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2nd Kiel Food Science Symposium

Mai 22-23, 2012
Kiel, Germany

Speaker abstracts - Keynote lectures

Speaker abstracts

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Speaker abstracts
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Bioactive peptides from food proteins

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Food proteins contain a large range of peptide sequences which when released either during processing or by digestion have potential to beneficially modulate biological function. Proteins from dairy and marine (e.g., fish, shellfish and algae) origin are rich sources of biologically active peptides. Much interest has focused on the discovery, identification and valorisation of food-protein derived bioactive peptides with a view to their application as disease prevention agents. Peptides having beneficial effects on the immune, cardiovascular and nervous systems, for example, have been reported from milk and marine proteins. Food protein-derived peptides having hypotensive effects have been the subject of significant research effort. This is understandable given that hypertension affects ~25% of the global adult population. This overview will focus on bioactive peptides from dairy and marine sources. Many challenges still exist with respect to the widespread utilisation of these peptides as disease prevention agents. These include the need for more human intervention studies and a detailed understanding of the mechanism of action connected to the different bioactive effects.

Functional food from marine resources

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Vast amounts of marine based raw materials are still in large part underutilized. Major opportunities exist with these raw material sources as they are rich in various natural and highly functional compounds, which with proper extraction, isolation and processing techniques can find use in various foods, speciality feeds, neutraceuticals, cosmeceuticals and even medical products. The market for natural products is growing very rapidly, particularly products which possess bioactive properties which can have positive effects on health and performance. The past few years have seen significant advances in the isolation and production of novel ingredients from underutilized raw materials. This includes the production of fatty acids, enzymes, cartilage compounds such as chondroitin sulfate, glucosamine, functional fish proteins, bioactive fish peptides and various seaweed based compounds, to name a few. Some of these ingredients have very unique functions compared to their non-marine counterparts, and display very high activity. The industry is realizing that very significant value addition can be achieved with underutilized raw materials. However for this industry to become successful and compete in the marketplace, continued and significant support of research and development is needed, as well as patience. Particular attention to marketing strategies is also important for these ingredients to stand out in the marketplace.

EHEC in food – current state of knowledge

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The group of Shiga toxin-producing *Escherichia coli* (STEC) is highly diverse. More than 400 serotypes of STEC have been isolated from human patients and even more STEC types were isolated from food, animals and the environment. In most countries, the search for STEC in food is limited to a few serotypes, mainly O26, O103, O111, O145 and O157 strains, known as enterohemorrhagic *E. coli* (EHEC). In Germany, by legal rules all STEC from humans and food are regarded as potential pathogens. Interestingly, classical EHEC are rarely isolated from food samples in Germany and many STEC strains that are frequent in food do not play an important role as human pathogens. Similar to classical EHEC, these STEC produce potent Shigatoxins (Stx) but lack adhesion factors such as the locus of enterocyte effacement (LEE) to colonize humans efficiently. By combining classical serotyping with molecular subtyping of virulence genes we could identify STEC strains that are closely associated with certain food producing animals. We could show that most food products are contaminated with STEC originating from the producer animals. Food of bovine origin was found as most frequently contaminated with EHEC types than food from other sources.

The outbreak with enteroaggregative haemorrhagic *E. coli* (EAHEC) O104:H4 has caused a paradigm shift in regard to human pathogenicity of STEC strains. In contrast to other STEC, EAHEC O104:H4 strains are of human origin. EAHEC have substituted the EHEC-LEE colonization system by the aggregative adherence mechanism (AA-fimbriae) which enables EAHEC to colonize humans efficiently and to cause severe illness in the patients. Current detection systems for human virulent EHEC are merely based on the presence of *stx*- and LEE (*eae*) genes. Genetic markers for detection of human virulent EAHEC, such as *aggR* were successfully employed in our laboratory for identification of EAHEC as new emerging human pathogens.

Health risk of parasites in fish

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Allergies to foods are a significant public health concern throughout the world, affecting in the general population up to 4 percent of adults and 8 percent of children. The allergic response is usually mediated by the interaction of serum IgE antibodies to allergenic proteins and causes a range of symptoms, from mild discomfort to life-threatening anaphylactic shock. While estimates vary from country to country, approximately 1% of the population is estimated to suffer from seafood allergy, which is more common in teenage and adult life than very early childhood.

Often, so-called 'adverse reactions' to seafood are misdiagnosed as "seafood allergy" when they are actually caused by contaminants. Worldwide, increasing allergies to an emerging food-borne parasite, Anisakis simplex, have been linked to the ingestion of this nematode, which causes human Anisakiasis ('herring worm disease').

Parasitic nematodes of the genus Anisakis infect mostly fish and can and induce immune reactions such as gastrointestinal reactions, urticaria and life threatening anaphylactic reactions. Seven different Anisakis morphotypes can be differentiated genetically using mutation scanning to allow specific identification. In addition already ten different allergenic proteins have been identified, but little information is available for other parasite groups infecting fish such as tapeworms and liver flukes.

Fish-borne parasitic infections have been limited in the past to low-income populations mostly in developing countries. On the contrary current growing international markets for seafood and demographic changes and eating habits have transformed this picture radically. The World Health Organization (1995) has estimated the number of people at risk, including those in developed countries, is more than half a billion. Compared to other well studied parasitic diseases, studies into fish-borne parasitic zoonoses have been very limited. Better quality control of potentially contaminated seafood, with Anisakids and related parasites, is only established by closer integration of Food Science, Medical Science, Parasitology and Public Health, ensuring better protection and education of consumers.

Speaker abstracts

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Peptidome profiling of milk for the identification of bioactive peptides

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Bioactive peptides, which have been detected in many different nutrition products, represent valuable ingredients of functional foods. In milk, bioactive peptides are often encrypted within the sequence of milk proteins and released by enzymatic hydrolysis or microbial fermentation. Several bioactive sequences have been identified in milk proteins, including antihypertensive, opioid, immunomodulating, antimicrobial and antithrombotic peptides[1]. However the components of milk low molecular weight peptide fraction are largely unidentified and their role as potential bioactive constituents is unknown.

The goal of the study was a systematic structural mapping of the low molecular weight (< 5000 Da) peptide fraction of milk in order to evaluate its bioactivity. Thus, peptidome analysis from the peptide fraction was performed. Matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry gave a rapid overview of the peptide profile. With the use of the tandem mass spectra, acquired on a MALDI-TOF/TOF-MS or a ESI-LTQ-Orbitrap-mass spectrometer coupled on-line to ultra-performance liquid chromatography (UPLC), the sequences were retrieved from SwissProt Database with Sequest of Mascot.

Direct MALDI-TOF-MS displayed 60 prominent signals and the 57 most abundant peptides were identified by MALDI-TOF/TOF and UPLC-ESI-LTQ-Orbitrap mass spectrometry. Prefractionation by reversed phase HPLC lead to 174 identified peptides and Offgel -pI based- separation prior to mass spectrometry to 181 identified peptides. Altogether a database of 248 milk peptides was established.

The identified peptides are mainly derived from selected regions of α_{S1}-, α_{S2}- and β-casein, especially from the protein termini. Some of the cleavage sites could be assigned to endogenous milk proteases. Literature data base search revealed 22 bioactive peptides in the newly identified milk peptidome, including ACE-inhibition or blood pressure lowering effects, antimicrobial, antioxidant and immunomodulatory properties, interaction with calmodulin or bitter taste. The established milk peptide data base can now be combined with functional assays to identify new bioactive peptides.

[1] Clare DA, Swaisgood HE (2000) Bioactive Milk Peptides: A Prospectus. J Dairy Sci 83: 1187 - 1195

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Application of electro-membrane cross-flow filtration for peptide fractionation

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In the food industry membrane filtration is a current process for concentrating proteins with a high-throughput. Generally, the selectivity of membrane filtration is too small for the fractionation of small peptides from feed solutions. Electro-membrane cross-flow filtration (EMCF) may offer the potential to fractionate peptides from complex protein hydrolysate based not only on size but also on electric charge. With this technique positively or negatively charged peptides can be transported from the feed to the permeate side, whereas the antipodal charged peptides are retained.

The objective was to increase the efficiency of separation regarding maximum yield of small peptides. Food grade micellar casein was hydrolyzed by trypsin to produce a standard casein hydrolysate, which was used for EMCF. The electrical enhanced membrane filtration experiments were performed on a modified plate-type ultrafiltration module made of polyvinylchloride, with embedded platinised titanium electrodes. The polarity of electrodes was set with the cathade at permeate side to fractionate positively charged peptides from the casein hydrolysate. The impact of polarity and electrical field strength on yield and relative purity of the target peptides permeation flux, ph and conductivity in the permeate was studied. HPLC-analysis of feed solution, permeate and retentate was monitored. Electro-membrane cross-flow filtration improved separation of small peptide fractions from feed solution as compared to standard ultrafiltration with a 5 kDa membrane. The permeation of small peptides was significantly ($p = 0.05$) enhanced by the superimposed electrical field strength of 1667 V m^{-1} and 2330 V m^{-1}.

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Characterization of peptides after *in vitro* gastrointestinal digestion of bovine κ-casein A, B and E

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Introduction

Cow´s milk allergy is a common food allergy affecting 2.5% of children less than 2 years and approximately 0.5% of adults. κ-casein (CN) belongs to the major allergens in cow´s milk. Within κ-CN the genetic variants A, B and E are described resulting from substitutions of amino acids at positions 136, 148 and 155. Their allergenic potential has not been investigated until now. The aim of this work was to determine the arising peptides of the κ-CN variants A, B and E after an *in vitro* gastrointestinal digestion and to identify resistant regions, which are described as IgE-binding epitopes.

Methods

κ-CN A, B and E from milk of cows homozygous for these variants were digested with an *in vitro* gastrointestinal digestion model. The resulting peptides were characterized with MALDI-TOF MS and ESI-MS/MS and compared with known epitopes.

Results

69 common peptides were identified in κ-CN A, B and E with molecular masses ranging from 402 to 3123 Dalton. Peptide 125-137 occurred in all variants, but in consequence of the substitution with variable masses. Further peptides were only present in κ-CN E (152-155, 147-160), κ-CN B (143-154, 134-150) or κ-CN A (146-157 or 148-159) as well as in both variants A and E (136-149, 117-137). The peptides 136-149 (κ-CN A, E) and 134-150 (κ-CN B) correspond to the IgE-binding epitope 137-148, which has been reported as one of the major IgE-binding epitopes of κ-CN A.

