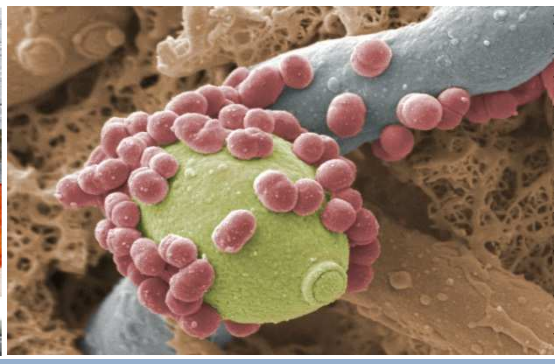
MRI
Max Rubner-Institut
Federal Research Institute
of Nutrition and Food





3rd Kiel Food Science Symposium

May 20-21, 2014
Kiel, Germany

Speaker abstracts - Keynote lectures

Speaker abstracts

Poster abstracts

Speaker abstracts
- Keynote lectures -

Delivery of milk peptides in functional foods

Dolores O'Riordan
University College Dublin, Dublin, Ireland

Peptides derived from milk have been studied extensively for their health benefits. Studies have shown that milk peptides have the potential to modulate heart disease, infection, immunity and growth/development. However despite this strong evidence for the health benefits of milk peptides there are very few commercial milk-peptide enriched functional food products in the market place.

In many instances, there has been poor correlation between potential health benefits demonstrated *in vitro* and their translation to *in vivo* trials. Although the reasons for this are multi-factorial, a major contributing factor is that the bioactive peptides become degraded in the human digestive system and lose the bioactivity observed in laboratory studies. In this presentation developments in encapsulation systems including those recently developed in our laboratory, to protect the bioactives as they transit through the gut will be described. In addition to degradation due to digestion, milk peptides may also lose their physiological potency when they are subjected to the food processing regimes used in the manufacture of functional foods. Encapsulation may also play a role in protecting milk bioactives in food systems.

One of the key challenges in utilising milk peptides in functional foods is to overcome their deleterious effect on the taste and aroma of end products. Milk peptides tend to have very bitter tastes and in addition their volatile components results in an aroma profile considered offensive by many consumers. In this presentation approaches taken to characterise the flavour profiles of milk hydrolysates and strategies to ameliorate their profile will be presented.

Determination of *in vivo* bioactivity of functional food products and nutriceuticals in human studies

Matthias Laudes
University Medical Center Schleswig-Holstein, Kiel, Germany

Almost 10 years ago the health claims regulations were initiated by the EU in order to ensure that a so called “functional food product” exerts beneficial effects on human health besides nutrition. Different types of claims have been defined, such as “function” (e. g. weight control), “risk reduction” (e. g. alteration of cardiovascular risk factors such as LDL-cholesterol) or claims referring to children’s development. The European Food Safety Authority (EFSA) is responsible for evaluating the scientific evidence supporting health claims. However, until now it still remains a major challenge to generate such scientific evidence. This keynote lecture aims to summarize different approaches and phenotyping procedures to examine potential beneficial effects of food products on human health. A special focus will be set on metabolic parameters (glucose, lipid metabolism, body composition), eating behaviour/appetite regulation, the microbiom as well as factors of the innate and adaptive immune system. Finally, on a molecular level a novel cytokine system belonging to the wnt-signalling will be presented which serves as a future phenotyping marker for metabolic inflammation in human intervention studies.

Faith in food: authenticity and traceability

Saskia van Ruth[1], Edoardo Capuano[2], Martin Alewijn[2]

[1] University and Research Centre, Wageningen, The Netherlands
[2] RIKILT, Wageningen, The Netherlands

The food supply chain has become a very extensive network. With global sourcing and optimized for low-cost production, it has become a fragile network susceptible to fraud. Economically motivated food adulteration is a major concern not only for consumers, but also for producers, processors, distributors, retail, food service and authorities. Food adulteration has been practiced forever, but has become more sophisticated in the recent past. These illicit activities result in considerable monetary losses worldwide and erode consumer confidence.

