT-2004-502988), EU LIPGENE (FOOD-CT-2003-505944) and Polish MNiI (2P05A 132 28) projects.

O1.1.10

Ghrelin in newborns – a Molotow cocktail?

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Ghrelin is a 28-amino acid acylated peptide isolated from human and rat stomach. It has also been detected in other tissues in various animals and human, where it facilitates various functions. Ghrelin and its receptor have been found in fetal and neonatal gastrointestinal tract, in milk and in colostrum. The most important function of this hormone is to stimulate food intake, growth hormone secretion, intestinal motility and gastroprotection. Intravenous or intraventricular ghrelin injection stimulated the increase in the body weight of weaned rats and pigs whilst in suckling rats it was found ineffective. In newborn piglets treated with ghrelin the decrease in overall body and pancreas weights, and increase in stomach weight were observed. The reduction in small intestine length was significant when compared to control. The aim of present study was to evaluate the influence of ghrelin on the intestinal mucosa remodeling, via evaluation of the mitotic and program cell death indexes among the enterocytes. Studies were conducted on newborn piglets. Animals were randomly divided into control (intragastric 5 ml 0.9% NaCl every 8h for 6d) and ghrelin group (intragastric 7.5 or 15 µg/kg b.wt. ghrelin every 8h for 6d). The animals were kept in “artificial sow” system and fed with milk formula for 6d. Ghrelin in higher dose (15 µg/kg) caused a significant increase in apoptotic index (p<0.05)
whereas the in lower dose of ghrelin apoptotic index was significantly lower (p<0.01) when compared to control. Simultaneously both doses caused a significant decrease in mitotic index (p<0.001). We also observed that treatment with ghrelin caused dose dependent increase in the expression of TGF-β1. Also the characteristic packet of apoptotic cells were observed.

Acknowledgements: PBZ-KBN-093/P06/2003; university grant 504-02310015.

O1.1.11

Flexible tools – food safety objectives

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Government and the food industry each has in important role to play identifying, assessing and managing risks associated with the consumption of food and drink. Considerable advances have been made in the area of quantitative risk assessment as a means of obtaining a more accurate evaluation of risk potential. The main principles of risk assessment can also be adhered to in descriptive or deterministic risk assessment approaches. Food management systems must be designed to apply to many different types of food chains, varying in structure, complexity, logistics and operational features. The interactions within any food management systems are likely to be dynamic, depending on changes in the food supply chain.

Risk assessment comprises four key stages: hazard identification, exposure assessment, hazard characterization and risk characterization. The final stage results in a risk estimate, for example a measure of the level of risk in a given population size associated with a particular food on food category. If a risk assessment process is going to influence the establishment of in food safety objectives.

Posters

P1.1.1

Bee pollen and bee bread as natural sources of bioactive dietary components.

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Bee pollen is a natural product consisting of mixed pollen collected from flowers of different plant species. It is advantageous to human diet and beneficial to health. Besides its extraordinary nutritive value due to high and diverse content of sugars, proteins, lipids and dietary cellulose, it is known as a rich source of biologically active substances. Among these are glycosides, phenols, flavonoids, carotenoids, tocopherols, phytosterols, polyunsaturated lipids as well as amino acids, micro- and macroelements, vitamins and enzymes. The described unique composition makes bee pollen a valuable substrate with broad use in pharmacology, medicine and nutrition. Bee bread, a pollen-derived storage stock product for bees, is formed in hives upon bee pollen lactic fermentation with bee-secreted enzymes.

The study was carried out to evaluate bee pollen and bee bread usefulness as therapy-oriented dietary supplements. The content, physico-chemical parameters, bioactive properties and nutritional potential of several samples originating from different sources were determined. Apart from pollen-mixed types, a rape pollen-rich sample was used. As for bee bread, the experiments were done both on natural bee hive-generated product and on laboratory-fermented pollen under controlled conditions.

Analytical methods involved UV/VIS spectroscopy, fractionation with liquid column chromatography (HPLC, FPLC) and electrophoresis, qualitative and quantitative gas chromatography with mass-spectrometry detection (GCMS), and antioxidant activity determination with electron paramagnetic resonance (EPR) using low-frequency (1.2 GHz) L-band spectrometer.

By means of extraction with solvents of different polarity: water, methanol, ethanol, aceton, hexane, several fractions were obtained that were enriched in certain substances like sugars, vitamins, glycosides, phenols and flavonoids, carotenoids, fatty acids and phytosterols. Every fraction was also checked for its antioxidative action as verified with EPR by the ability to reduce stable free radicals (TEMPO, DPPH).

The results indicate that bee pollen and bee bread are valuable products exhibiting heterogeneous content with high level of biologically-active components. Antioxidant activities were present in both ethanolic and aqueous extracts and were comparable with that of a green-tea standard. Thus, it is suggested to consider the studied bee products as diet-supplementary preparations applied according to the principles of therapeutic nutrition.

P1.1.2

Role of fatty acids in endothelial cell differentiation

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Introduction: It is well recognized that nutrients exert biological effects regulating gene expression. Fatty acids, change the activity of transcription factors, or directly
influence the gene transcription or mRNA stability, thus participate in cell maturation and fate.

**Aim:** Comparison of the saturated, monounsaturated, and polyunsaturated fatty acids to modulate cultured endothelial cell and its progenitors gene expression.

**Methods:** Human endothelial cells (HUVEC) isolated from umbilical vein using collagenase digestion method and were grown in EBM medium with supplement and antibiotics. AC133+ cells were isolated from human cord blood using magnetic microbits (Miltenyi Biotech). Cells were grown in EBM medium with supplement of SDF and VEGF and antibiotics. After 6 days cells which expressed VE cadherin (EPC) were used for study. Influence of the 24-hour incubation with non-toxic (1-30 mM) palmitic acid (PA), oleic acid (OA), arachidonic acid (AA), eicosapentaenoic acid (EPA) on the gene expression was measured using oligonucleotide chips (Affymetrix), and confirmed by real-time PCR. The effect on cell proliferation was measured by BrdU incorporation. Chemotaxis was performed using Boyden Chamber System (Becton Dickinson). Angiogenic potency was investigated by the tubule formation assay in the in vitro 3D matrigel model. To measure the activity of the main kinases the Fast Activated Cell-based ELISA Kits were used.

**Results:** Unsaturated fatty acids increased gene expression related to homing of progenitors as well as to genes characteristic for adipocytes. The activity of ERK kinase was not observed after incubation of used cells with all fatty acids, what is in consistence with the lack of influence on cell proliferation by FAs. However all used FAs in non-toxic concentrations activated EPC and HUVEC cell migration connected with the activation of p38-kinase. The reduced activity of FAK kinase was observed (except PA in HUVEC), what may be connected with reduction of cell-matrix adhesion.

**Conclusion:** The dietary FA strongly influence the phenotype of EPC from proangiogenic towards adipogenic fate. Supported by the WL 194/F/L, 501/NKL/46/L.