Conclusion

The *in vitro* gastrointestinal digestion of κ-CN A, B and E resulted in a wide range of peptides, which are partially long enough to contain IgE-binding epitopes. Since the described epitopes refer to κ-CN A and differences between the epitopes of variant A, E and B could be demonstrated, the allergenic potential of the peptides will be investigated in ongoing studies with microarray immunoassays.

Oxidation of marine lipids in foods

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Marine lipids such as fish oils are frequently used in foods to enhance the content of omega-3 long chain poly unsaturated fatty acids EPA and DHA. However, EPA and DHA are particularly prone to oxidation. The oxidation products may have undesirable health effects, e.g. malondialdehyde is one of the best known mutagenic and cancerogenic aldehyde formed during endogenous lipidoxidation *in vivo*.

To prevent lipid oxidation of highly unsaturated fatty acids it is required to consider chemical aspects (e.g. antioxidants) and physical (e.g. interfacial properties) of the food.

In oils LCPUFAs are stabilized by the addition of tocopherols to fulfill dietary requirements in terms of vitamin E. However, further addition of antioxidants such as lecithin and rosemary extracts is necesseary to stabilize the oil during storage.

In dispersed systems lipid oxidation occurs at the interface, e.g. at the surface of oil droplets in an o/w emulsion. Therefore it is an aim to design a droplet surface that acts as barrier towards oxygen and metal catalysts

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In vitro fish cell culture paves a sustainable way to fish related products

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Seafood contains valuable proteins and high quality fats and is therefore interesting in several respects. Especially the consumption of highly unsaturated omega-3 fatty acids - in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) - has been proven to be beneficial for human health. The majority of EPA and DHA for human consumption originate from marine fish resources. Since these natural fish stocks continuously decrease and accumulation of heavy metals in fat tissue of fishes along the food chain are major problems, new sources for omega-3 fatty acids need to be explored.

We investigated the ability of a highly proliferative *in vitro* fish cell culture to produce nutraceutical grade omega-3 fatty acids and found out that cultured Sturgeon cells *(Acipenser oxyrinchus)* convert short chain polyunsaturated fatty acids into long chain highly unsaturated fatty acids *in vitro*. The addition of α-linolenic acid (ALA) during cell culture and the reduction of cell culture temperature afterwards caused an increase of EPA and DHA in cell biomass. However, *in vitro* culturing of large quantities of anchorage dependent cells is currently cost-intensive and requires a high number of disposable materials. Thus, an industrial production is not yet feasible. One of our main challenges is to develop new bioreactors suitable for fish cell culture, which allow cost-effective production of cell biomass to enable access to fish derived products with low ecological damage.

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Enzymatic synthesis of galacto-oligosaccharides from lactose – comparison of different β-galactosidases from *Kluyveromyces lactis, Aspergillus oryzae and Bacillus circulans*

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In a number of publications health-promoting effects for galacto-oligosaccharides (GOS) are described. Bifidogenic and immunostimulating effects as well as protection against bacterial and viral infections are mentioned. Cow milk, however, has only low concentrations of oligosaccharides (MOS). The aim of the current study is to synthesize GOS enzymatically from lactose and to characterize the oligosaccharides in terms of composition and nutritional properties.

For GOS synthesis raw milk was skimmed, milk proteins were separated via ultrafiltration (NMWCO 5000 Dalton) and edible grade lactose was added up to 40% (w/w) in the permeate. After pH settings, β-galactosidases from *K. lactis*, *A. oryzae* or *B. circulans* were added. The reaction was carried out at 40°C and samples were taken hourly. The sugar composition was characterized by high-pH anion-exchange chromatography with pulsed amperiometric detection (HPAEC-PAD) and online electroscopy ion trap mass spectrometry (IT-MS).

Mono-, di-, tri-, tetra- and pentasaccharides were identified in the GOS samples by mass spectrometry. Depending on the applied enzyme distinct differences in the composition of GOS were determined. One main trisaccharide was detected after enzymatic incubation with the β-galactosidases from *K. lactis* (approx. 10%), *A. oryza*e (approx. 15%) and *B. circulans* (approx. 20%), respectively. Due to the different HPAEC retention times of the diverse main trisaccharides, the preferred formation of different glycosidic bound oligosaccharides was identified. This is supported by studies described in the literature [Martínez-Villaluenga et al. JFCA, 21, 540-544 (2008) and Gosling et al. JAFC, 57, 11570-11574 (2009)] where it is stated that β-galactosidases from *A. oryzae* and *K. lactis* preferably generate β1-6GOS and β-galactosidases from *B. circulans* produce GOS predominantly by β1-4bonds.

The highest yields of GOS were achieved by the application of the β-galactosidases from *B. circulans.* Therefore, this enzyme seems to be the most promising for further studies. To evaluate the physiological effectiveness of the GOS samples and to elucidate the structure-activity relationships also anti-inflammatory and bifidogenic investigations are planned.

Bioactivity of *Palmaria palmata* hydrolysates *in vitro*

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Studies with macroalgae have revealed the presence of various endogenous nitrogenous bioactive compounds. Some macroalgal species, particularly the red seaweeds, can contain significant levels of different proteins (up to 47% (w/w)) which represent a potentially rich source of bioactive peptides encrypted within their primary structures. While a limited number of protein-derived bioactive peptides have been characterised, macroalgal proteins still remain an essentially untapped resource for biofunctional peptide mining. The objective of this study was to investigate the cardioprotective, anti-diabetic and antioxidant activity of macroalgae protein hydrolysates using *in vitro a*ssays of biomarkers for the above activities. Crude fractions of aqueous and alkaline soluble proteins were extracted sequentially from milled freeze-dried *Palmaria palmata* using distilled water and 120 mM NaOH, respectively. Aqueous, alkaline and combined aqueous and alkaline protein fractions were hydrolysed with the food-grade proteinase preparations; Alcalase® 2.4L, Flavourzyme® 500L and Corolase PP®. Control protein samples, without added proteolytic activity, were treated in the same manner. Freeze-dried protein hydrolysates were then assessed for angiotensin converting enzyme (ACE), renin, dipeptidyl peptidase (DPP) IV inhibitory activity. The ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) was also assessed. The different protein hydrolysates were shown to have a range of ACE (IC_{50}, 0.15-2.34 mg/ml)), renin (0 - 49.94 % inhibition at 1mg/ml) and DPP IV (IC_{50} (1.65- 4.60 mg/ml)) inhibitory activities. In general, hydrolysates of aqueous protein extracts generated with Alcalase® 2.4L and Corolase PP® displayed highest *in vitro* enzyme inhibitory activity. Furthermore, the same enzyme-protein substrate combinations had high antioxidant activity in the FRAP and ORAC assays. These results show that the nature of the *Palmaria palmata* protein fraction and proteolytic enzyme preparations used to generate hydrolysates therefrom can significantly affect the observed *in vitro* cardioprotective, anti-diabetic and antioxidant response. Moreover, this study identifies the potential of macroalgal proteins as substrates for the enzymatic generation of biofunctional peptides for utilization as functional foods ingredients.

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Identification of microorganisms that may contribute to the safety and quality of traditional foods and beverages consumed in the Black Sea region[1]

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The main aim of the EU-funded BaSeFood project is to investigate the healthy properties of traditional foods and beverages of plant origin. The quality and safety of foods are largely dependent on the level of microbial contamination.

In this study, the major plant ingredients of a series of prioritized traditional foods and beverages were collected from Georgia, Bulgaria, Russia, Turkey and Ukraine. The microbial species (strains) isolated from the products/plants were identified using semi-automatic biochemical and serological tests and MULDY techniques. The bacteria were assigned to one of three categories: beneficial (A), detrimental of environment and human origin (B1) and classical “foodborne” pathogens (B2).

Beneficial species isolated from fresh green parts of plants primarily included *Streptococcus lactis*, *Enterococcus faecalis and faecium* (carrot and hot peppers from Bulgaria), *Actinomyces israeli* (kale from Turkey), *Bifidobacterium longum* (parsley) and *Lactobacillus acidophilus* (elder flowers) in Ukrainian samples.

The dominant opportunistic pathogenic bacteria (category B1) were mainly *species of Klebsiella pneumoniae and oxytoca* (rose petals), *Enterobacter cloacae* (carrot), *Proteus vulgaris/mirabilis* (sorrel, dill, parsley, from Ukraine and Bulgaria), *Str. agalactiae* (nettle and corn, from Georgia and Turkey). Some of the bacteria isolated were plant specific (e.g. *Serratia odorifera biogroup 1,* carrot and *Pantoea agglomerans,* tomato).

Salmonella typhi were identified in sorrel (Ukraine), *Shigella flexneri* ABC from kale, crop and green beans (Turkey), and *Listeria monocytogenes* from bread (Georgia).

The density of bacteria with potential beneficial properties was significantly higher in the traditional fermented foods and drinks that were analysed: *L. fermentum, B. breve* and *L. acidophilus* (in boza), *B. dentinum* (in fermented beans, Turkey), *A. israeli, L. plantarum* and *casei* (kvass, Russia).

Key microorganisms relevant to quality and safety of foods prioritised within BaSeFood project had been detected.

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Designing safe processes for the dairy industry

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In the dairy industry, aerobic spore formers such as *Bacillus cereus* and other species are a hazard to the safety and quality of the final product. They are very heat-resistant and can survive the thermal treatment applied to the product. In order to have safe processes inactivation parameters of spores are determined and processes are designed according to these. Inactivation curves are often examined in small-scale batch heating systems instead of continuous heating systems as used in the dairy industry. Information is lacking on whether the data from batch systems can easily be transferred to continuous heating processes. Hence, the aim of this study was to compare inactivation kinetics determined of batch and on continuous heating systems and to identify, if present, reasons for differences between the two systems.

To reach that aim, the inactivation of spores of *Bacillus amyloliquefaciens* was examined in a small-scale batch system (volume: 1.5 mL) and in a continuous heating system (volume flow rate: 150 L/h). Inactivation kinetic parameters were calculated and compared. The recording of the temperature profile in the continuous heating system was stepwise ameliorated. Incorporation of the whole temperature profiles of the heating process in the calculation showed that the kinetic parameters determined with the two systems converged. However, when only the holding times of the temperature profiles were considered, significant differences were observed. In conclusion, one reason for the observed differences between the systems is the usage of the incorrect and not the exact temperature profiles when calculating the inactivation parameters.