Dairy products and fish are two commodities rating high on the food fraud list. Although paper trailing is very useful, analytical data can underpin the authenticity of the products but simultaneously substantiate quality aspects. In the past adulteration involved mainly removal, replacement or extension of major or minor constituents. Nowadays, consumers are more concerned with how and where foods are produced, especially in view of social and environmental sustainability. The history of the product, e.g. its provenance, the production system, and the processing applied has become important and allow for a new form of adulteration. In the presentation an overview of analytical methodology dealing with the authentication of the compositional authenticity will be provided. Furthermore, other authentication aspects related to how and where dairy products and seafood was produced will be discussed.

Microbial hazard in seafood and use of bioprotective bacteria to guaranty the safety

Françoise Leroi
Ifremer, Nantes, France

Estimates of foodborne illnesses are always uncertain because most illness go unreported. However, in countries where a surveillance system exists, around 10 to 20% of all outbreaks are attributed to seafood and some of the largest food poisoning outbreaks have been associated with marine products. The microbial hazards in seafood due to bacteria present in the aquatic environment or in the handling and process chain will be presented. For indigenous bacteria (*Clostridium botulinum, Vibrio* spp., *Aeromonas*, *Listeria monocytogenes,* histamine-producing bacteria, etc.), the hazard concern (i) products in which the growth of those bacteria is possible and which are eaten raw or insufficiently cooked; (ii) Scombroid and Clupeid fish stored at abuse temperature; (iii) shellfish which concentrate the bacteria and virus during their filter feeding. Other micro-organisms such as *Salmonella*, *Shigella*, *Echerichia coli*, *Staphylococcus aureus*, etc. can be introduced in the aquatic environment or during post harvest handling and processing.

Beside this review, the biopreservation technology will be presented. It is a biological struggle that consists in inoculating in food protective bacteria selected for their capacity to inhibit the growth of pathogenic microorganisms, thus improving the safety of the products. This natural technology can replace the use of chemical additives. This talk will present the strategy of protective culture selection, the mechanisms of action, the regulation context and examples of application in seafood, especially for prevention of risk associated to *L. monocytogenes* and histamine-producing bacteria.

Speaker abstracts

Natural and ligand-modified β-LG fibrils for functional emulsions

Julia Katharina Keppler[1], Yvonne Serfert[1], Stephan Drusch[2], Karin Schwarz[1]

[1] Institute of Human Nutrition and Food Science, Kiel University, Kiel, Germany
[2] TU Berlin, Germany

Whey proteins, most of all β-lactoglobulin (β-LG), are versatile ingredients for the functionalization of foods. They are used as emulsifying, gelling and foaming agents or as natural transporter for small hydrophobic compounds like fatty soluble vitamins, green tea catechins or isothiocyanates from cabbage. β-LG was also found to form fibrillar structures after heating for several hours in acidic conditions and at low ionic strength. Fibrils are long self-assemblies with a diameter of approximately 4 nm consisting of linked peptides. They were found to be useful as food structuring agents in gels, as thickeners or as coating material.

The influence of different pH-values and of covalent modification on β-LG fibrillation was tested and it was found that the properties and stability of fibrils can be significantly changed. Following this, ligand-modified β-LG fibrils were used as emulsifying agent. Their emulsification capacity and stability was evaluated and compared with β-LG treated in different ways.

Development of a soft cheese with added bio-value

Giovanna Contarini[1], Falvio Tidona[1], Domenico Carminati[1], Salvatore Francolino[1], Aurora Meucci[1], Lucia Monti[1], Valeria Pelizzola[1], Giorgio Giraffa[1], Milena Corredig[2]

[1] Consiglio per la Ricerca e la sperimentazione in Agricoltura, Centro di Ricerca per le Produzioni Foraggere e Lattiero Casearie CRA-FLC, Lodi, Italy
[2] Department of Food Science, University of Guelph, Guelph, Canada

A low-calorie rindless soft cheese from half skimmed milk was produced using *Streptococcus thermophilus* with functional properties. Two strains of *S. thermophilus* were selected for their ability to produce hetero-exopolysaccharides (EPS) mainly composed of glucose, galactose and rhamnose but in different molar ratio. These strains had a different technological impact: the first one produced stringy EPS with very high water holding capacity, while the second one showed a more texturing effect. The combined use of these strains as starter culture made it possible to obtain a cheese with about 10% of fat, 15% of proteins and 70% of moisture. Despite the high level of hydration, the structure of the cheese was preserved and the lack of fat was successfully compensated by the EPS, exerting an important role in mouth feel, as well as on the cheese yield. Moreover, one strain of *S. thermophilus* was found to produce folic acid, thus contributing to the natural enrichment of the product with vitamin B9, an essential nutrition component in the human diet involved in many metabolic pathways. The innovative cheese developed appears slightly creamy with pleasant sensorial characteristics and meets the interest of new consumer's demand, beyond the mere nutritional requirements.