**P1.1.3**

Transgenic potatoes with over-expression of enzymes of the flavonoid synthesis route as a source of bioactive substances in diet

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The studies on the safety of application of dried potato tubers with over-expression of chalcone isomerase (CHI), chalcone synthase (CHS), dihydroflavonol reductase (DFR) in the diet, prepared from transformers, obtained as a result of modification of *Solanum tuberosum* L. cv. Desiree plants included comparison of chemical composition and nutritional experiments on growing Wistar rats. In the tubers of transgenic plants, there was found a differentiation of concentration of secondary metabolites, having a character of antioxidants (compounds from flavonoids’ group), of ascorbic acid and of the compounds with anti-nutritional effect – steroid glycoalkaloids (substances specific of the plants from solanaca group). The in vivo studies were aimed at evaluation of potential allergenicity and toxicity of genetically modified plants with a changed (as a result of transgenesis) concentration of bioactive substances. A systematic administration (4 weeks) of considerable GMO quantities in the diet (30% DM of semi-synthetic mixture) did not affect negatively the growth rate of rats and favourably changed metabolism of lipids (decrease in the level of total cholesterol and of triglycerides in blood). It was found that the administration of GMO-containing diets was connected with the differentiation of rat immunological system reaction, change of antioxidant status of the selected organs, changes in histological picture of liver and activity of liver enzymes. Value of almost all evaluated animal health parameters was, however, found within the limits of values, characteristic of healthy animals. It was, therefore, recognized that transgenic potatoes with over-expression of enzymes of flavonoid synthesis route (CHI, CHS, and DFR) were a health-safe component of diet.
show that mitotic index is significantly increased in the BS group, which was associated with the up-regulation of the EGF receptor. The DNA damage, on the other hand, was significantly higher in the RS group. Interestingly, we showed that the extent of apoptosis gradually increased from CTRL to RS and BS. It was markedly higher in BS, but due to large variations between the individual rats, no significance has been found. Increase of the apoptosis ratio was related to the up-regulation of the estrogen receptor, suggesting the action of phytoestrogens. Concluding, the influence of soybean consumption on the intestine mucosa depends rather on its promitotic action than on proapoptotic properties. Thermal processing of soybean markedly influences the intestinal epithelium remodeling. The increased mitosis in animals fed with boiled soybean dissembles the negative effect of bioactive compounds on the small intestine structure.

Acknowledgements: PBZ-KBN-093/P06/2003; university grant 504-02310015.

P1.1.5

Pathways of death in neonatal epithelium

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Understanding the molecular mechanisms of programmed cell death in the intestinal epithelium is the basis of intervention in the development and remodeling of gut epithelium during postnatal early life as well as remodeling and regeneration after injury. In our study the piglet model was utilized due to similarities in growth, composition and development to children. In our earlier studies we observed “packets” of dieing enterocytes (groups of several neighboring cells). This suggested presence of auto/paracrine factors involved in promotion of cell death signal. Our studies showed that TGF-β1, the cytokine expressed in cells (both macrophages and epithelium) that ingest the apoptotic bodies, played a major role in this process. “Packets” expressed this cytokine in combination with active promoter caspases. We observed high expression of TGFRII in close vicinity of cells with high expression of TGF-β1, localized mostly on the basal layer, but also on the luminal plane and in between enterocytes. This explained the luminal transmission of death signal observed earlier. The link between TGF-β1 and cell death proved to be RunX protein. It is up-regulated by the action of secondary TGF-β1 messengers – the Smad cascade, and facilitates the shift in the balance between pro- and anti-apoptotic proteins from Bcl-2 family, as was confirmed by scanning cytometry. The second, direct death signal comes from TNFα, cytokine involved in apoptosis induction via DISC. Its expression generally colocalized with TGF-β1 and always with regulatory caspase 8, but no “packets” expressing this cytokine were ever observed. We hypothesize that TGF-β1 is the major auto/paracrine factor in the small intestinal mucosa, sensitizing the enterocytes for the signal of TNFα, the direct trigger of cell death.

Acknowledgements: PBZ-KBN-093/P06/2003; university grant 504-02310015.

P1.1.6

Food safety and the emerging new food products and food technologies

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The concept of food safety objective has been proposed to provide a target for operational food safety management, leaving flexibility in the way equivalent food safety levels achieved by different food chains. Performance objectives and performance criteria are two new concepts proposed recently to complement that of food safety objectives with respect to food safety control and control measures and process criteria regarding operational food safety management. Food safety management systems such as Hazard Analysis Critical Control Points (HACCP) and the pre-requisite systems Good Manufacturing Practice (GMP) and Good Hygiene Practice (GMP) have provided the professional players in the food supply chain with excellent tools. Many different food professionals are involved in the chain of food production, e.g. from primary production, distribution, processing and manufacture, packing, retail, to food service and preposition in the home by consumers. Food operations do not need to completely change the way they manage food safety now a number of new concepts have been introduced in the food control.

P1.1.7

Relationship between seasonal variation in nutrient intake and the 17 beta-estradiol level in menstrual cycle of women at pre-menopausal age

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Introduction: Level of 17 beta-estradiol in circulation is connected to fecundity as well as to the risk of breast and endometrial cancer. Recent trials proved that dietary modification can favorably alter the level of endogenous sex hormones.

Methods: To determine impact of nutritional status on the 17 beta-estradiol concentration in plasma, the study was conducted among young women living in urban area who filled out dietary questionnaires in summer and winter seasons. At the same time, they donated blood
samples, twice in follicular and luteal phase of menstrual cycle in each season for determination of 17 beta-estradiol concentration in plasma. The dietary data were computerized and energy and nutrients intake were estimated using the recently updated Polish food tables. For the ANOVA break down analysis women were stratified according to seasonal and menstrual cycle phases groups.

**Results:** Mean values of 17 beta-estradiol concentration did not show statistically significant differences between both phases in summer, whereas 17 beta-estradiol concentration was significantly higher in luteal phase as compared to follicular phase in winter (p = 0.019). The energy intake and carbohydrates did not influence 17 beta-estradiol concentration. The protein intake decreased this concentration only in follicular phase (p = 0.024). The effect is not specific for protein origin. The fat intake enhanced 17 beta-estradiol concentration level in luteal phase in winter season (p = 0.06). High tea intake and catechins enhanced 17 beta-estradiol concentration in follicular phase in summer (p = 0.045 and 0.01), but not in winter.

**Conclusion:** Seasonal variation in nutrient intake can affect the estradiol level.

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**P1.1.8**

**Changes of selected food components in the intestinal tract and its influence on human faecal microflora**

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Food is considered as a major source of exposure to ingested contaminants. Intestinal microflora is the main component of the digestion tract and plays an important role in digestive functions. The role of colonic microflora remains generally unclear regarding both polyphenols degradation and formation of phenolic compounds and other food components as well as microorganisms responsible for that action and mechanisms involved.

The aim of presented research was to analyse changes in microbial composition and metabolic function of colonic microflora as well as interaction of intestinal microflora with food components. Extrudated products from legumes seeds-Red Kidney bean (chips) were selected for this project.