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Survival of Shiga toxin-producing and generic *Escherichia coli* during ripening of semi-hard raw milk cheese

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Prevalence of Shiga toxin-producing *Escherichia coli* (STEC) among raw milk cheeses at retail level has been reported by several studies. Investigations on survival of STEC during production and ripening of raw milk cheeses may assist the development of control measurements. In this study five different *E. coli* strains, including three STEC strains, were analysed for their behaviour during production and ripening of artificially contaminated semi-hard raw milk cheese. The strains used were previously isolated from raw milk cheese and selected based on phenotypic traits and stress response abilities. Prior to the cheese production process the strains were inoculated into raw milk at two different contamination levels (10^1 and 10^3 CFU/g). Raw milk cheeses were produced according to semi-hard raw milk cheese recipe while using two different cooking temperatures (40°C and 46°C). Artificially inoculated *E. coli* strains were monitored separately during production and 16 weeks ripening period. An increase in bacterial loads of about 3.5 log from raw milk to fresh cheese occurred during the manufacture before counts were decreasing over the ripening period. At both contamination levels significant differences in the behaviour of the strains were found. The two generic *E. coli* strains were able to survive in higher numbers than the STEC strains. However, at the end of the ripening period in 6 of 16 cheeses made at lower contamination level STEC were still present at more than 10 CFU/g while detection of STEC after enrichment was possible in almost all cheeses.

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Fermentation failures and flavour deficiencies caused by heat-resistant bacteriophages attacking *Leuconostoc* starter cultures

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Bacteriophages of lactic acid bacteria are still the primary cause for fermentation failures in the dairy industry. They can cause loss of failure of viability and affect both – acidification cultures as well as aroma cultures. *Leuconostoc* strains are important for the production of flavour compounds like diacetyl in several dairy products. They have also other essential functions, e.g. production of gas resulting in hole-formation in cheese. Contamination with *Leuconostoc* bacteriophages in dairies is due to the presence of *Leuconostoc* strains in raw milk. Some heat resistant bacteriophages may survive pasteurization and then be present on dairy products. The aim of this study was to test *Leuconostoc* phages for their heat resistance and to select a thermo-resistant *Leuconostoc* phage in order to provide data for designing heating processes of milk and whey. The test phage was also used to assess phage-derived influences on the organoleptic properties of cream cheese.

A total number of 77 dairy *Leuconostoc* phages, either isolated from milk products provided by dairies or obtained from starter culture manufactures, were tested for their heat resistance[1]. Heat inactivation experiments showed that 15% of the phages were still active after a heat treatment of 85 °C for 1 min. For one of the most thermoresistant phages (i.e. *Leuconostoc pseudomesenteroides* phage P793), kinetic parameters of inactivation were determined. Results showed that pasteurization was not sufficient for a 3-log inactivation of phage P793. In addition cream cheese was produced using milk either contaminated with *Leuconostoc* phages or without contamination. The products were evaluated by a triangle test method. A significant difference of the cream cheese samples was observed. In the presence of *Leuconostoc* phages, a reduction of flavour compounds was noted in the samples.

[1] Atamer, Z., Ali, Y., Neve, H., Heller, K.J. and Hinrichs, J. (2011) Thermal resistance of bacteriophages attacking flavour-producing dairy *Leuconostoc* starter cultures. International Dairy Journal 21 (5): 327-334.

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Microbiological characterization of Iranian raw milk cheese "Lighvan" with reference to food safety

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Lighvan is considered the most popular Iranian raw milk cheese made from raw ewe's milk or a mixture of ewe's and goat's milk following ancient cheesemaking technologies without addition of starter. This study aimed to compare the microbiological quality and safety of fresh (curd and day 30) as well as ripened (day 90) cheese as a tool to verifying possible associations between microbial populations, and the detection of the dominant lactic acid bacteria (LAB) with potential antagonistic activity against foodborne pathogens in ripened cheese. Fresh and ripened cheese samples were collected and submitted for the analysis of LAB, mesophilic count, total coliforms, molds & yeasts and coagulase-positive Staphylococcus (CPS). The samples presented high counts of mesophilic aerobes, total coliforms, and LAB, and also high and significant correlation indices between these populations both in fresh and ripened cheeses but with specific trend in each. In total, 95 isolates were identified from the counting plates of M17 (21 isolates), MRS (39 isolates) and KKA (35 isolates). The highest number belonged to *Enterococcus faecium* (22.44%), *Lactococcus lactis ssp. lactis* (20.4%), *Lactobacillus plantarum* (18.36%) and *Enterococcus faecalis* (14.28%). Some particular LAB, such as *Enterococcus faecalis* and *Enterococcus faecium*, have been claimed to have the ability of bacteriocin production. Further investigation is necessary to prove the antimicrobial potential of the autochthonous microbiota originated from raw milk and also from other sources such as processing and storage environment of Lighvan cheese.

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Upstream microfiltration of whey: Improvement of the hygienic quality of whey protein concentrates and optimization of ultrafiltration-performance

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Whey proteins are gaining an increasing importance in food industry due to their techno-functional properties as stabilizers and emulsifiers in foams and emulsions. Since they have to be extracted and concentrated from sweet and acid whey, ultrafiltration processes are commonly applied. The efficiency of ultrafiltrations is limited to the permeability of the membrane and thus to the extent of deposit layer built-up.

A pasteurization of whey is actually mandatory for the microbial stabilization of the concentrates but is usually not carried out since reactive particle species are formed that lead to an increase in membrane fouling.

Whey pre-treatments developed for an enhancement of the ultrafiltration flux in lab-scale so far are often complex and do not reduce the microbial load to a sufficient extent. Hence, these processes are not applied in industrial practice. To develop an effective process that can be easily integrated in whey processing, a fundamental knowledge on the molecular fouling mechanism is required.

Therefore, the present study focuses on colloidal protein-deposit and protein-membrane interactions during deposit formation of single whey constituents in lab- and in pilot-scale. It was found, that fouling during whey filtrations is caused by two major particle species. Whey protein aggregates formed during heat-treatment of the curd catalyse fouling when they undergo a surface denaturation during deposition on the membrane. A second fouling species are native or partially aggregated casein micelles forming casein-gels on the membrane.

Based on these findings, an upstream separation of these fouling species by means of microfiltrations were carried out leading to a significant increase in downstream ultrafiltration-performance. Membrane fouling was minimized. A synergistic effect of an upstream microfiltration was the reduction of the microbial load in the processed whey leading to an improvement in hygienic quality of the whey protein concentrates.

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Characteristics and antioxidative activity of fish sarcoplasmic protein-fructose Maillard reaction products

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The oxidation reaction directly affects food quality and is commonly associated with the formation of toxic compounds. The synthetic antioxidants presently used by the food industry are effective in preventing rancidity but their safety is often questioned. The Maillard reaction products (MRPs) occurred in food products can be used as a natural preservative. The use of new sources of protein with the reactive amino groups from industrial waste could be a promising means to lower the cost of production. Fish sarcoplasmic protein, a by-product from fish processing, especially washing step of surimi production, is one of a potential source of protein for Maillard reaction. Therefore, MRPs were prepared by heating 2% low molecular weight (LMW) or high molecular weight (HMW) fractions from treadfin bream (TB; *Nemipterus peronii*) or mackerel (M; *Rastrelliger kanagurta*) sarcoplasmic protein and 2% fructose at 100ºC, pH 8.0 for 12 h. The pH values of all MRPs were found in the ranges of 5.51-5.97. MRPs derived from TB-LMW showed the highest values of both UV absorbance at 294 nm and browning intensity (A420) ($p<0.05$). All MRPs had a high fluorescent intensity and reducing power except for that derived from TB-HMW. All MRPs exhibited a strong chelating activity (85-90% chelation). MRPs prepared from the M-LMW possessed the highest DPPH° scavenging activity ($p<0.05$). The highest hydrogen peroxide scavenging activity was found in MRPs prepared from M-HMW ($p<0.05$). The results indicated that the characteristics and antioxidative effect of MRPs depended on types of sarcoplasmic proteins used and the MRPs derived from M-LMW tended to have the highest antioxidant capacity. Consequently, the MRPs derived from fish sarcoplasmic protein especially from M-LMW fraction and fructose could be used as a natural antioxidant to retard the oxidation of lipid in food products with related to health benefits.

Composition, sensory assessment and parasites of Grey gurnard

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Grey gurnard (*Eutrigla gurnardus*) is a widely distributed demersal fish throughout the North Sea. The stock has increased since the late 1980s and the fish is ranked among the 10 dominant fish species in the area. It is caught as by-catch of the bottom trawl fish and presently mainly used for fish meal and oil production. The objective of this study was to investigate the potential of this species for human consumption. The studies focused on larger specimen, which can be consumed as whole fish or fillets. Quality evaluation of fish as human food should include the determination of the processing yields, of the composition (positive and unwanted compounds) as well as sensory assessment, biological and chemical changes throughout the year, a survey on parasites of human health significance and the storage characteristics fresh and deep frozen. The fat content of the fillets ranged between 2.7 % and 3.8 %, the protein content was 19 to 20 % and the amount of n-3-fatty acids was around 1 g / 100 g product weight. The sensory quality was rated as high by an experienced panel. Anisakis simplex was found to be the only parasite of health significance. Every fish was infected and the abundance in viscera and flesh was high. The distribution in the flesh was studied in detail and the removal of the belly flaps reduces the presence of Anisakis larvae in the edible part considerably.

Investigations on quality changes during prolonged frozen storage of whole Grey gurnard are under progress.

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A Real-Time PCR assay for large-scale screening of farmed marine fish for the presence of anisakid nematode larvae

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Norway is among the world's leading producers of farmed marine fish. The main species is Atlantic salmon with a live weight sales turnover of > 900 000 metric tons in 2010, while rainbow trout, Atlantic cod and halibut are produced at lesser volumes. Since more than 90% of the annual Norwegian fish production is bound for various international markets, strong focus is put on food safety in order to continuously retain consumer confidence. This includes control of fresh, i.e. unfrozen, products for the possible presence of *Anisakis* larvae. However, the currently recommended nematode detection methods are rather labour-intensive and based on visual inspection, thus being less suitable for routine large-scale monitoring of fresh fillets of cultured fish. Since NIFES acts as national reference laboratory for quality reducing or potentially human pathogenic parasites in seafood, we have established an *Anisakis simplex*-specific Real-Time PCR assay which targets a consensus 96 bp fragment of the cytochrome c oxidase II gene. The underlying premise was that only whole undamaged larvae would be present in fresh and unprocessed fillets of farmed fish. Thus, the dilution taking place during the initial homogenisation step of whole fillets or fish sides prior to parasite DNA extraction, still allows a rapid and reliable screening – on a YES or NO basis – of large quantities of samples in a comparatively short period of time.