Hygienic quality of mare milk

Karin Knappstein, Hans-Georg Walte, Philipp Hammer
Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel, Germany

Mare milk is associated with positive health effects and is therefore usually marketed as raw milk. For shipment the milk is deep frozen or lyophilized. In this case the demands for certified raw milk have to be applied in Germany. The evaluation of the hygienic quality of milk is based on the detection of specific pathogenic micro-organisms and total bacterial count in whole milk. Somatic cell count in composite milk samples is used as criterion for udder health. Milking of mares requires that the foals are not weaned but only separated from their mothers for a few hours before milking. Milking is performed between once per day and to three times per day by hand or machine milking.

The aim of the own investigations was to determine, whether results of routine and reference methods for determination of somatic cell count and total bacterial count comply each other. Investigations were performed on seven farms during one lactation period. Different breeds of mares were included to determine influence factors. In addition samples from udder halves of all lactating mares were investigated.

A good correlation between reference and routine method was determined for somatic cell count. For measuring total bacterial count the routine method is not applicable, because the low bacterial counts present in mare milk could not be differentiated by flow cytometry. In addition it became clear that SCC is not sufficient to determine udder health in mares, but bacteriological investigations are necessary to prevent contamination of mare milk by pathogenic microorganisms. Purulent infections of foals were determined as a cause for post secretory contamination of bulk milk with *Streptococcus equi ssp. zooepidemicus*.

Antioxidant and prebiotic potential of fenugreek seeds in modulating colorectal cancer

P. Nisha, K. B. Arun, G. C. Akhil

Agroprocessing and Natural Products Division, CSIR-National Institute for Interdisciplinary Science and Technology, Kerala, India

The present study was undertaken to evaluate the potential of fenugreek seeds (*Trigonella foenum-graceum*), a spice with amazing therapeutic and medicinal properties, as a source of bioactive compounds and prebiotic dietary fibre in the management of colorectal cancer. The seeds were sequentially extracted with hexane, ethyl acetate, and methanol. Dietary fibre was extracted and evaluated for its prebiotic efficacy using standard protocols. Antioxidant activity were assessed in terms of TPC, TFC, CUPRAC, FRAP, Metal chelating activity and its ability to scavenge DPPH and NO radicals. Phenolic compounds present in extracts were identified by HPLC. MTT and scratch-wound assay were performed in HT 29 cell lines to analyze the anticancer potential of the extracts.

From various antioxidant assays it was found that the methanol extract was more active and efficiently scavenged free radicals, especially NO (IC_{50}-56.54 µg/ml) and was rich in poly phenols like syringic acid, p-coumaric acid and ferulic acid. The methanol extract was toxic to HT 29 cell lines (IC_{50}-294.26 µg/ml) and reduced the area of migration up to 1.8% at 250 µg/ml.

Fenugreek seeds were found to be a very good source of dietary fibre with a soluble dietary fibre (SDF) content of 44.1±4.8 g/100g dry seeds. The morphology of dietary fibre isolated from the seeds was determined by SEM and its prebiotic efficacy was evaluated using *Lactobacillus casei* and *Bifidobacterium bifidus* species. The SDF isolated from fenugreek seeds could effectively promote the growth of probiotic species selected for the study as compared to the negative control and positive controls (inulin and oligo fructo saccharides). The short chain fatty acids produced during fermentation were quantified by gas chromatography. The results from our study conclude that fenugreek seeds are a good source of antioxidant prebiotic dietary fibre which can modulate colon cancer and can maintain a healthy digestive system.