The digestion tract model, which comprises 3 parts: stomach, small intestine and large intestine, was used for controlling and regulating the environment in which digestion processes occurred. In each parts of the model the total amount of soluble nitrogen, carbohydrates, phenolic compounds (mg gallic acids) and endproducts of microflora metabolism (short-chain fatty acids) were determined by HPLC. Changes in microbial composition were controlled during the digestion process.

Faecal flora was isolated from feces of 3 human volunteers. The intestinal model was inoculated with bacterial flora at concentration of 10^6 cfu/ml. Growth of Lactobacillus, Bifidobacterium, Enterococcus and Enterobacteriacae was enumerated on selective media. The amount of bacteria after digestion in the large intestine reached 10^8 cfu/ml for other groups of microorganisms. The main microbial metabolic end products observed in the digested material were short-chain fatty acids. The lactic acid was the main product detected in the digested Red Kidney bean product.

It was noticed that after each stage of digestion process there was an increase of soluble nitrogen. The most sharp increase was shown in stomach. After each stage of digestion process there was an increase of carbohydrates. Only after 21 hours of digestion in large intestine the decrease of carbohydrates was found.

The highest amount of phenolic compounds after the digestion process was determined.

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**Evaluation of the antagonistic properties of natural, antibacterial substances extracted from herbs**

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Herbs, are commonly known natural products exhibit antimicrobial activity. This properties allow to investigate their potential use as natural antimicrobial or preservative additives for food and feed.

To evaluate the antagonistic properties of natural substances extracted from herbs, the following six genus of herbs were examined for their antimicrobial activity: melissa (*Melissa officinalis* L.), hyperici (*Hypericum perforatum* L.), celandine (*Chelidonium majus* L.), chamomile (*Chamomilla recutita* Rausch.), sage (*Salvia officinalis* L.) and echinacea (*Echinacea purpurea*). The antibacterial activity of herbs extracts was investigated against four test bacteria: *Escherichia coli*, *Enterococcus faecium*, *Bifidobacterium animalis* and *Lactobacillus plantarum*. The test microorganisms were isolated from human large intestine.

The herbs extracts were prepared as water and 10 and 30% water – ethanol (v/v) solution. The extracts were obtained by shaking for 30 min at 150 rpm at room temperature and sterilized by microfiltration (filtrated through 0.45 µm Millipore filters). The antibacterial activity expressed as growth inhibition of the tested bacteria was evaluated by three methods: classical paper disc diffusion method, serial dilution method as well as the electrical impedance measurement.

The 10 and 30% ethanol solutions, allowed to obtain extracts with higher antibacterial activity in comparison to water extracts. The highest inhibiting properties were observed when 30% ethanol solution was used to extraction. Hyperici and sage were noted as herbs exhibited the highest antibacterial activity. *Enterococcus faecium* and *Escherichia coli* were more sensitive than *Bifidobacterium animalis* and *Lactobacillus plantarum*. 
According to the research, it was assumed that for the search and evaluation of the antagonistic properties of natural antibacterial substances, the serial dilution method as well as the measure of medium electrical impedance changes during growth of microorganisms in the presence of the evaluated extracts was more precise and sensitive comparing to the classical paper disc diffusion method.

P1.1.10

In vitro cytotoxicity of aronia juice to human cancer cells

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The objective of the present study was to examine the effect of aronia juice (Aronia melanocarpa Elliot) after digestion in gastrointestinal tract and transport across intestinal cell monolayer \textit{in vitro}; on the human intestinal (Caco-2 and HT-29), breast (MCF-7) and liver (HepG2) cancer cells and leukaemia (HL-60) cells, in terms of the proliferation, cytotoxicity and genotoxicity.

The AJ digestion \textit{in vitro} was carried out by sequencing changes of pH value and addition of digestive agents (porcine pepsin, pancreatic extract and bile salts) and faecal bacteria culture. The mixture obtained after digestion in gastrointestinal tract was sterilized by filtration (0.22 µm) and transported across intestinal cell monolayers. For transport experiments, the intestinal cells were grown on the polycarbonate Millicell PCF membranes (0.4 mm pore size) for 21 and 14 days for Caco-2 and HT-29 cells. Exponentially growing cancer cells were treated with the digested and transported aronia juice for 48 hours. Cell proliferation was monitored by cell counting. Cytotoxicity and genotoxicity of aronia juice was determined by performing MTT assay and using comet assay, respectively.

The obtained results showed that AJ inhibits of cancer cells proliferation in a concentration-dependent manner. Aronia juice was found to suppress the growth of intestinal Caco-2 and HT-29 cells with an IC\textsubscript{50} of 0.9 and 2.4% v/v, respectively. AJ cytotoxicity was reduced by low AJ permeability in Caco-2 cell monolayer. During the transport across HT-29 cell culture, the better bioavailability of the AJ bioactive compounds was observed. It was reflected in higher antiproliferative potency of transported AJ against tested cancer cells. Treatment of the MCF-7, HepG2 and HL-60 cells with AJ transported across Caco-2 and HT-29 monolayers caused, respectively, 10-25% and 40-50% growth inhibition.

The Grant K 094/P06/2003 from the State Committee for Scientific Research, Warsaw, Poland, supported the study.

Panel II: Food processing and functional properties of foods

Oral presentations

O1.2.1

Proteomics in nutrition research: principles, technologies and applications

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Every nutritional process relies on the interplay of a huge number of proteins encoded by the respective mRNA molecules that are expressed in a given cell, organ or organism. Alterations of mRNA levels and in turn of the corresponding protein levels (although this not necessarily changes in parallel) are critical parameters in controlling the flux of a nutrient or metabolite through a biochemical pathway. Nutrients and non-nutrient components of foods, diets and lifestyle – including physical activity – can affect essentially every step in the flow of genetic information from gene expression to protein synthesis and protein degradation and thereby alter metabolic functions in the most complex ways. In contrast to transcript profiling, proteomics technologies determine the level of the functional units, namely the proteins. Proteome analysis (“Proteomics”) allows both, changes in protein expression patterns, and the identity of the proteins themselves to be displayed and determined simultaneously. Moreover for individual proteins, post-translational modifications that may be crucial for functions or even amino acid substitutions (polymorphisms) can be detected. The potential value of proteomics for nutritional science has been recognised for some years but in contrast to large scale transcriptome analysis that is already used by the nutrition research community, and has led to numerous published studies, only a very few reports have used proteome analysis as a tool in nutrition research. I shall review and summarize recent technological developments and describe the principles of “classical” proteomics based on two-dimensional gel electrophoresis in combination with peptide mass fingerprinting \textit{via} MALDI-TOF MS for protein identification. Studies on the effects of nutrients and non-nutrient compounds of foods on the proteome of human cells and in animal studies will be used to show the power of proteomics in biomarker discovery. More recently, methods for human body-fluid proteomics (plasma, urine) as well as from human platelets and peripheral mononuclear cells have been improved that will allow to apply proteomics technologies also in human intervention trials.