Speakers abstracts
- General aspects -

Reduce risks using a Quantitative Microbial Risk Assessment approach

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In the last decade food safety has become of increasing importance for food companies. The producers are held responsible for the safety of their products. However, the safety of products is challenged by new developments, such as the increasingly complex supply chain, minimal processing and reduction of sugar and salt.

The food industry has to manage the risks and must be able to show the safety of their processes. This is currently done with HACCP analyses, but these analyses do not take into account the whole chain and especially the interaction between different steps. Secondly, risks are often very low, making it impossible to identify them by sampling methods. Therefore, NIZO food research has developed an approach to evaluate the potential risk of a contamination, based on a Quantitave Microbial Risk Assessment (QMRA). The approach consists of a stepwise description of the process, identification of the key micro-organisms involved and modeling based on Monte Carlo simulations.

The process can be described by the chain of different steps, including pre- and post-processing steps. Process and product parameters and their variance are described based on information supplied by the producer. Growth and inactivation kinetics of key micro-organisms including variance are based on scientific literature or experimental data. Contamination data are preferably obtained from industrial practice. Using Monte Carlo simulations, it is possible to identify the risks in a chain and relations between input and output variables. In additional sensitivity analyses, the most influential parameters are identified. Using this information, different scenarios can be run subsequently to evaluate measures to reduce risks (e.g. changes in processing conditions, storage times).

This approach has been applied by NIZO in various projects with different dairy processes. In the presentation an example analysis will be shown which demonstrates the methodology, the sensitivity analysis and potential scenario analyses.

Ethical aspects of nanotechnology in the area of food and food manufacturing

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Nanotechnology is one of the key technologies of our age. There are great expectations for nanotechnology in a number of fields, both in terms of general technical and technological development and in relation to food technology in particular.

Since risks are associated with every human action, we need to investigate the background and interconnections with regard to possible advantages and disadvantages (i.e. risks). As far as nanotechnology is concerned, however, we face a fundamental problem: normally a risk is calculated according to the formula 'risk = danger x exposure' and is thus based on experience of comparable cases (extent of damage) and on probability considerations (likelihood of occurrence). The problem with nanotechnology is that, firstly, there are no real comparable cases and, secondly, the possible – i.e. supposed – risks appear to be simply endless.

The risk debate surrounding nanotechnology will be crucially important to its future. The reason for this is that there are still many unanswered questions related to health considerations and environmental compatibility. The strengths and advantages of nanotechnology are also the cause of its potential risks. Although nanoparticles are not dangerous per se, the switch to the nanodimension changes the properties of many materials even though their chemical composition stays the same.

Although there is no question about whether nanotechnology should be used in the food industry, its further development doubtlessly needs to be accompanied by critical, constructive and ethical analysis. And consumers' acceptance of nanotechnology in future will depend above all on the fact, that he must gain direct and immediate personal benefits from the use of nanotechnology in the area of food and nutrition and he must be able to see these benefits.

Poster abstracts

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Poster 1

Identification of ACE-inhibitory precursor peptides from fermented camel milk (Camelus dromedarius) produced by *Lactobacillus helveticus* or *Lactobacillus acidophilus* using HPLC-MS

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Objective

Aim of this study was (i) to characterize peptides by HPLC-MS released from camel milk fermented with *Lb. helveticus* or *Lb. acidophilus*, and (ii) to match peptide sequences with those established to exhibit angiotensin-converting-enzyme (ACE) inhibitory activities.

Methods

Whole camel milk was collected from dromedaries ('Majaheim' breed) from a farm located in Al-Jawf city in the north region of Saudi Arabia. The pH and acidity of camel milk were 6.54 and 0.14%, respectively. Milk was sterilized using an Arnold steam sterilizer and then inoculated with *Lb. helveticus* (LMG11445) or *Lb. acidophilus* (LMG11430) strains and incubated anaerobically at 37°C for 127 and 112h, respectively. Samples were filtered through 3 kD cutoff dialysis membrane and fat was removed from the permeate by extraction with hexane. Aliquots of 5 ml were separated and sequenced by on-line HPLC-MALDI-TOF-MS. Sequences were searched against SWISS-PROT database using MASCOT.

Results and Discussion

Lb. helveticus released three peptides of the sequences ***1*** (LSLSQF, SLSQF, or SQF)KVLPVPQ, three peptides of the sequences ***2*** (TDLEN, DLEN, or LEN)LHLPLPL, and one peptide of the sequence ***3*** KVLPVVPQQMVPYPQ.
Peptides are released by cleavage of proteins by bacterial proteases. Sequences in parentheses result from successive cleavage of *N*-terminal amino acids by amino acid peptidases. Sequences underlined correspond to established ACE-inhibitory peptides. All peptides are released from β-casein of *Camelus dromedarius* milk. Peptide ***1*** corresponds to amino acid positions 55-67, peptide ***2*** to positions 20-31, and peptide ***3*** to positions 61-73
Lb. acidophilus released two peptide ***4*** FQEPFPDPVR and peptide ***5*** VLPFQEPVPDPVRG. Peptide ***4*** corresponds to positions 85-94 and peptide ***5*** to positions 82-91 in β-casein. The lower proteolytic activity of *Lb. acidophilus* is probably due to its slower growth. Although the characterized peptides represent longer sequences they are considered as precursors of the established ACE-inhibitory peptides underlined.

Conclusions and Perspectives

Under the fermentation conditions described potential precursor peptides of those having known ACE-inhibitory activity were detected. All peptides contain C-terminal sequences for which bioactivities have been established. *Lb. helveticus* is proteolytically more active in comparison to *Lb. acidophilus*. Fermented milks from 'camels' (*Camelus dromedarius*) are considered as potential candidates for ACE-inhibitory peptides but fermentation conditions have to be optimized together with the selection and use of proteolytically more active single or mixed bacterial starter cultures.

Acknowledgement

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Poster 2

Quantification of four bioactive di- and tripeptides in fermented milk after derivatisation with dabsyl chloride

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Food matrices are complex systems consisting of proteins, carbohydrates, fatty acids and a variety of minor components. Therefore the application of advanced analytical methods such as, high performance liquid chromatography (HPLC) with mass spectrometry (MS), capillary electrophoresis (CE) or nuclear magnetic resonance (NMR) is needed for analysis and correct quantification of such particular bioactive compounds [1]. However, peptides such as the tripeptides IPP and VPP are difficult to detect, because the amount of UV light absorbed by peptides is directly related to the number of peptide bonds [2] and, the mass spectrometric identification of native di- and tripeptides is also challenging [3].

In our study we established a HPLC method using pre-column dabsyl chloride derivatisation for the separation and quantification of antihypertensive di- and tripeptides in fermented milk products. The derivatised peptides Val-Pro-Pro (VPP), Ile-Pro-Pro (IPP), Leu-Pro-Pro (LPP) and Phe-Pro (FP) were separated on a RP-C_{18}-column coupled with VIS-spectrometry (436 nm) or mass spectrometry (ESI-MS in selected ion monitoring), respectively. Due to the derivatisation of the peptides, base line separation was achieved and the peak width was improved. The VIS-spectrometry did not allow a serious quantification of these peptides since more than one peptide co-eluted under one single peak. However, when applying LC-ESI-MS with a single quadrupole an accurate quantification of the derivatised peptides was done. In commercial Evolus® milk, 6.9 mg L^{-1} for VPP, 6.1 mg L^{-1} for IPP, 0.8 mg L^{-1} for LPP and 3.2 mg L^{-1} for FP were determined. In fermented skim milk (*Lactobacillus helveticus*, 37 °C, 48h) lower concentrations of these peptides occurred (0.7, 0.6, 0.0 and 2.2 mg L^{-1}, respectively).

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Poster 3

Metabolite profiles of intestinal and lactic acid bacteria growing on different sugars

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People suffering from non-alcoholic fatty liver disease (NAFLD) show - without any consumption of alcohol - all signs of a typical alcohol-induced fatty liver. So far the elicitor of NAFLD remains unclear. However, alcohol produced by the intestinal microbiota has been discussed to be involved in the development of the disease. In order to take a closer look at this problem, a simple fermentation model was established for evaluating strains intestinal and lactic acid bacteria. So far, we analysed Anaerostipes caccae, Bacteroides thetaiotamicron, Bifidobacterium longum, Escherichia coli, Lactobacillus acidophilus, Lactobacillus fermentum, Lactobacillus plantarum and Lactobacillus reuteri with respect to their metabolic activities. Medium for colonic bacteria (MCB), supplemented with different sugars like glucose, fructose, lactulose, arabinose, ribose and inulin was used under anaerobic conditions.The results obtained generally reflected the anticipated metabolic activities. Lactate was the major metabolite for all lactobacilli strains. High amounts of ethanol were observed in L. fermentum and L. reuteri, grown in MCB supplemented with either glucose or fructose. Lactate and acetate were the major metabolites of B. longum, however succinate and acetate were the major metabolic substances of B. thetaiotamicron. On the other hand, butyrate was only the major metabolite of A. caccae in all tested sugars except inulin. E. coli showed mixed acid fermentation with different amount of ethanol from all tested sugars except inulin. Fermentation of lactulose and inulin, two potential prebiotics, could reduce the production of ethanol by the intestinal bacteria. We now plan to conduct co-fermentation experiments to see, whether combinations of different bacteria will change the overall metabolic profiles in general and the production of ethanol in particular.

Key words: lactobacilli, Bacteroides thetaiotamicron, fructose, prebiotic, probiotic, intestinal microbiota

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Poster 4

Lactolipos – liposomes as nutraceutical carrier for application in functional dairy products

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Liposomes can be used for the fortification of foods with nutraceuticals for enhanced bioavailability as well as targeted and controlled release. Furthermore liposomes can provide protection against chemical and physical degradation processes in food matrices.