Obesity and allergy risks by infant formula feeding

Bodo C. Melnik
Department of Dermatology, Environmental Medicine and Health Theory, University of Osnabrück, Osnabrück, Germany

Obesity and allergies are major, frequently related diseases in industrialized societies. Human breast milk is the most important functional food that facilitates adequate, species-specific postnatal growth associated with life-long metabolic, adipogenic and immunologic programming tightly controlled by the human lactation genome. Milk´s functionality resides primarily in milk-mediated transfer of essential branched-chain amino acids, which activate the amino acid-sensitive kinase mechanistic target of rapamycin complex 1 (mTORC1). Recent evidence of molecular medicine underlines that mTORC1 orchestrates amino acid-dependent regulation of growth, metabolic programming and adipogenesis. In comparison to all other human body fluids, milk transfers highest amounts of exosomal microRNAs to the infant, which is believed to play an essential role for the postnatal development of the immune system.

In contrast, artificial infant formula feeding bears the risk of excessive and uncontrolled intake of amino acids that may over-stimulate mTORC1 but lacks functionally important microRNAs, such as microRNA-155, which are of crucial importance for the maturation of the immune system and for allergy prevention. Remarkably, recent studies confirm that intake of fresh unpasteurized cow´s milk has an atopy-preventive effect in farm children.

Evidence based on translational research will be presented that infant formula feeding promotes the development of both obesity and allergy by excessive intake of amino acids and deficient supply of regulatory microRNAs.

Infant formula feeding is thus a public health concern and requires correction of both amino acid/mTORC1- and microRNA-dependent pathways for adequate metabolic and immunologic programming. Postnatal feeding is a matter of postnatal programming that occurs during the most vulnerable period of human food consumption and relies on optimized and species-specific functionality of infant food. There is only one food system developed by mammalian evolution that obviously meets all functional requirements for disease prevention: human breast milk.

Kiel Food Science
Symposium 2014
Kiel ◆ Germany ◆ May 20-21 ◆ 2014

MRI
Max Rubner-Institut
Federal Research Institute
of Nutrition and Food

Identification of differences in the allergenic potential of epitopes from α_{s1}-casein variants in cow and other ruminant species by microarray immunoassay

Maria Lisson[1], Natalija Novak[2], Günter Lochnit[3], Georg Erhardt[1]

[1] Department of Animal Breeding and Genetics, Justus-Liebig University, Gießen, Germany
[2] Department of Dermatology and Allergy, University of Bonn, Germany
[3] Institute of Biochemistry, Medical Faculty, Justus-Liebig University, Gießen, Germany

This study investigated the influence of the genetic polymorphism on the IgE-binding properties of epitopes from the bovine α_{s1}-CN variants A, B, C, D, E, F, G, H, and I. In addition, differences in IgE-binding between epitopes of cow, sheep, goat, water buffalo, and camel were determined. On the basis of the IgE-binding epitopes previously identified for α_{s1}-CN of cow and of the corresponding peptides from goat, sheep, water buffalo, and camel, a set of 77 peptides was commercially synthesized and tested by means of microarray immunoassay for IgE-binding by using sera from humans with cow milk allergy.

In the 5 α_{s1}-CN variants A, B, C, E, and I, the amino acid substitutions and deletion affected the immunoreactivity of 5 immunodominant epitopes leading to an abrogation or increase or decrease of IgE-binding.

The majority of sera showed IgE-binding to α_{s1}-CN peptides of cow and the homologous counterparts of sheep, goat, as well as water buffalo, whereas peptides of camel were barely recognized. However, in most sera, epitopes from the non bovine species displayed lower immunoreactivities compared with those from cow. Moreover, IgE antibodies of individual sera reacted only with peptides of sheep or goat or water buffalo, or both, but not with the corresponding peptides of cow, even when the peptides were highly similar.

The results of this study demonstrated that genetic variants in cow milk differ in their allergenicity and should, therefore, be taken into account into the search for a suitable protein source for patients with cow milk allergy. Furthermore, it was confirmed that milk from sheep, goat, and water buffalo harbor an allergenic potential due to cross-reactivity with α_{s1}-CN peptides from cow and, consequently, milk from these species cannot be used as a safe alternative for cow milk in the nutrition of allergic subjects.