Abstracts


O1.2.2

Condition for implementation of biotechnological achievements in agriculture

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Abstract: Rapid development of biotechnology and a wide application of its results influence significantly the economy of the present world. In this respect, countries paying attention to and supporting the biotechnology development are preparing ground for future rapid development and domination on world markets. This process requires, however, careful coordination of basic research, innovation practices and finally implementation of the results on industrial scale. In many countries, the last step is quite often restrained or even stopped due to different reasons. A good example is agriculture where new achievements in biotechnology are implemented at different pace, in different countries. This is leading to future problems in the development of the sector among others its competitiveness and competitiveness cooperating industries.

The presentation will concern different legal, social, scientific and technical aspects influencing the implementation of biotechnological achievements in agriculture, with particular emphasis placed on Poland and other EU countries.

O1.2.3

Starch of native and roasted buckwheat grains – structure, chemical composition and functionality

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Abstract: More than ¼ of population in the world suffers from the metabolic diseases depend on a diet, the incidence of tumors or hypertension is still rising also. These facts provoke the interesting of functional food or its components which show a influence on human organism and healthy activity. Starch is the storage polysaccharide component of buckwheat grains. Generally starch is recognize as a significant source of energy for human however the buckwheat starch is a low energy component. Moreover it could be the positive interact with gut microflora what indicates that buckwheat products may be the prebiotic substrates for a gastrointestinal tract.

The aim of this study was determine the nature and functional properties of laboratory isolated starch from the native and the roasted buckwheat grains of Kora variety (Fagopyrum esculentum).

The buckwheat starch to be examined by scanning electron microscopy resulted the starch granules of non treated grains are spherical, polygonal, irregular shape, whereas granules from the grains upon thermal processing (roasting 160°C, 30 min) are oval, irregular, some breakings or conglomerates were noticed. The size of granule distributed between 2–6 x 10⁻⁶ m, but after roasting the slight damages on the surface were indicated Chemical characteristics confirmed a low content of ash and protein, relatively a high content of resistant starch and dietary fibre. These compounds, that act physiological functions, are important in diet applying for prevention of civilization diseases, e.g. diabetes, hypertension, obesity. The amylase fraction of starch was changing during thermal process indicating respectively: the native 17.1%, the roasted 12.4%. The ability to absorb of water and oil at 25°C was tested. Both starches characterized the balanced hydrophilic-hydrophobic interactions, results range: 1.36–1.51 g water/g d.m. for water binding capacity or 1.26–1.44 g oil/g d.m. for oil absorption. The swelling power and the solubility of starch granules analysed in interval 60–95°C effect the increase of these functional properties with growing temperature. The swelling power was high in spite of small size of granules, but the solubility was low in comparison with other cereal starches.

O1.2.4

The chemotactic activity of beta-carotene in endothelial cell progenitors and HUVEC: the microarray analysis


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Abstract: Endothelial cells and its progenitors play an important role in angiogenesis, essential for organogenesis, tissue remodeling but also for inflammatory response, carcinogenesis in all periods of our life. In our study we concentrated on the direct effect of beta-carotene (BC) on human umbilical cord originated endothelial progenitors (EPC) and human umbilical vein endothelial cells (HUVEC).

Methods: BC uptake was measured by HPLC method. The effect on cell proliferation was measured by BrdU incorporation. Chemotaxis was performed in the Boyden’s chamber The influence on tubular-like structure formation was investigated by the 3D assay in matrigel in vitro as well as in vivo model. Changes of gene expression were analyzed using microarray hybridization method. The quantitative gene expression was estimated using the real-time PCR method.

Results: We have demonstrated that BC-in the physiological range of concentrations, found in human blood, is a potent activator of chemotaxis of endothelial cells. The microarray data analysis revealed that the genes involved...
in cell/cell; cell /matrix adhesion; matrix reorganization, and activation of chemotaxis were the most affected by BC genes in HUVEC and EPC. These results were also confirmed in in vivo angiogenesis model. Conclusion: BC in the physiological concentration range stimulates early steps of angiogenic activity of endothelial cells by activation of cellular migration as well as matrix reorganization and decrease of cell adhesion.  
Sponsored by the EU F5 DLARFD QLTR-2001-00183 and STEC QLK3-CT-2002-30307 projects.

**O1.2.5**

**Separation of betalains from cacti fruits of Hylocercus polyrhizus by preparative high-speed countercurrent chromatography (HSCCC)**

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Betalains are very potent antioxidants and are used in various applications in the food industry due to their colorant properties [1]. Furthermore, these compounds can support anticarcinogenic therapies. Betalains are water soluble nitrogenous pigments consisting of two groups of compounds, red-violet betacyanins and yellow betaxanthins. Betalains are present in plants of the Caryophyllales order (e.g. in Beta Vulgaris L.). Some vine cacti species belonging to the subfamily Cactoideae of the tribe Cactae have been recently investigated and betacyanins were identified in their fruit pulp [2-4]. A potential source of betacyanins are fruits of Hylocercus species, known as red pitaya or pitahaya, which are medium-large berries bearing large green or red scales. Preparative isolation of betalains is still problematic since these compounds are rather labile especially at elevated temperature. Our current investigations on betalains are focused on isolation of the pigments by high-speed countercurrent chromatography (HSCCC) which is emerging and powerful technique for preparative isolation of natural compounds from plant extracts [6]. The aim of this study was to perform a separation of betalains from Hylocercus polyrhizus fruit extracts.

The pigments were extracted from 100 grams of freeze-dried H. polyrhizus fruit flesh with H₂O: MeOH (20:80). The extract was rotovaporated under reduced pressure at 25°C, freeze-dried and cleaned-up by solid phase extraction on a C18 column with elution by MeOH: H₂O (0.3% TFA) (95:5, v/v). Separation of 700 mg freeze-dried pigment extract was carried out on a "high-speed countercurrent chromatograph" (HSCCC) model CCC-1000 (Pharma-Tech. Res. Corp., U.S.A.) applying the biphasic solvent system n-BuOH: ACN: H₂O (0.7% TFA) = 5:1:6 (v/v). The flow rate was 3 mL/min and the rotation velocity was set to 850 rpm [1]. The HSCCC system was operated in the "head-to-tail" mode [1]. The resulting fractions were analysed by LC-ESI-MS/MS. The fractions contained 6 main betacyanins (betanin, phyllocactin and hylocerenin as well as their C15 isomers) and other minor pigments characterised recently [4]. The decarboxylated derivatives were observed in the more lipophilic fractions. The first fraction contained a bulk of the most polar compounds which were very well separated from betacyanins by HSCCC.