Lactolipos aims to produce coated liposome systems as carriers for phenols and n-3 fatty acids for the application in fuctional dairy products like yoghurts and milk drinks. To design carriers of continuous quality different methods for liposome production and finishing treatment were compared with respect to size, narrow size distribution, zeta potential and encapsulation effiency (EE). cTEM images were conducted for confirmation of size measurements based on dynamic light scattering (DLS).

As an example of a possible polyphenole encapsulated in soy liposomes quercetin (Quc) was chosen. Liposomal solutions were produced by thin-film evaporation method (TFM) with subsequent probe-sonication, high pressure homogenization or membrane extrusion for downsizing or by ethanol-injection method. EE was determined by size exclusion chromatography with Sephadex and subsequent HPLC analysis. The results show that TFM liposomes without downsizing are large and polydisperse while extruded liposomes are approximately 120 nm in diameter and very homgenous. Sonicated liposomes are of similar size whereas homogenized and ethanol-injection particles are even smaller. Taking into account that the ethanol-injection-method leads to residual ethanol in the liposomes extrusion and sonication are the methods of choice. A very homogenous particle distribution is confirmed by cTEM.

The optimal phospholipid to quercetin ratio resulting in a high EE and load was evaluated for sonicated liposomes. Over a period of eight weeks liposomes remain stable in size, zetapotential and EE when using 50mM sodium acetate buffer at pH 5.

In the future, the developed liposomal systems will be stabilized by a biopolymer coating and incorporated into a dairy product.

Poster 5

Milk proteins as nanotransporters

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Nanotechnology raises the interest of the food industry because of its versatile applicability. In fact, various nanostructures are naturally present in food, such as proteins. However, many people associate nanotechnology with intricate abstract structures possessing a high hazard potential. From the perspective of consumer protection the possible use of nanoparticles in the food area is still being discussed controversially. Nevertheless, there are simple ways to use natural and safe nanostructures for food technological purposes. Milk proteins for example are nano structures that bind poorly water-soluble substances within the milk such as retinol and fatty acids and keep these compounds in solution. Due to the described binding ability of many proteins, it is only a small outlay to bind bioactive food constituents to milk proteins and use them as nano carriers in food. These milk nano carriers cannot be perceived due to their small size. They can also increase the solubility of the bound substance (as can be shown for fatty acids and retinol), protect it against degenerative processes (for example: it can reduce the oxidation of polyphenols like epigallocatechin gallate in solutions) and exert taste or odor-neutralizing activity (as can be seen for allyl isothiocyanate). Additionally, by binding to a nanocarrier, an increase in the bioavailability of the substances bound to the nanocarrier is conceivable. All milk proteins show different binding capabilities primarily for poor water-soluble substances such as fatty acids, vitamins, polyphenols and aroma compounds. The possibility to use milk proteins as a natural nano carrier will be explained in the following using β-Lactoglobulin as example.

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Poster 6

Production of bioactive peptides by lactic acid bacteria – increasing the yield by different approaches

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Milk proteins are composed of caseins (80%) and whey proteins (20%) and have been shown to be a source of bioactive peptides, which are released by enzymatic hydrolysis. Bioactive peptides may exhibit various beneficial health effects such as anti-inflammatory, antihypertensive, antioxidative, antimicrobial, antithrombotic, immunomodulatory or mineral-binding activities.

We intended to detect different proteolytically active lactic acid bacteria by means of an *in-vitro* assay, where casein labelled with a fluorescent dye as sole substrate source was applied. Solely homofermentative lactococci and lactobacilli with increased proteolytic activity were detected. When conducting fermentations under optimal growth conditions in a 10 l scale at a constant pH value for 8-10 hours, cell counts were in a range of 10^{10} to 10^{11} cfu/ml. Disassociation of the cell-bound protease with subsequent purification was performed employing ion exchange chromatography. The enriched enzyme was used for hydrolysis of milk proteins and liberation of bioactive peptides. Due to several amino acid auxotrophies, lactic acid bacteria depend on an efficient proteolytic system consisting of a protease and three different peptide transport systems (Opp, Dpp, DptT). To increase the yield of bioactive peptides by living cultures, we carried out random mutagenesis for inactivation of the oligopeptide transport system (Opp), located in the cell membrane.

Poster 7

Identification and Quantification of milk oligosaccharides

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The aim of this work was to characterize oligosaccharides (MOS) from goat, sheep, mare and camel milk in relation to those from human and bovine milk. Because of their assumed beneficial prebiotic and anti-infective effects, MOS are substances of particular interest in human nutrition. However, due to their complexity, MOS with structures identical to those in human milk are not available as dietary ingredients. Therefore MOS from non-bovine milk may be an attractive source for potential application in human nutrition. For this reason, raw milk samples from different mammals were analyzed.

The milk samples were defatted by centrifugation at 4°C and the proteins were removed through a membrane with a NMWCO of 10 kDalton. Size exclusion chromatography was used to separate the MOS from lactose, monosaccharides and other undesired analytes. The measurements were performed by high-pH anion-exchange chromatography (HPAEC) with pulsed amperiometic detection (PAD) and parallel on-line electrospray ion-trap mass spectrometry (IT-MS). This allows a direct characterization of the composition and structure of the MOS fractions without derivatization. Six oligosaccharide standards were used for the quantification of the MOS.

The results demonstrate that milk samples from different species are unique in relation to the type and content of MOS. As expected, the highest amount of MOS was found in human milk. The MOS content in sheep, mare, goats and camel milk was approximately 20 times smaller and more similar to the amount in cow milk. Nevertheless, the structure and amount of oligosaccharides in milk of different origin can vary significantly.

To provide a better understanding of the relation between structure and possible health benefits of MOS, further studies will deal with the prebiotic and anti-inflammatory properties of oligosaccharides from different species.

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Poster 8

Bioactive peptides from milk protein as functional food ingredient - focus on antioxidant and ACE-inhibitory peptides

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Introduction

Bioactive peptides are protein fragments which may exert a biological activity in the body, regardless of the nutritional value. Most of the bioactive peptides consist of 2 to 20 amino acid residues. In milk and milk products, a number of bioactive sequences have already been identified. Whereas ACE-inhibitory peptides with hypotensive activity have been thoroughly examined [1], antioxidant peptides from milk proteins need further investigations [2, 3, 4]. In the frame of the network of excellence FoCus (Food Chain Plus, *www.focus.uni-kiel.de*), besides others, bioactive peptides from milk proteins will be generated and characterised.

Materials and methods

Proteolysis with β-casein / β-lactoglobulin (both attained on pilot plant scale) and different enzymes were carried out by the pH-stat method for 4 hours and the resulting proteolysates were separated into fractions by ultrafiltration at different NMWCO (5 kDa & 1 kDa). All samples were screened by a cell-based assay for getting information about possible anti-inflammatory effects. Furthermore, all fractions were screened for radical scavenging activity (TEAC) and complex-forming ability (FRAP). Some fractions were also screened for ACE-inhibitory activity. The IC50 values were calculated.

Results and discussion

When comparing different proteolysates, which were generated by trypsin or an alkaline protease from *Bacillus licheniformis*, the ultrafiltrated fractions > 5 kDa showed good complex-forming properties, whereas the peptides < 5 kDa indicated improved radical scavenging properties. Regarding the ACE-inhibitory activity, the lowest IC50 values were calculated for the fractions < 1 kDA (approximately 50 mg/l). With a cell-based assay, the proteolysate from β-casein and trypsin indicated anti-inflammatory potential. In prospect, selected fractions will be analysed by ESI-LC-MS/MS to identify respective peptide sequences.

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Poster 9

Innovative approaches to realize the production of dairy based pharmaceutical active peptides

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To date, large-scale processes to obtain and enrich individual biofunctional peptides with special therapeutic character out of natural sources are not feasible. Though different milk proteins are known to be potential precursors for functional peptides the targeted release of these specific peptides is not possible. Particularly within β-Lactoglobulin (β-Lg), the major whey protein, several peptides with different biofunctionalities like antibacterial and hypocholesterolemic activity as well as angiotensin-I converting enzyme (ACE)-inhibitory properties are latent and can be released by enzymatic hydrolysis.

However enzymatic hydrolysis results in a mixture of numerous different mostly unpredictable peptides. Since some of the peptides may have undesired effects, e.g. bitterness, processes to produce desired peptides with high recovery and purity are needed.

Therefore two approaches were investigated. On the one hand thermal pre-treatment of the substrate was examined as a new possibility to control enzymatic hydrolysis. Thereby conformational changes within the protein structure were utilised to prevent the hydrolysis of undesired, and increase the accessibility of the enzyme to desired peptide bonds. On the other hand a selective chromatographic high through-put process was developed to separate the hydrolysate to obtain peptide fractions enriched with desirable peptides.

Due to the combination of these two strategies the production and separation of peptides with bactericidal, hypocholesterolemic and ACE inhibitory properties was facilitated.

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Poster 10

Structure of lupin protein isolates and their application into food products

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As already proven for soy protein, lupin proteins have shown to exhibit health-promoting effects. The most described effect is their ability to prevent arteriosclerosis by reducing the concentration of circulating LDL and VLDL [1]. Besides, lupin proteins offer excellent techno-functional properties, such as water binding capacity, foaming- and emulsification properties [2].

Focus of the present study was the development of a specific fat-like protein isolate from seeds of *Lupinus angustifollus* L. Its production was based on a salt-induced extraction followed by a dilution of the protein extract in water facilitating proteln precipitation [2,3]. Its application in a low-fat formulation of truffle fillings exhibited a testy praline wlth appropriate sensorial properties showing the potential of lupin proteins for fat replacement. However, the reason for this fat-like behaviour has not been investigated up to now.

Different lupin protein isolates were produced varying production parameters, which are known to influence the conformation of proteins. Fundamental differences in their physical structure were found by physical and microscopic analyses. An unfolding and irreversible denaturation of the proteins after isoelectric precipitation was attended by a rough and curdy texture. In contrast, the fat-lilte mouthfeel of the product obtained by dilutive precipitation was found to be caused by a rnicellar structure arangement of the lupin protein. The micellar protein isolate showed a flexible behaviour and the lowest denaturation degree among all protein lsolates indicating high bio-functionality.

Techno-functional and rheological properties of lupin protein isolates were studied to bridge the gap between treatment procedures, structure formation and resulting protein behavior. Comparatively, the micellar protein isolate featured lowest storage modulus, lowest protein solubility and forefront emulsifying capacity. In contrast, chemical composition of the different lupin protein isolates revealed similar leading to the assumption that physlcal causes might play a major role for differences in texture and techno-functional properties.