Study on stable oxygen isotopes $^{18}O/^{16}O$ for milk identification

Alexander Kolesnov, Olga Maloshitskaya, Irina Filatova
Research Laboratory of Food Quality & Technology, Moscow National University of Food Production, Moscow, Russian Federation

The IRMS/SIRA technique with isotopic equilibration method for measuring of oxygen ($^{18}O/^{16}O$) isotope ratios was first applied to study a biological fractionation of oxygen isotopes in an aqueous phase of milk from stall and free grazing cows and commercial milks from retail.

The method applied revealed high sensitivity towards milk quality. The average oxygen isotope ratios expressed as $\delta^{18}O_{VSMOW}$ in analyzed fresh milk vary from -8.11 ‰ (milk from stabling cows) to -5.45 ‰ (milk of free grazing cows). To evaluate the extent of the biological fractionation of oxygen isotopes $^{18}O/^{16}O$ ratios were measured in surface and underground water in the Moscow region. Compared to water from this region (average $\delta^{18}O_{VSMOW}$ = -11.52 ‰), the aqueous phase of milk is found to be enriched with ^{18}O oxygen isotope.

The study results of domestic and import commercial milk samples with declared name “Natural drink milk” from retail show that values of the $\delta^{18}O_{VSMOW}$ vary over a wide range - from -11.69 ‰ to -1.42 ‰. It allowed to draw the conclusion that a part of samples of commercial milk didn't correspond to the declared name and contained water with not biological fractionated stable oxygen isotopes.

These results of the study are of practical importance for determination of origin, quality, and detection of adulterations of milk and other products (e.g. milk drinks, natural sour milk products).

Food analysis to check quality, safety and authenticity by full-automated ^{1}H-NMR

Markus Link, Manfred Spraul, Hartmut Schaefer, Fang Fang, Birk Schuetz
Bruker BioSpin GmbH, Rheinstetten, Germany

Full-automated high resolution ^{1}H-NMR spectroscopy offers unique screening capabilities for food quality and safety by combining non-targeted and targeted screening in one analysis (15 - 20 minutes from acquisition to report). Full-automated high resolution ^{1}H-NMR (400 MHz) has found its way into the quality control of food and beverages over the last years. The advantage of full-automated high resolution ^{1}H-NMR is its absolute reproducibility and transferability for laboratory to laboratory, which is not equaled by other methods currently used in food analysis. NMR reproducibility allows statistical investigations e.g. for detection of variety, mixing of varieties, geographical origin and adulterations, where smallest changes of many ingredients at the same time must be recorded. Reproducibility and transferability of the solutions shown are user-, instrument- and laboratory-independent. Sample preparation, measurement and processing are based on strict standard operation procedures which are substantial for this fully automated solution. The non-targeted approach to the data allows detecting even unknown deviations, if they are visible in the ^{1}H-NMR spectra of e.g. fruit juice, wine, edible oils or honey. The same data acquired in high throughput mode are also subjected to quantification of multiple compounds. The fully automated ^{1}H-NMR methodology will shortly be introduced and then results on fruit juices, wine and edible oils will be presented and the advantages of the fully automated ^{1}H-NMR solutions shown. The method has been proven on fruit juices and wine, where so far unknown frauds could be detected. In addition conventional targeted parameters are obtained in the same analysis. This technology has additionally the advantage that NMR is completely quantitative and concentration calibration only has to be done once for all compounds.

Consumers' perception towards traceability of fishery products and its relationship with quality: across-national comparison

Domingo Calvo Dopico[1], Rogério Mendes[2], Helena Silva[2], Stefano Mariani[3], Andrew Griffiths[3], Belgees Boufana[3], Véronique Verrez-Bagnis[4], Marc Jérôme[4], Ute Schröder[5], Kristina Kappel[5], Julie Maguire[6], Marc Shorten[6], Amaya Velasco[7], Ricardo Pérez-Martín[7], Carmen G. Sotelo[7]

[1] University of A Coruña, A Coruña, Spain
[2] Instituto Nacional de Recursos Biológicos, Portugal
[3] University of Salford, UK
[4] Ifremer, France
[5] Max Rubner-Institut, Germany
[6] Indigo Rock Marine Research Station, Ireland
[7] Instituto de Investigaciones Marinas CSIC, Spain

Companies from the fishing sector are facing an ever-more globalised and competitive environment, with growing demand from consumers for better quality. Meanwhile, the phenomenon of globalization has highlighted the need for knowledge and monitoring of products offered to the consumer. One of the tools that help firms to track the path of a food product throughout the fishing chain is traceability. However, most of consumers do not recognise what the term 'traceability' means. Similarly, there are very few studies that have investigated within the fishery sector, from consumer behaviour point of view, the relationship between quality and traceability.