**O1.2.6**

**Cell culture-based system construction for analysis of selected gene response on nutrients**

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Intestinal epithelial cells (enterocytes) and liver cells (hepatocytes) are major cells making first contact with ingested nutrients. The enterocytes play crucial role in intake of digested food posing a selective barrier but also play important role in defense and immunological processes. The hepatocytes metabolize and store nutrients but also detoxify and produce compounds. We would like to describe a construction of in vivo system for analysis of selected gene response to nutrients. The system is composed of human transformed enterocytes and hepatocytes (Caco-2 and HepG2 cell lines, respectively) stably transfected with reporter vector. The reporter gene coding for the secreted embryonic alkaline phosphatase (SEAP) is under control of microsomal epoxide hydrolase 1 (EPHX1), quinone NAD(P)H dehydrogenase 1 (NQO1), or prostaglandin-endoperoxide synthase 2 (PTGS2) promoter sequences. EPHX1 is biotransformation enzyme that catalyzes the activation and detoxification of exogenous chemicals such as polycyclic aromatic hydrocarbons. NQO1 serves as a quinone reductase in connection with conjugation reactions of hydroquinones involved in detoxification pathways as well as in biosyn-
thetcic processes. PTGS2 is an inducible gene that may have a role as a major mediator of inflammation and/or a role for prostanoid signaling in activity-dependent plasticity. The HepG2 cells are co-cultured at the basolateral side of Caco-2 cells grown and differentiated on culture plate insert membrane. The SEAP enzyme is thermostable allowing heat-inactivation of the endogenous and serum-derived activity. The enzyme is secreted to culture medium permitting continuous analysis of reporter gene transcription level. Its activity is measured colorimetrically or with chemiluminescent assay (more sensitive and not affected by dye-compounds). The system was developed to analyze the EPHX1, NQO1 and PTGS2 gene transcription level in response to plant antioxidants and adhesion of intestinal microbes, as well as other nutrients.

**O1.2.7**

**Bioactive substances in sow diet influence the development of intestine epithelium in their offspring**

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Change to the intestinal nutrition after birth is the reason for the huge remodeling event in the gastrointestinal tract. The most important changes take place in small intestinal epithelium i.e. growth of mucosa layer and enterocyte maturation, associated with the decline of apical canalicular system (ACS) in the enterocyte. Dramatic growth of the mucosa in early life is a result of colostral molecule incrustation to the enterocytes (via ACS), as well as an increase in cell number. The later emerges as the mitosis to programmed cell death (PCD) ratio favors the proliferation. Delayed or incorrect development of small intestine in neonatal piglets is the one of the crucial reasons contributing to debilitate animal growth or reduce survival rate, especially in the intensive rearing systems. The aim of present study was to investigate the effect of bioactive substances fed to sows on the development of small intestine mucosa in their offspring. Sows were randomly divided into control (C = non supplemented) and experimental groups (S = supplemented). The blend of bioactive substances (polyphenols, antioxidants, PUFA and limiting amino acids) was created to mimic the content of these substances in wild boar milk. Piglets from two groups – control (C) and treated (S) sows – were sacrificed at day 1, 2, 4, 7 and 14. Samples taken from mid-jejunum were evaluated for mitosis (Ki67, present only in proliferating cells), apoptosis (active caspase 3, characteristic for late, irreversible phase of apoptosis), autophagy (MAP I LC3, the only reliable marker of autophagy, that occurs in autophagosome membranes), DNA damage (p53, protein recognizing damaged DNA), TNF-α and TGF-β1 (major cytokines that are involved in the induction, regulation and promotion of apoptotic signal in the epithelium). Mitotic index was slightly decreased (ns). The autophagy index at day 2 was higher in S (p<0.001). The overall mitotic to programmed cell death ratio increased in early life in S group quicker than in C, which was in parallel to increase in mucosa thickness observed in histometry. The highest differences between the groups were in the DNA damage index, where dramatic decrease in p53-positive cells was observed in favor of S group (p<0.001). Adding up the facts, the presented work strongly suggests that bioactive substances in sow diet have a positive effect on the small intestinal epithelium growth and maturation in their offspring.

**Acknowledgements:** PBZ-KBN-093/P06/2003; university grant 504-02310015.

**O1.2.8**

**Effect of husked and naked oats grain on blood lipid markers of laboratory rats**

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Oat grains of *A. sativa* var nuda (Polar and STH 6102) and *A. sativa* L. (Krezus and STH 735) were investigated, harvested in 2005. The chemical compositions of oat isolates and amino acid composition of proteins, as well as the nutritive values were estimated. Significant differences (P<0.05) were observed among naked and husked oat isolates in their crude fibre, crude fat and crude protein. All the samples examined were characterised by a lysine and isoleucine while the level of methionine was satisfactory for the most of grains. EAAI and the total amino acid content in protein were higher in naked oats than in husked oats.

The experimental part of the biological studies was carried out in experiment on laboratory rats (Rattus Norvegicus) of the Wistar strain. Content of triglyceride, cholesterol, LDL and HDL in blood were determined. Significant differences were determined using analysis of variance and Duncan’s multiple range test. There were no significant differences between groups in LDL, HDL and triglyceride. However, naked oats in the diets decreased the cholesterol and lipid level in blood.

**Posters**

**P1.2.1**

**Dietary 5-n-alk(en)ylresorcinols in cereals and cereal products**

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Effect of husked and naked oats grain on blood lipid markers of laboratory rats: Control and experimental groups were divided into the control (C) and treated (S) groups. Each of the groups was randomly divided into control and experimental groups. The food consumed by the rats was provided as normal laboratory diet (C) or oat grains of *A. sativa* var nuda (Polar and STH 6102) and *A. sativa* L. (Krezus and STH 735) supplemented with 20% of oat grain isolates (S). After 8 weeks of experimental period, the rats were sacrificed and their blood samples were collected. The content of triglyceride, cholesterol, LDL and HDL in blood were determined. Significant differences were determined using analysis of variance and Duncan’s multiple range test. There were no significant differences between groups in LDL, HDL and triglyceride. However, naked oats in the diets decreased the cholesterol and lipid level in blood.
Food containing whole grain cereals have been identified as providing significant health benefits over refined grains and have been linked to a decrease of diabetes, obesity, heart disease and some cancers [1]. The idea to use 5-n-alk(en)ylresorcinols (AR) as biomarker for whole grain intake was made when characteristic position of this compounds was estimated in cereal grains [2,3]. AR are found mainly in the outer layers (bran fraction) of cereal, which means that they are largely missing in refined flour and conventional cereal products such as white bread and most breakfast cereals [4]. The AR content of rye and wheat grain samples (intact or milled), as well as of whole wheat and rye bran and cereal food containing wheat and rye was determined. The method of extraction included ethyl acetate and acetone as extraction solvent, 48 h as extraction time and 40 ml as extraction volume per gram of sample. The extracts were analysed (without further purification) by standard colorimetric method with diazoniun salt Fast Blue BxBF₄ [5]. The average total AR content in whole wheat grains was about 2050 nmol/g and 2400 nmol/g in whole rye grains, what is in agreement with a previous study [2,3]. The AR content in cereal food commonly consumed, varied widely from nondetectable levels in white flour to more than 2200 nmol/g in some whole grain rye products. The relative composition of AR homologues was measured by RP-HPLC. The ratio of the homologues C17:0/C21:0 was up to 1.3 in rye and below to 0.3 in wheat, indicating that it can be used to distinguish between those two cereals. The C17:0/C21:0 ratio was altered in cereal products containing a mixture of whole grain wheat and rye or their bran. In this case the ratio varied between 0.3 and 1.3. AR appear to be good marker of whole grain wheat and rye in food, and their analysis may be an objective way to identify food rich in whole grain wheat and/or rye or their bran.