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Poster 11

β-Casein hydrolysis using *Lactobacillus helveticus* peptidases: Generation of X-Pro and X-Pro-Pro peptides

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The proteolytical system of lactic acid bacteria is a rich source of endopeptidases and exopeptidases with various specificities such as the aminopeptidases (e.g. PepN; EC 3.4.11.2) and the proline-specific exopeptidases (e.g. PepX; EC 3.4.14.11)[1]. A potential application for the proline specific peptidase PepX is to produce bioactive dipeptides like the ACE-inhibitory dipeptides YP and FP by cleaving milk proteins.

In our studies we investigated the aminopeptidases PepX and PepN of *Lactobacillus helveticus* ATCC 15009 in i) the crude cell free extract and in a ii) partially purified peptidase mixture. Both enzyme preparations were separately used for *β*-casein hydrolysis (30 ml scale, 37 °C, 96 h). The operational stabilities of PepX and PepN were significantly higher when they were partially purified resulting in residual activities of 78% and 70%, respectively (in crude extract: 12% for PepX and 54% for PepN). Both casein hydrolysates obtained were directly tested for their potential ACE inhibiting properties using an *in vitro* assay. Here, the generation of hippuric acid was quantified by HPLC with UV detection at 228 nm. In both cases an ACE inhibiting effect was measured. With i) an ACE inhibition of the *β*-casein hydrolysate of about 16.7% ± 2.1% was measured whereas with ii) the *β*-casein hydrolysate was more effective showing an ACE inhibition of about 23.2% ± 0.4%. Both *β*-casein hydrolysates were analysed by HPLC with MS detection after derivatisation of the peptides with dabsyl chloride. Nearly all possible di- and tripeptides (17 peptides) with the structure X-Pro or X-Pro-Pro in *β*-casein were found.

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Poster 12

Conjugated linoleic acid (CLA) content of various greek fermented dairy products

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Conjugated linoleic acid (CLA) is a mixture of positional and geometrical isomers of linoleic acid (LA) with conjugated double bonds. The major CLA isomer in natural products is 9-cis, 11-trans, also known as rumenic acid (RA), which is considered to be the biologically active isomer. At present there is great interest in CLA, because of its potentially beneficial biological effects [1]. Dairy products are the major source of CLA in animal foodstuffs, where its content ranges from 3 to 9 mg/g of fat [2].

In the present study the content of CLA in several traditional yogurts and fermented dairy products (Ariani, kefir, sourmilk) that are found in the Greek market, was examined. Although previous studies [3], [4] have shown that Greek cheeses are an excellent source of CLA, there is no literature available for Greek fermented dairy products. CLA content in cow, sheep and goat milk yogurts was 0.6-0.61, 0.56-0.74 and 0.04-0.32 % of total fatty acids, respectively. The variation in CLA amounts was larger in cow milk Yogurt samples than in sheep or goat milk yogurts. In general, low-fat milk yogurts showed lower values of c-9, t-11 CLA content on lipid basis compared to full-fat yogurts. Ariani, kefir and sourmilk contain 0.49, 0.56 and 0.48 (% CLA of total fatty acids), respectively. According to previous studies, Feta (brine cheese), Graviera (long aging time cheese) and Manouri (whey cheese), contain 0.74, 0.77 and 0.56 (% CLA of total fatty acids), respectively. Since the above dairy products are widely consumed by the Greek population, it was interesting to determine and to compare their CLA content. Results showed that most of the Greek dairy products are an excellent source of CLA, although CLA seems to depend on geographical, seasonal and milk origin factors.

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Poster 13

Evaluation of Angiotensin-converting enzyme inhibitory activity in lactic acid bacteria isolated from indigenous milk products of Orissa

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A total of one hundred (100) isolates randomly picked from one hundred and fifty (150) samples (collected from several indigenous milk products of different places of Odisha) on MRS and MRS-BCP media. These isolates were initially selected for screening the potential bioactive peptide producing lactobacilli isolates. The gram positive, catalase and indole negative, non motile isolates were stored at -80°C. These isolates were matched against reference lactobacillus strains. Eighty (eighty) isolates were matched with preliminary characteristics of lactic acid bacteria as per Bergey's manual. Out of eighty isolates, only eight (8) isolate demonstrated a clear zone of proteolysis in milk agar plates. These microorganisms were further evaluated for in vitro Angiotensin-converting enzyme (ACE) inhibitory activity. The IC_{50} (the volume that inhibits the enzyme by 50% under assay conditions) value was observed in the range of 14.6 - 80 µl for different isolates. The isolate KSBT 17 showed IC_{50} value of highest potency (ca. 14.6 µl) that means lowest dose required for 50% inhibition of ACE inhibitory activity. The isolate KSBT17 was identified as *Lactobacillus fermentum* by 16S rDNA sequencing and biochemical analysis.

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Poster 14

Physicochemical and gelation characteristics of tropical mackerel surimi

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Dark muscle fish have been paid more attention as a potential alternative raw material for surimi production due to the limited fish resources, especially white muscle fish. Mackerel was one of the most abundant dark-fleshed species caught in the Gulf of Thailand. The use of this small pelagic fish for surimi production is one of a major challenge ways to transform the underutilised fish protein resources into human foods, particularly protein gel-based products. However, the characteristics of surimi gel depended on the properties of myofibrillar proteins, which were affected by the species and freshness of the fish, as well as on the processing parameters. Therefore, the objective of this study was to investigate the characteristics and gel-forming ability of surimi from three species of mackerel caught in Thailand. From the results, the highest whiteness with the lowest redness index corresponding to the lowest myoglobin content especially its oxidised form, metmyoglobin, was found in short-bodied mackerel (*Rastrelliger brachysoma*) surimi ($p<0.05$). Frigate mackerel (*Auxis thazard*) surimi contained the highest lipid content ($p<0.05$). The pH of all surimi was in the range of 6.58-6.80. The highest sulfhydryl content and Ca2+-ATPase activity was found in natural actomyosin extracted from short-bodied mackerel surimi ($p<0.05$). The highest TCA-soluble peptide content was found in frigate mackerel surimi gels ($p<0.05$). Kamaboko gel of short-bodied mackerel surimi exhibited the highest breaking force with the lowest expressible drip ($p<0.05$). Heating regime had no effect on deformability of gels from Indian mackerel (*Rastrelliger kanagurta*) and short-bodied mackerel but not for frigate mackerel. The highest metmyoglobin content with the lowest whiteness was found in frigate mackerel surimi gel ($p<0.05$). Therefore, short-bodied mackerel was the best suited for the production of surimi with superior functional attributes including whiteness and gel-forming ability.

Poster 15

Dietary intervention with different PUFA significantly affects fatty acid- and micronutrient profiles of beef and beef products

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The study investigated the dietary impact of ALA (α-linolenic acid) *vs* LA (linoleic acid) on fatty acid- and micronutrient composition of beef tissues and the extent of diet- and processing-induced changes by lipid- and micronutrient composition of beef products made thereof [German Corned beef (GCB), scalded sausage]. Beef and beef products were obtained from German Holstein bulls (n=29) which either received a control diet consisting of maize silage and concentrate with soybean meal (41%), (n=15), or an experimental diet of grass silage and concentrate plus rapeseed cake (12%) and linseed oil (3%), (n=14). The study revealed that upon an ALA *vs* LA intervention the sum of saturated fatty acids in beef (longissimus muscle) decreased by approximately 25%, whereas the amounts of ALA (by 2.6 times), EPA (by 2.3 times) and Σn-3 LC-PUFA (by 1.7 times) were significantly elevated. The amount of CLAcis-9, trans-11 in beef did not differ between feeding groups, whereas the n-6/n-3 FA ratio was significantly lower in beef of ALA (2.3±0.1) than LA (5.8±0.1) fed animals. Trace element (Fe, Cu, Zn, Se) concentrations were not affected by the diet. Experimental diet significantly increased ß-carotene contents, and the g-tocopherol contents were decreased. During beef processing, n-3 FA (ALA, EPA, Σn-3 FA) from beef were found to be product-specifically transferred into the corresponding beef products. ALA and Σn-3 LC-PUFA contents were found be by 1.4 and 1.5 times higher in GCB from ALA than LA fed animals. The n-6/n-3 FA ratio was significantly lower in GCB produced from ALA (4.0±0.4) than LA fed animals (5.9±0.4). The trace elements contents were not affected by the diet; however g-tocopherol contents were decreased by experimental diet. Scalded sausage was the only beef product for which no significant effect of a dietary FA intervention of beef bulls was obtained.

Poster 16

Applications of chitosan and chitooligosaccharides as food additives, films and coatings

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The effect of chitosan and chitooligosaccharides as health promoting food additives in wheat bread on its shelf life, moisture migration and mechanisms of staling, mechanical and barrier properties of chitosan films, and the effect of chitosan edible coatings on vitamin C and polyphenols content changes in cherries during storage are analysed.

It was found using DSC method that chitosan affects redistribution and state of the water in bread crust and crumb. Content of freezing water in bread crumb decreases more intensively in the presense of chitosan. Redistribution of water affects intensity of Maillard reactions in bread crust.

The possible mechanisms including prevention of amylose lipid complexation, acceleration of dehydration from both starch and gluten, adsorption of chitosan onto the starch surface and increase of moisture migration rate from crumb to crust are proposed to explain increase of the rate of crumb firming. Chitosan oligosaccharides increase bread crumb staling rate to a much lesser extent than does middle molecular weight chitosan.

It was found that water vapour uptake decreases with storage time at room temperature and increases significantly with storage time in freezer at -24 0 C and at +4 0C. Mechanical properties of chitosan based films improve to a great extent as a result of storage at low temperatures. Drastic increase of tensile strength and decrease of elongation at break and water vapour uptake was found when thickness of chitosan films is 20 mkm or less.

The loss of vitamin C during storage of non-treated cherries and synthesis of vitamin C in cherries coated with chitooligosaccharide edible coatings has been observed. Total phenols in cherries increased after 7 days refrigerated storage. The changes were more pronounced for samples coated with high molecular weight chitosan edible coatings.