Based on this preliminary research, and aware of the fishing industry's required response to these challenges, we want to research how consumers perceive the traceability. Particularly, we want to reach three main objectives. Firstly, understand consumers' perception of the term 'traceability' and its relationship with quality. Secondly, know the best indicators to enable the consumer to recognise the traceability of fish products. Third, assess the consumers' expectations towards the introduction of a traceability system and willingness to pay a price premium.

To find the answers to those objectives an e-questionnaire – see at http://www.economicas.udc.es/labelfish_2014 – is being carried out in Spain, Portugal, France, United Kingdom, Ireland and Germany. Fieldwork began on January 7 and will run until February 7. Currently we have already collected more than 1500 observations.

This study may provide a very interesting contribution. If we properly comprehend what the consumer understands by traceability and how it relates to quality, we can improve not only the quality assurance for the end consumer but also to help those in charge of the value chain (e.g. fishermen, processors, distributors) to identify and differentiate better fish (or seafood) products.

Fish species determination using NGS and real-time PCR approaches

Ilka Haase, Tobias Janke, Katrin Juling, Rainer Schubbert,
Eurofins Genomics, Ebersberg, Germany

Molecular biological methods become more and more important for the control of fish authenticity with respect to (i) species identification; (ii) catch area authentication and (iii) the differentiation of aquaculture derived fish from wild catch derived fish. For species identification, Sanger sequencing of several barcoding regions (e.g. Cytb, COI and 16 S) is the state of the art analysis. More and more fish sequences are deposited in public databases like NCBI (National Center for Biotechnology Information), BOLD (Barcode of Life Data Systems) or FISH-BOL (Fish Barcode of Life Initiative) and help to clearly identify the fish species present in a sample by sequence comparison. The success of this approach is strongly dependent on the quality of the obtained sequence data. Apart from DNA fragmentation in processed samples, the overlay of several sequences in samples with more than one fish species mainly limits the applicability of the Sanger sequencing method. Eurofins Genomic is therefore developing next generation sequencing (NGS) methods for the species identification in mixed fish samples to overcome this limitation. NGS enables the analysis of complex sequence mixtures, but is up to now mainly applied for metagenomic studies of microbiological communities in the research sector. However, constantly declining costs in the NGS field makes the methods attractive also for routine analysis of food in the future. Using this methodology, also fish meals will be able to be analysed (e.g. also for the addition of by-catches).

Apart from that, real-time PCR tests are introduced for the semi-quantitative analysis of several fish species selected according to their economical relevance in Europe and their use in mixed food products.

The oral presentation will give an overview on the ongoing developments of methods for fish authentication at Eurofins Genomics.

Tuna and other scombridae fish authentication by DNA analysis

Asadatun Abdullah[1,2], Hartmut Rehbein[1]

[1] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Hamburg, Germany

[2] Department of Aquatic Product Technology, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Bogor, Indonesia