P1.2.2

Sources of carotenoids and their use in the food industry

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Carotenoids concentrates made from carrots (Daucus carota L. var. Nevis F1) were produced in two application forms – as lyophilised powder of carotenoids and another form was carotenoids concentrate dried on corn starch. They were used for application into pasta (eggs and non-eggs pasta). Wholemeal flour (Tr. aestivum), Tr. durum flour (semolina) and whole grain flour from spelt (Tr. spelta) were treated with lyophilized carotenoids concentrate (LCC) or carotenoids concentrate dried on corn starch (SCC). Pasta products (eggs and non eggs) enriched by LCC or SCC were evaluated with sensory and objective methods. The best acceptability was for variants with addition of SCC (20 g into 480 g flour) into eggs and non-eggs pasta. Addition of 20 g of SCC into whole grain of Tr. spelta, showed better colour of fortified pasta compared to control.

Objective evaluation of eggs variants with LCC use showed that these pasta products did not create any sediment. Cooking water absorption of non-eggs pasta with addition of SCC was higher compared to eggs pasta with addition of LCC. This natural dyestuff didn’t show negative influence on another objective properties of pasta, what can be positively evaluated.

P1.2.3

Indole derivatives concentrations in mycelial culture and fruitbodies of Xerocomus badius

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Whereas in every day life fungi are perceived as a food-stuff of debatable nutritional value, both the ages-long folk medicine tradition and the contemporary phytochemical research suggest at their enormous therapeutic potential. Xerocomus badius (Boletaceae) is a very popular, edible species of mushroom in Poland. The aim of the work was optimization of their culture conditions in terms of the highest yield and analysis of chemical composition of mycelia. The mycelial cultures were maintained on liquid medium according to Oddoux. Qualitative and quantitative analysis of the indole derivatives was also determined in fruiting bodies grown under natural conditions in order to compare these data with results of analysis of mycelium cultured in vitro. From the fruiting bodies and from mycelial cultures methanol extracts were prepared. Than this extract were purified by TLC method on aluminum plates, covered by silica gel (DC-Alufolien, Kieselgel F-254, Merck). After purification extracts were analyzed with using chromatographic (TLC, PTL, HPLC) and densitometric methods. The following indole metabolites were identified: 1,1-d-kynureine sulfate, hydroxtryptamine, l-tryptophan, tryptamine, melatonin in methanol extracts of mycelial cultures and only hydroxtryptamine in methanol extracts of fruit bodies of Xerocomus badius.

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P1.2.4

The influence of culture parameters on carotenogenesis in cells of Phaffia rhodozyma yeast

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Carotenoids are commonly applied as additives in feed, food, pharmaceutical and cosmetic industry. Among them special attention should be paid to astaxanthin, an indispensable ingredient of compound feeds used in commercial salmon, rainbow trout and shrimp culture. It is responsible for the characteristic reddish pink colour of their tissues. Astaxanthin exhibits also high antioxidant activity, higher than those of β-carotene and α-tocopherol. The potential commercial source of this pigment seems to be Phaffia rhodozyma yeast. But employing of wild Phaffia rhodozyma strains to astaxanthin production is not economical because of low level of pigment synthesis. In order to reduce astaxanthin production costs, two kinds of investigations are conducted. One is connected with mutagenization of wild strains and second with optimization of environmental agents influencing carotenogenesis.

The aim of this study was to evaluate the effect of pH and culture time on the production of carotenoids by a strain of Phaffia rhodozyma CBS 5626. Yeast cultures were carried out on YM medium, in Erlenmayer flasks (150 r.p.m.) for 96 h at 22°C, at illuminance of 600 lux. The initial pH of medium was adjusted to 4.0, 5.0 or 6.0. Every day the pH of culture was adjusted to the initial level and the culture was fed with glucose solution. The pH of medium, yield of yeast dry matter, number of yeast cells and their vitality, concentration of reducing substances [1] and total carotenoids were controlled every 24 h. The method described by Sedmak et al. [2] was applied to isolate carotenoids from cells of analyzed yeast, using DMSO (55°C) and next acetone in order to pigment extraction. In the obtained extract the amount of carotenoids was determined by spectrophotometry (λ=484 nm).

A significant effect of time and culture medium pH on carotenoid synthesis by yeast of Phaffia rhodozyma CBS 5626 was shown in the experiment (α=0.05). The highest pigment yields both in terms of yeast dry matter as a culture volume unit (1L) were obtained in culture carried out with pH regulation to the level of 6.0 at 168 h. The lowest carotenoid yields were obtained in case of culture carried out with pH regulation to the level of 4.0 at 4.0 pH. In this culture the highest biomass yield was obtained. Noticed results indicate, that in order to obtain the high carotenoid yields, the Phaffia rhodozyma culture should be carried out in two stages e.g. at the beginning at pH=4.0 and than at pH=6.0.


P1.2.5

Simple and economic method of non-alcoholic beer production

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The original and investmentless method of production of non-alcoholic beer, which contains to 0.5% ethyl alcohol by volume was developed. The ability of special strains of yeasts to ferment only saccharose, glucose and fructose from brewery wort were used. The optimum conditions of fermentation and maturation of beer were developed. The technology was implemented and used to industrial production of alcohol-free beer.

Beer is isotonic drink, because his osmotic pressure is similar to osmotic pressure of blood. It is good, natural source of mineral salts, which come from water and malt. The existing, recommended methods of production of alcohol-free beer require expensive investments, and it is high the cost of their exploitation. The final product contains very little ethyl alcohol, but it loses part of aroma volatile products producing during fermentation.

P1.2.6

Assessment of selective qualitative indicators in amaranth

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Four specieses of amaranth (A. hypochondriacus, A. paniculatus, A. cruentus and A. caudatus) where observed in relation to qualitative indicators of their quality. It follows from the research, that seeds of observed specieses show high content of fat (55.7–77.2 g/kg) and fibre (39.3–81.8 g/kg). We evaluated high content of iron (375.1–651.7 mg/kg), manganese (37.2–46.9 mg/kg) and zinc (33.5–39.5 mg/kg). The amaranth flour contained all essential amino acids, whose average values exceeded severalfold the amino acid values of brain flour. The highest content of leucine (10.61 g/kg), isoleucine (6.77 g/kg), phenylalanine (7.79 g/kg), treonine (7.65 g/kg), valine (8.01 g/kg), glycine (14.90 g/kg), asparagine acid (15.80 g/kg) and serine (12.98 g/kg) was found out in species A. hypochondriacus. The species A. paniculatus showed the highest content of histidine (5.51 g/kg). The highest values of lysine (9.50 g/kg) and arginine (19.44 g/kg) was evaluated in A. cruentus. On the base of our results we can summarised, that in comparation with species of amaranth in view of observed indicators A. hypochondriacus reached the better parameters.
Optimization of the culture conditions of *Sarcodon imbricatus* for mycelial growth and nitrogen compounds production

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*Sarcodon imbricatus* (L.P.Karst) belongs to *Aphyllophorales* (Basidiomycetes). This species is under legal protection in Poland.