Poster 17

In vitro evaluation of Irish seaweed extracts as inhibitors of key enzymes linked to type 2 diabetes

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Hyperglycaemia, a condition characterised by an abnormal postprandial increase in blood glucose level, has been linked to the onset of type 2 diabetes. Hydrolysis of carbohydrates such as starch is the major source of glucose in the blood. Pancreatic α-amylase and intestinal α-glucosidase are the key enzymes involved in this hydrolytic process and it is believed that their inhibition could provide a therapeutic approach for diabetes. Inhibitors of the enzymes delay carbohydrate digestion and prolong overall digestion time, causing a reduction in the rate of glucose absorption and consequently attenuating the rise in blood glucose.

To date, numerous polyphenols have demonstrated anti-diabetic properties through inhibition of carbohydrate-digestive enzymes. The objective of this research was to evaluate polyphenolic extracts of different Irish seaweeds for α-amylase and α-glucosidase inhibitory effects. The enzymes, α-amylase (0.5 mg/ml) and α-glucosidase (0.1 U/ml), were incubated in the presence of the extracts and their activities were assessed over time. Fifteen seaweed extracts with the highest polyphenol yield were chosen and, following an initial screen at 2000 µg/ml, three extracts were identified as inhibitors. These extracts were then tested at lower concentrations. At 100 µg/ml, extracts ISCG0084, ISCG0051 and ISCG0115 had the same inhibitory effect on α-amylase as the pharmacological inhibitor acarbose. Moreover, only 1 µg/ml of the extracts was required to decrease α-glucosidase activity to < 50%, which was 100 times more potent than acarbose.

A major drawback of pharmacological inhibitors is side effects such as abdominal distention, meteorism and diarrhoea. Such effects are caused by the excessive inhibition of α-amylase resulting in the abnormal fermentation of undigested carbohydrates in the colon. Given that these extracts have a lower inhibitory effect against α-amylase and a stronger inhibitory activity against α-glucosidase they offer the ideal conditions for an effective therapy for postprandial hyperglycemia with minimal side effects.

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Poster 18

Quantitative Population-Epigenetics in screening and development of Biologically Active Compounds

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The potential for using Biologically Active Compounds in Agriculture and for Functional Food is far-reaching. Yet despite extensive research, only a few chemical compounds have achieved practicability. Lack of success may be attributed to two factors: up to now, either such biologically active compounds have not been detected or, screening and development procedures are not adequately sensitive to detect effects on quantitatively inherited characters.

This publication propounds to render screening more efficient by taking into account laws of inheritance. The argument undertakes to show that statistical (epi-)genetic theory as a basis for developing screening methods may be appropriated with the same facility as is done in plant and animal breeding schemes. The research discipline and the treatment subject are the same for both the breeder and the investigator of regulatory agents, save each treats different sides of the same coin (organism). The breeder endeavours to improve the genotype -- for him environments are 'fixed' effects; the "environmentalist" is not able to augment the genotype -- one strives to intervene in the environment by effecting a specific phenotypic expression with a biologically active compounds (i.e. functional food) within the 'norm of reaction' inherent in the genotype.

Likewise index selection based on statistical epigenetic theory can be used to improve efficiency in screening compounds for potential to enhance quantitative agricultural characters such as yield, stability and resistance to unfavourable environmental influences (e.g., water stress, cold temperatures, disease resistance) -- as well indeed, for potential in pharmacological intervention.

Ecological and Evolutionary Epigenetics is a new field of frontier research at the intersection between molecular genetics and evolutionary ecology. The term 'Epigenetics' is used only for about ten years. The statistical Quantitative Population-Epigenetics theory was published with *STAUSS, R., 1992: Genetic analogues in chemical screening. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, Stuttgart, 99(6), 653-656* and *STAUSS, R., 2012 Quantitative Population Epigenetics. Z. Pflanzenkrankh. Pflanzenschutz (in press)*.

Poster 19

Fraunhofer EMB – Technical Center for applied food research (TFAL)

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In the year 2011, the Fraunhofer Institution for Marine Biotechnology (EMB) established a food technical center for application-oriented research on materials of marine origin with a high potential for use in the food industry (TFAL).

As part of its research, Fraunhofer EMB deals with potential products and their application in the food and dietary supplement industry.
For example, research framework is focused on fish cell flour rich in omega-3 fatty acids or proteins and polysaccharides from macroalgae and the products of integrated mutlitrophic aquacultures (fish, clam and seaweed). The TFAL helps to extract these substances professionally, efficiently and economically from respective raw materials and make them available to food-, dietary supplements- and animal feed industries.
From new formulations and application possibilities to new procedures for obtaining appropriate active compounds, the TFAL is an innovative and competent partner for industrial research and science.

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Poster 20

Effects of Omega-3-fatty acid or resveratrol supplementation on cognitive functions in mild cognitive impairment - study concept and preliminary results

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Background

Mild cognitive impairment (MCI) is viewed as a prodromal stage of Alzheimer's disease. Evidence from epidemiological studies suggests that high intake of fatty fish as well as regular physical and mental activity may protect against age-related cognitive decline. However, controlled intervention studies involving MCI patients, which investigated neuroprotective effects of such dietary and lifestyle interventions are lacking.

Objectives

We investigate the effect of dietary interventions (Omega-3 fatty acid and resveratrol supplementation) on cognitive performance in patients with MCI of the amnestic type. The study will answer fundamental questions about intrinsic mechanisms of diet-mediated effects on learning in the elderly impaired brain, including neurochemical pathways.

Methods

This randomized, double-blind placebo-controlled study will be carried out to test the efficacy of dietary interventions on functional and structural integrity of the brain. Participating subjects will be randomized to three groups receiving fish-oil (1.320 mg EPA/d + 880 mg DHA/d), resveratrol (200 mg/d), or placebo (2.000 mg/d olive oil) for six months. Memory performance will be tested using different cognitive scales, including e.g. MMSE, ADAS, and VLMT. Magnetic resonance imaging will be used to monitor cerebral gray matter volume and white matter integrity. Moreover, underlying mechanisms will be elucidated by measuring a set of peripheral blood measures (insulin, inflammation markers, neurotrophins, lipid profile, and Omega-3 Index). Genotyping for common learning- and metabolism-relevant polymorphisms will also be conducted.

Results

The study is on-going, 30 MCI patients (n = 16 male, n = 14 female) aged 70.4 ± 6.4 years have already been included. At baseline mean Omega-3 Index was 6.5 ± 1.4%. About 80% of the patients had an Omega-3 Index below 8%. The initial Omega-3 Index was not associated with the intake of DHA and EPA assessed by a 7-day food record. Current readings and history data will be available soon and presented.

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Poster 21

Bioactive properties of *Echinacea pallida* harvested in the different time and influence of its extracts on oxidative stability of sunflower oil

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The objective of this study is to determine the effect of harvesting time on the total phenolic contents (TPC), antiradical (ARA) and antioxidant activities (AOA) of leaves, stems and flowers of *Echinacea pallida (EPA)* as well as influence of *EPA* extracts on oxidative stability of sunflower oil.

Echinacea was harvested in June and August. It was differentiated to flowers, leaves and stems. Plant extracts were obtained with 80% methanol as a solvent. Folin-Ciocalteu, DPPH and phosphomolybdenum methods were used for determination of TPC, ARA and AOA, respectively. *Echinacea* extract was obtained by methanol and added to sunflower oil. Oxidative stability of the oil was determined by rancimat apparatus.

The TPC of the *EPA* harvested in June and August, were observed as 121.93±1.65-148.08±1.03, 30.45±0.87-45.26±0.49 and 34.85±0.40-63.25±0.75 mg GAE/g for flowers, leaves and stems, respectively. The ARA were determined as 190.03±1.85-232.50±3.83, 57.59±0.68-82.88±0.83 and 59.00±0.27-85.63 ±0.77 mg (BHA)/g for flowers, leaves and stems, respectively. The AOA were observed as 162.37±1.44-187.69±1.43, 123.04±0.06-134.58±0.59 and 111.80±0.85-128.65±0.7 mg(AAE)/g for flowers, leaves and stems, respectively. The stabilization factor of *EPA* on oxidative stability of sunflower oil was observed 111.8, 117.10, 124.56 by 500, 1000 and 2000 ppm extract addition, respectively. It was found higher than factor for 100 ppm BHA (108.95).

The highest content of ARA and TPC in different plant parts were sequenced as: flowers > stems >leaves also for AOA: flowers > leaves > stems. The bioactivity of the plant parts increased as the harvesting time longer. *Echinacea* was found as effective for inhibition of lipid oxidation in sunflower oil.

Keywords: *Echinacea pallida*, Harvesting time, Bioactivity, Rancimat, Oxidative stability

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Poster 22

Effects of dietary *Cannabis sativa* on fatty acid composition of quail (*Coturnix coturnix japonica*), meats and eggs

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This study was carried out to determine the effects of Japanese quail diets which contain *Cannabis sativa*, on fatty acid composition of quail eggs and meats. Quails were divided into two groups according to their sexuality, male and female groups which were divided into four sub-groups. These four sub-groups were fed by four different type of diet. The first group's diet did not contain *C. sativa* which is used as control. The other groups contain 5%, 10% and 20% *C. sativa.* After five weeks feeding period male and female quails were slaughtered and the fatty acid composition of the quail meats was determined. The ω-3 fatty acid composition of the male quails increased from 0.76% (control) to 4.67% by feeding of 20% *C. sativa* containing diet. This increasing ratio was found higher than the females group which were determined as 2.54% (control) to 4.57 (20% *C. sativa* containing diet)

The other four groups were fed by mentioned diets by two weeks and quail eggs were collected and fatty acid composition of the eggs was determined. The ω-3 fatty acid levels of all supplemented diet groups were higher than that of control group. While the amount of α-linolenic acid was increased, arachidonic acid was decreased by this feeding. α-Linolenic acid composition of the enriched eggs was increased from 0.4% (control) to 1.77% (20% *C. sativa* containing diet). Accordingly, the ratio of arachidonic acid was decreased from 1.31% (control) to 0.8% (20%) by feeding of 20% *C. sativa* containing diet.

It was observed that when C*annabis sativa* amounts was increased in quail rations, omega-3 fatty acid amounts in meat and egg yolk were improved.

Keywords: *Cannabis sativa*, Quail, Meat, Egg, *Coturnix coturnix japonica*, Omega 3

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Poster 23

Food safety and quality assurance in the industrial production of traditional mesophilic dairy cultures

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This study presents how classic and modern tools hand in hand with rigorous hygiene and technological control tests are applied to ensure high product safety and quality in the industrial production of traditional "undefined" mesophilic starter cultures.