Fast and reliable analytical methods for the identification of species of processed fish are needed to ensure the correct trade of products. Nowadays, DNA-based methods for seafood authentication are performed predominantly through polymerase chain reaction (PCR) analysis. Tunas and other scombrids landed in Indonesia and processed for export are not analyzed by DNA-based identification techniques at present. Despite of many studies performed over the last years, problems in the identification of processed tunas still exist. In order to provide a PCR based-based authentication system for tunas, 27 reference samples of tunas from Indonesian waters were collected and identified using the mitochondrial cytochrome c oxidase subunit I (COI) gene, as a genetic marker for "DNA barcoding" and cytochrome b (cyt b). Additionally, 12 tuna products were analyzed to assess the reliability of method for processed fish. Furthermore, the nuclear gene encoding parvalbumin was tested as an alternative to the mitochondrial (cyt b and COI) genes, as the intron sequences of nuclear genes may express high variability between fish species. From the parvalbumin gene sequence of *Thunnus albacares* (GenBank accession number FN 544082), two primer pairs, located in intron 1 - exon 1 (forward) and exon 3 (reverse), were constructed to obtain amplicons of 670 and 785 bp in size. Restriction fragment length polymorphism (RFLP) analysis of the 670 and 785 bp amplicon allowed the differentiation of the closely related tuna species *Thunnus albacares* and *T. obesus*. Another pair of primers (located in intron 2) amplifying a shorter sequence (227 bp) could be used to distinguish *Thunnus* species from skipjack (*Katsuwonus pelamis*) and bullet tuna (*Auxis rochei*). Our findings demonstrated that the COI and cyt b gene could be more reliable used as a tool for Indonesian commercial tuna products authentication, if sequencing was combined with character-based identification using differences at certain nucleotide positions. Moreover, the parvalbumin-based PCR systems turned out to be a useful completion of the results obtained from mt-DNA gene analysis.

Keywords: COI, cyt b, parvalbumin gene, intron, species identification.

Preliminary results on the prevalence of parasites in fish sampled during French scientific surveys

Véronique Verrez-Bagnis[1], Aliouat-Denis[2] Cécile-Marie, Gay Mélanie[3], Thébault Anne[3], Cuzzucoli Diane[3], Jérôme Marc[1], Kolypczuk Laetitia[1], Seesao Yuwalee[3], Bruzac Sandrine[1], Certad Gabriela[2], Standaert Annie[2], Follet Jérôme[2], Bourgau Odile[3], Le Fur Bruno[4], Gantois Nausicaa[2], Delplace Linda[4], Dei-Cas Eduardo[2]

[1] Ifremer, Nantes, France
[2] Institut Pasteur
[3] Anses
[4] Pfi Nouvelles Vagues

Some parasites of marine fish, such as nematodes (*Anisakidae*), are zoonotic pathogens that have an impact on public health. Given the increasing consumption of seafood in France and especially of raw fish, the project Fish-Parasites aims at: (i) identifying parasites in fish most commonly consumed, (ii) exploring the potentially pivotal role of host species, geography and other factors on pests, (iii) providing technical strategies to improve the detection of the parasite in fish fillets, (iv) creating a platform for identification of parasites in fish and (v) providing continuing education.

Fifteen fish species were selected based on a risk analysis that took into account the French consumption, the level of consumer exposure and the level of infestation by anisakids (literature data). Fish were sampled either on boats during research campaigns or inland (fish markets or fishing companies), and dissected to collect the parasites. Nematodes were identified to species using molecular identification. All data on sampling, fish individuals, and parasites were collected in a database developed for the project.

Here, only the data regarding the infestation level of fish sampled during research campaigns are described. Parasite prevalence is strongly dependant on the fish species and on geographical areas (Mediterranean Sea, Atlantic Ocean and Channel). In total, 474 fish over the 831 fish sampled during the scientific campaigns harbored at least 1 macroscopical parasite, 53% were infested by at least 1 nematode, and 27% had at least 1 nematode in their fillets. Prevalence and nematode species data will be analysed to determine the potential structuring role of some factors on the distribution of nematodes.

Effects of high pressure processing on the sensory and microbial status of fresh catfish fillets and mild smoked rainbow trout fillets

Ruth A.L.P. Mengden, Günter Klein, Annika Boulaaba, Nadine Sudhaus
Institute for Food Quality and Food Safety, University of Veterinary Medicine, Hannover, Germany

Fish and fish products enjoy increasing popularity throughout the world not least due to their healthy image. At the same time there is high demand for safe products with high quality. Particularly fish products like fresh catfish fillets and mild smoked rainbow trout fillets are perishable and vulnerable for (re-) contamination with pathogens. High pressure processing technology (HPP) enables to prolong shelf-life and to maintain quality features of food.