The aim of this study was to optimize of submerged culture conditions for biomass increments and production of nitrogen compounds such as amino acids, indole derivatives and amine.

*S. imbricatus in vitro* cultures was initialized from fruit bodies taken from natural state. The optimal medium composition for submerged culture was determined. To investigate the effect of carbon and nitrogen source on hyphal growth, the mycelium was cultivated on the medium containing various nitrogen (ammonium nitrate, hydrolyzate of casein, yeast extract, malt extract, and sodium nitrate) and carbon (glucose, fructose, maltose, sucrose, lactose) sources. Additionally the optimum initial pH of culture was determined.

The optimal medium composition for biomass increments was 5% glucose, 1% hydrolyzate of casein, 0.3% KH₂PO₄. Maximal growth of biomass was observed at initial pH 6.0.

The presence and content of various amino acids, indole derivatives and amines was determined using HPLC and TLC methods. The largest contents of leucine, lysine, arginine, alanine, methionine, histidine were detected. Among indole derivatives high of contents of tryptophan and tryptamine and low content of melatonin was found in mycelial culture. Any amines were quantified in the culture.

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Pannel III: Probiotics

Oral presentations

O1.3.1

Probiotics – anticancer activities and effects on gene expression

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While a myriad of healthful effects have been attributed to the probiotic lactic acid bacteria, perhaps the most controversial remains that of anticancer activity. Reports in the literature, regarding the anticancer effects of lactic acid bacteria, fall into the following categories: *in vitro* studies and *in vivo* studies in laboratory animals; dietary intervention studies in human volunteers and epidemiological studies correlating cancer and certain dietary regimes. However, it must be emphasised that, to date, there is no direct experimental evidence for cancer suppression in humans as a result of consumption of lactic cultures in fermented or unfermented dairy products. However, there is a wealth of indirect evidence, based largely on laboratory studies, in the literature (1) and this will be summarized in my presentation. At present, the results from the epidemiological studies do not appear to support the results from experimental studies. The reason for this is unclear but might be explained by differences in bacterial strains, with the strains being used in the experimental studies surviving better in the gastrointestinal tract than the strains present in fermented dairy products. It should also be emphasized that great care must be exercised in extrapolating the results of *in vitro* and animal studies to the human system. It must also be pointed out that the precise mechanisms by which probiotic bacteria may inhibit colon cancer are presently unknown and these will be discussed.

However, even with these reservations in mind, the use of lactic cultures for human cancer suppression holds promise and deserves more scrutiny. The latter should involve carefully designed human dietary intervention studies to corroborate the wealth of experimental studies. I will report on such an intervention study which was recently completed as part of an EU funded project “Synbiotics and Cancer Prevention in Humans” (2).

Finally, studies are presently underway in our laboratory to study the effect of probiotic bacteria on gene expression *in vivo* in animal models (colon of germfree mice) and in cell culture models (colonic epithelial cells). Interest is focused on genes relevant to tumorigenesis and obesity. Some recent findings will be presented.


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O1.3.2

How to develop probiotics as functional foods?

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Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit to the host. While probiotics have proven benefits, the optimism associated with their use is counterbalanced by the fact that many so-called “probiotic” products are unreliable in content and unproven clinically. Therefore much remains to be done to gain the acceptance of the broader medical community. To ensure safety and reliability of probiotics in food several recommendations and legis-
latory activities have been initiated last years. These include operating standards for the evaluation of probiotics in food issued by FAO and WHO and the latest directive of the EU on health claims. Obviously, probiotics registered as functional foods or food supplements subject for uniform procedures required by the EU regulatory bodies or FDA. Generally, a candidate microorganism strain should be identified by phenotypic and genotypic methods, characterized functionally for its probiotic activities to gain knowledge on mechanisms of its activity and its safety. These in most instances include multiple tests giving evidence that the strain is able to adhere and multiply on the human (or animal) body surfaces and to compete with pathogens. However, most of the actual and future applications of probiotics as functional foods indicated to ameliorate or prevent infections and immune dysfunctions or even metabolic disorders require demonstration of interactions between probiotic bacteria and molecular mechanisms of the host impaired in these conditions. In such cases, animal models can provide substantiation of in vitro effects and determination of probiotic mechanism. The principal outcome of efficacy studies on probiotics should be proven benefits in human trials, such as statistically and biologically significant improvement in conditions symptoms, signs, well-being or quality of life, reduced risk of disease or longer time to next occurrence, or faster recovery from illness. Each should have a proven correlation with the probiotic tested by demonstration of colonization of test persons by the probiotics strain(s) under investigation. Critical evaluation of clinical trials on efficacy of probiotics shows, however, multiple examples of unreasonable usage of products containing bacteria without proven probiotic activities or already registered probiotics which, however, have been designed for other indications.

O1.3.3

Estimation of bactericidal and bacteriostatic effect of biological active spice extracts

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Spices are used in food technology not only to enhance the flavor and aroma but also as an alternative approach to the preservation of food products. The knowledge on the influence of species on bacterial activity, both pathogenic and beneficial (favourable) gastro-intestinal microflora, is still improved and supplemented.

In the performed experiments four test bacteria strains and five kinds of spices were evaluated for their antagonistic properties. The test microorganisms: *Escherichia coli*, *Enterococcus faecium*, *Bifidobacterium animalis* and *Lactobacillus plantarum* were isolated from human large intestine.

The water extracts obtained from: basil (*Ocimum basilicum*), oregano (*Origanum vulgare*), lovage (*Levisticum officinale*), cumin (*Carum carvi* L.) and majoran (*Origanum majorana*) were evaluated from the point of view of bactericidal and bacteriostatic activity against the applied test microorganisms.

The spices extracts were prepared by shaking in water for 30 min at 150 rpm at room temperature and sterilized by microfiltration (filtrated through 0.45 µm Millipore filters). The evaluation of biological activity of the extracts was performed by paper disc diffusion method, serial dilution method as well as nephelometric method. The highest spices amount used for analysis was the extract from 50 mg in 1 cm² of water and the next serial dilutions were successive: 25, 12.5, 6.25, 3.125 and 1.56 mg/cm². The inhibition effect was observed after 24 h (*Escherichia coli* and *Enterococcus faecium*) or 72 h (*Bifidobacterium animalis* and *Lactobacillus plantarum*) of incubation at 37°C.

It was evaluated that *Escherichia coli* and *Enterococcus faecium* were more sensitive than *Bifidobacterium animalis* and *Lactobacillus plantarum*. The highest antibacterial activity, presented as inhibition of growth of tested microorganisms was observed when oregano and lovage were added to the culture medium. Oregano water extracts demonstrated bactericidal effect against to *Escherichia coli* strain and lovage water extracts – bacteriostatic activity against all the tested microorganisms. The antimicrobial activity was observed only in the case of use of the highest extracts concentration.