Traditional starter cultures, primarily originating from spontaneous fermentation of foodstuffs, have been used for more than 100 years in the industrial production of soft and semi-hard cheeses and fresh fermented milk products in North and West Europe.

Typically these cultures comprise a high diversity of strains of one and more species of the genera *Lactococcus* and *Leuconostoc*. The high strain diversity offers huge advantages in terms of high phage robustness and the production of broad range of flavour and gas profiles.

Although the exact number of different strains is not specified, type and ratio of the genera and species are precisely identified. A specific oligonucleotide-based, two-channel DNA micro-array is applied to identify and monitor the species composition and relevant technological genes such as for phage resistance, aroma-production and texture formation in the processing of the cultures.

DNA-fingerprinting of culture isolates and whole cultures, provides information on the minimum number and diversity of the strains in the culture. Furthermore, DNA fingerprints of the whole culture guarantee the sustainment and consistent quality of the traditional cultures.

Thanks to modern molecular biological technologies, such as the Realtime-PCR, micro-array and DNA-fingerprinting techniques, the composition of the so-called undefined mesophilic cultures today is far from unknown. In addition comprehensive hygiene control protocols guarantee the absence of undesired pathogenic contaminants. Given the highest possible degree of safety and product consistency, traditional "undefined" mesophilic dairy cultures will remain an important source in the production of high-value, high-quality fermented milk products.

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Poster 24

Production of bacteriocins by *Enterococcus* spp. isolated from traditional, Iranian, raw milk cheeses, and the detection of their encoding genes

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Strong bacteriocins, or bacteriocins with a wide range of activity against pathogens and spoilage microorganisms, are actively sought for use as natural food preservatives. This work reports the inhibitory activity of 96 enterococcal isolates from two Iranian, raw milk cheeses against five indicator organisms (including *Listeria innocua*). Forty eight isolates inhibited at least one indicator in spot agar assays. Of these, 20 isolates corresponding to 15 different strains were shown to produce bacteriocin-like substances in liquid cultures. PCR analysis revealed the genes coding for enterocins (enterococcal bacteriocins) A, B, P or X, or their combinations, in all but one of these 15 strains. In addition, the gene coding for enterocin 31 was detected in two strains. No amplification was obtained in one strain when using specific primers for all 13 bacteriocin genes sought. Three different enterocin genes were identified in most strains, and four in one strain. Although the concomitant production of bacteriocins is still to be verified, producers of multiple enterocins could be of great technological potential as protective cultures in the cheese industry.

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Poster 25

Atypical bacteriophages attacking *Streptococcus thermophilus* - an underestimated risk for thermophilic starter cultures?

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Many dairy strains of *Streptococcus thermophilus* starter cultures (required for production of yoghurt, mozzarella and *Swiss*-type cheeses) are susceptible to infection by lytic bacteriophages. *S. thermophilus* phages have been isolated and characterized world-wide and are currently grouped into two distinct subgroups on basis of their unrelated phage structural proteins. Representatives of both phage groups cannot be differentiated morphologically, as they reveal the same morphotype (i.e, *Siphoviridae* phages). For their simultaneous detection and differentiation, a PCR system has been established on basis of DNA regions of their non-related *mhp* genes coding for the major head protein. This multiplex PCR system allows both the detection of lytic phages in whey and product samples and furthermore the identification of prophages in lysogenic cultures as well.

When the multiplex PCR tool was tested with a broad set of lytic phages, one phage (i.e., phage P738) failed to generate a PCR amplicon. By electron microscopy it was shown that this new phage differed morphologically from all other *S. thermophilus* phages. It is notable that phage P738 also exhibited a number of unusual physiological characteristics unrelated to other *S. thermophilus* phages. In order to improve the PCR-based phage detection system with respect to this new phage isolate, DNA sequence analysis was performed for the region flanking the major head protein gene. Surprisingly, the P738 phage genome revealed high DNA homology to *Streptococcus pyogenes* phages, therefore phage P738 represents a new type of *S. thermophilus* phages. As judged by its uncommon physiological characteristics and DNA sequence analysis, this new phage has crossed the host species barrier and originates from an *S. pyogenes* host background. The standard multiplex-PCR for comprehensive and reliable detection of *S. thermophilus* phages was finally updated with a DNA primer pair specific for the *mhp* gene of the new phage.

Poster 26

Incidence of aflatoxin M1 in human milk and animal milk from Jordan

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This study was undertaken to determine the presence of aflatoxin M1 (AFM1) in animal milk and the exposure of infants to aflatoxin M1 (AFM1) and lactating mothers to aflatoxin B1 (AFB1), using AFM1 in breast milk as a biomarker for exposure to AFB1. A total of 100 samples of fresh animal milk (cows, goats, camels and sheep) and fermented milk (buttermilk) and 100 samples of human breast milk were collected during 2010-2011 years. An enzyme –linked immunosorbant assay (ELISA) was used for the analysis of milk samples. AFM1 was detected in all animal milk samples with average concentration of 56.17 ng/kg (range 7.05-129.79 ng/kg) in fresh milk samples and 1079.57 ng/kg (range 47.97-2027.11 ng/kg) in fermented milk. The concentration of AFM1 in 70 samples from fresh and fermented milk were higher than the maximum tolerance limit accepted by European Union and USA (50 ng/kg).

For human milk samples, the average concentration of AFM1 was 67.76 ng/kg (range 9.71-137.18 ng/kg), the concentration of AFM1 in 95 samples were higher than the maximum tolerance limit accepted by European Union and USA (25ng/kg). Logistic regression analysis failed to show a relation between AFM1 and type and amount of dairy consumption, vegetables, fruits and meat. But it was a relation to the cereal consumption. The present study is the first one ever carried on the occurrences of AFM1 in milk consumed by the Jordanian population.

Keywords: Aflatoxin M1, animal Milk , human milk , ELISA, Jordan.

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Poster 27

Detection, quantification and genotyping of noroviruses in oysters implicated in disease outbreaks

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Noroviruses (NoVs) are a major cause of foodborne outbreaks of acute gastroenteritis in humans. Transmission of NoV is commonly linked to the consumption of oysters as they accumulate viruses through filter feeding in faecal-contaminated water. The NoV genogroups (G)I, GII and GIV infect humans. While GI and GII have often been verified as causative agents of oyster-transmitted illness, GIV is rarely detected and has so far not been confirmed in outbreaks related to oysters.

The aim of this study was to determine whether NoVs from oysters implicated in a disease outbreak were linked to the GI, GII and GIV found in the stool samples from infected patients. NoVs were quantified in the oyster samples using Taqman real-time RT-PCR and characterised by sequencing of the partial capsid region.

The results confirmed the presence of GI, GII and GIV in the oysters in levels between 101 - 103 detectable genome copies/g of digestive tissue. Sequences of the NoV strains found in oysters clustered with GI.3, GI.6, GII.7 and GIV.1, and showed 87, 79, 79 and 90% nucleotide identity to the respective genotypes found in patients. This discrepancy in sequence identity is not surprising and is probably due to the contamination of the oysters with multiple strains discharged with sewage. To our knowledge, this is the first study characterising NoV GIV in outbreak related oysters. Our results confirm the inherent challenges of establishing the linkage between NoV strains in patients and foods contaminated by sewage.

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Poster 28

Arsenic speciation of marine shellfish in Korea by HPLC-ICP-MS

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We speciated arsenic compounds in 6 species of marine shellfish (145 samples) from Korea using HPLC-ICP-MS and analysed the level of arsenic by ICP-MS. The total arsenic of ark shell, oyster, comb pen shell, abalone, mussel, and shortnek were 1.42, 2.13, 3.14, 3.27, 1.41, and 2.76 mg/kg, respectively. AsB, AsC, and DMA were detected in all samples. AsB had the highest proportion of the total arsenic. The AsB contents of ark shell, oyster, mussel, shortnek were 48, 40, 38, and 40%, and those of DMA were 9, 12, 9, and 14%, respectively. Pen shell had the highest AsB content with a proportion of up to 71%. A trace amount of As(III) was detected in only Pen shell with the level of 0.07±0.06 mg/kg. In case of Abalone, AsB speciation was identified only 5 ones among 23 samples, and AsC was more higher (19%) than the others. As(V) was not detected in all samples. We provided evidence that inorganic arsenic levels in marine shellfish of Korea are generally not a concern.

Key words: Arsenic compounds, As speciation, HPLC-ICP-MS, Korean shellfish

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Poster 29

Muscle-invading larvae of *Anisakis simplex* (*Nematoda, Anisakidae*) transfer specific spoilage bacteria into the flesh of fish which may affect the spoilage rate of minced fish products

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Commercially important fish species from northern temperate waters are, to varying degrees, infected with the larvae of the parasitic nematode *Anisakis simplex*. The parasite uses fish as transport host for transmission to the final host which usually are different species of whales. During the transport stage some larvae may enter the fish muscle by migration from the intestine of the fish. The fish intestine is densely populated with bacteria of various species, including members of the specific spoilage bacteria (SSB) of fish. The present study examined the role of muscle-invading nematode larvae on the bacterial contamination of the initially sterile fish flesh, and the extent of which the infection intensity might affect the shelf-life of minced fish products. The notoriously A. simplex-infected blue whiting (*Micromesistius poutassou*) was used as a model species. Muscle samples with or without *Anisakis i*nfections, along with the intestinal content of the fish, were collected and prepared for aerobic cultivation on Iron Agar Lyngby (IAL) for 3 days at 20 °C. The number of culturable bacteria present in *Anisakis*-carrying muscle tissue was four times higher than muscle samples without nematode larvae. The same trend was also found for the fish SSB, which may possibly result in reduced shelf life of the actual product. To test this hypothesis, an experiment was run on minced fish products based on lean fish without parasitic infections (farmed cod) stored for 15 days at 4 °C. Parasites collected freshly from the viscera of blue whiting were homogenized and added to 100 grams of minced fish flesh, representing 15, 30 or 50 parasites, respectively. Aliquots of these samples were withdrawn for cultivation on IAL at day 0 before and after the adding the parasites, and further every third day until day 15. The pH levels were measured at day 3, 9, 12 and 15. The number of bacteria in the parasite itself was also examined, and the colonies obtained on IAL were identified by sequencing. The findings of the study will be discussed.

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