Posters

P1.3.1

Estimation of bactericidal and bacteriostatic effect of biological active spice extracts

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See oral presentation abstract O1.3.3

P1.3.2

Influence of microencapsulation on the viability of *Bifidobacterium bifidum* at different pH

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Many strains from *Bifidobacteria* sp. are known to have probiotic properties. In the presented work we examined whether microencapsulation could have an effect on the viability of these probiotic bacteria during the gastrointestinal tract transit. In the work different methods of encapsulation were applied. Additionally, to increase the survival of the encapsulated bacteria alginate beads were used.
Specifically, we assessed the impact of microencapsulation with a liquid-core on the survival of *Bifidobacterium bifidum* DSM 20082, DSM 20239, DSM 20456, DSM 20215 at different pH of the culture media. Bacterial cell encapsulation was performed using the extrusion method as described by Yoo et al. and Dembczynski & Jankowski. Harvested cells were resuspended in cation starch containing 100 mM calcium chloride (liquid-core). The starch solution containing bacteria was added dropwise to 0.6% (v/v) of sodium alginate and capsules were incubated for 15 min in calcium chloride. Next, capsules were filtered off and washed with distilled water before the final experiments. *Bifidobacterium bifidum* in Medium 58 (DSMZ) were grown anaerobically at 37°C. The survival of bacteria at different pH was determined in the substrate by regulating and stabilizing using buffers its pH to the following values: 2; 3; 4; 5; 6; 7; 8. The applied bacterial inoculum contained 10⁷ cfu ml⁻¹ or g capsules. The number bacterial cells (cfu ml⁻¹) was measured at definite time intervals until no bacteria were detected in the medium.

Experiments revealed significant differences in survival between the four *Bifidobacterium bifidum* strains tested. At pH 2 after 48 hours the number of living *B. bifidum* cells dropped to zero while only the microencapsulated *B. bifidum* DSM 20456 strain demonstrated a longer survival. Microencapsulation was shown to increase the resistance of *B. bifidum* DSM 20082 and *B. bifidum* DSM 20215 in pH 3. Microencapsulated *Bifidobacterium bifidum* cells were found to live longer, especially in the pH 4–5 environment. The most favourable pH at which all *B. bifidum* strains demonstrated a high survival was pH 6. At pH 7–8 all strains, except *B. bifidum* DSM 20082, exhibited significant differences in survival of free and microencapsulated cells. In summary, microencapsulation with liquid core overall increased the resistance of *Bifidobacterium bifidum* at different pH of the medium; yet, differences between strains were observed.

**P1.3.3**

**Examination of changes in quantity of phenolic compounds and the survival of *Lactobacillus acidophilus* and *Lactobacillus plantarum* in bean-derived chips**

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Red bean has previously been demonstrated to be a good source of antioxidants. Here, we show the influence of *Lb. acidophilus* on antioxidant activity and quantity of phenolic compounds in Red Kidney bean products. The aim the research was to analyze changes in microbial composition as well as bacterial interactions with biologically active compounds of extruded Red Kidney bean products (chips). Extrusion parameters were as determined by Czarnecka et al. 1998. Chips were obtained from the bean-fermentation meal and ground maize mixed at the ratio of 1:1. Bacteria were added to the meal at an inoculum of 10⁷ cfu ml⁻¹ and the lactic fermentation process was carried out at 37°C for 18 or 24 hours for *Lb. acidophilus* and 18 hours for *Lb. plantarum*. Changes in microbial composition were controlled in the meal after fermentation by *Lactobacillus acidophilus* DSM 20079 and *Lactobacillus plantarum* T-106 as well as in extruded products (chips). Bacterial cells were enumerated by plating them on MRS solid medium after a 48 hour incubation period at 37°C. Total polyphenol content was analyzed by the Folin-Ciocalteau method, in which results are expressed in gallic acid equivalent per 1 g of dry mass. Antioxidant activity was established using ABTS⁺ reagent and results expressed in mg of Trolox per 1 g of dry mass.

The number of living *Lb. acidophilus* and *Lb. plantarum* cells after extrusion (in chips) was determined to be 10⁵ cfu/g of final product. The result allowed concluding that the extrusion process decreased the number of living cells for both strains. Moreover, the fermentation process using *Lb. acidophilus* was found to result in the lowest amounts of lactic acid and the final pH even after 24 h was higher than when *Lb. plantarum* was used. Both *Lb. plantarum* and *Lb. acidophilus* were determined to decrease the content of total polyphenols as well as reduce the antioxidant activity of the Red Kidney bean product. Yet, for *Lb. acidophilus* the level of total polyphenols and antioxidant activity was much lower. The content of phenolic compounds was recorded as 3.34 mg/g of flour, 2.95–3.14 mg/g of meal after fermentation, 1.06–1.27 mg/g of extruded product. Antioxidant activity was calculated to be 7.6–9.1 mg Tx/g of meal after fermentation and 3.26–5.67 mg Tx/g for extruded product. Furthermore, it was observed that when *Lb. acidophilus* was used for fermentation the antioxidant activity was higher for products than for flour.

In summary, this study has shown that application of the *Lb. plantarum* T-106 strain in the fermentation process is more advantageous as the level of phenolic compounds in extruded product (chips) was lower then when *Lb. acidophilus* DSM 20079 was used.

**P1.3.4**

**Structural investigations of exopolysaccharides isolated from bacteria of genus *Lactobacillus***

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Bacteria of the genus *Lactobacillus* are Gram-positive microorganisms known as natural inhabitants of mammalian gastrointestinal tract. A number of bacteria from that group show probiotic activity on the host, i.e. the beneficial influence on its health. Colonisation of the intestine by probiotic microorganisms is considered to be an important factor for antagonistic activity against enteropathogens, modulation of the immune system activ-
ity, increased healing of damaged gastric and intestinal mucosa or hypocholesterolemic action. The mentioned activities involve the process of microbial adherence to the intestinal mucosa; the essential role in this phenomenon play surface antigens of bacterial cell e.g. capsular polysaccharides and proteins of the cell wall. There is a growing interest in investigation concerning the use of purified exopolysaccharides as immunostimulatory substances or in the experimental medical treatment. Exopolysaccharides from probiotic bacteria, due to their rheological properties, find also their application in the dairy industry. During the investigation concerning the immunological activity of various polysaccharides from probiotic bacterial strains, the information about the molecular structure of the polysaccharide is essential. In this work the preliminary results of structural analysis of exopolysaccharides isolated from seven Lactobacillus strains: L. paracasei OXY, L. animalis murinus 116, L. animalis murinus 148, L. reuteri 130, L. reuteri 115, L. johnsonii 151 oraz L. johnsonii 142 are presented. Strains derived from mice produce more teichoic acid material than capsular exopolysaccharide, whereas these of human origin possessed mainly exopolysaccharide material.