The main objective of this study was to determine the effect of HPP on aerobic mesophilic counts, *Lactobacillus* spp. and *Pseudomonas* spp. of fresh catfish fillets and mild smoked rainbow trout fillets. Furthermore, samples were inoculated with *Listeria monocytogenes* and *Escherichia coli.* Additionally, the impact of HPP on sensory characteristics was investigated.

Six different pressure-time-combinations were used for HPP-treatment ranging from 200 MPa for 1 min to 600 MPa for 5 min and compared with untreated controls. Subsequently, samples were stored at < 8 °C for 7 days (catfish) and 41 days (trout), respectively.

The most intense treatment (600 MPa/5 min) reduced aerobic mesophilic counts and *Escherichia coli* (trout) as well as *Pseudomonas* spp., *Lactobacillus* spp. and *Listeria monocytogenes* (both fish samples) below detection limits.

Investigation of sensory parameters (appearance, texture, colour) showed differences between the two fish products. Catfish samples permitted gradations depending on HPP intensity and partly revealed a cooked appearance. However, no significant effect was demonstrated on trout samples.

In summary, HPP is a promising technology to prolong shelf life of mild smoked trout fillet. Regarding fresh catfish fillet HPP provides an opportunity to develop new types of food products.

Colorimetric determination of the total phenolic content in cold-smoked fish products - an in-house method validation study

Ulrike Zitz, Konrad J. Domig, Florian Wiederstein, Marija Zunabovic, Wolfgang Kneifel
Department of Food Science and Technology, University of Natural Resources and Life Sciences, Vienna, Austria

Cold-smoking of fish is an old tradition to preserve food, as anti-microbial and antioxidant components such as phenolic compounds are transferred to the food during smoking. Some of them contribute significantly to the typical smoke flavour. Hence, the smoking process has an essential impact to both food quality and safety of fish products resulting in a prolonged shelf-life. Within this context, some indications exist that smoking effects such as the phenolic content may inhibit growth of pathogens such as *Listeria monocytogenes*. Therefore, the quantification of phenolic compounds in cold-smoked fish products is of great relevance. However, European legislation lacks in providing any validated methods or even a standard method for testing smoked fish products for their phenolic content.

Based on a method briefly described by Cardinal et al. (2004), a spectrometric method for the determination of the total phenolic content in cold smoked salmon and trouts was developed and validated. For this purpose, the basic calibration line covering the interesting concentration range of 2 to 23 µg per aliquot was developed by using phenol as standard. Model adequacy was proven by testing for linearity and variance homogeneity. Additionally, several matrix calibrations were performed to investigate the evidence of systematic effects on the calibration line caused by the fish matrix. For this, different cold-smoked salmon and trout samples were selected to serve as matrix. Moreover, storage tests were performed to investigate possible influences on the total phenolic content of the samples. Therefore, sample aliquots of different fish sample homogenates were taken periodically over a storage period of 4 weeks. Furthermore, methodological improvements were done to simplify the working procedure and to enhance method robustness considerably to provide a suitable method qualified to fulfil the specified requirements.

Electrochemical inactivation of bacteriophages: generation of electro-activated solutions by means of cross-flow electro membrane filtration

Meike Samtlebe, Aline Holder, Christiane Merath, Jörg Hinrichs, Zeynep Atamer
Institute of Food Science and Biotechnology, Department of Dairy Science and Technology, University of Hohenheim, Stuttgart, Germany

So called electro-activated water is used as a new technology in the food industry, especially in the dairy industry after cleaning-in-place applications. The activation of water can be realized by subjecting a sodium chloride solution to an external electric field in a membrane cell. Two different electro-activated solutions can be obtained: a solution with oxidizing properties (anolyte), and a solution with reducing properties (catholyte). Anolyte is a sodium hypochlorite solution with a low pH and a high redox potential. Among several applications anolyte is successfully employed as disinfectant for surfaces after cleaning of processing equipment, and for the treatment of process and cleaning water. Besides, it has, compared to conventional disinfectants, a very high bactericidal, virucidal and fungicidal effect. Nevertheless, little research has been conducted regarding the inactivation of bacteriophages by electro-activated water.

In this study, a cross-flow electro-membrane filtration process has been proposed and set-up to produce electro-activated water with the aim of phage inactivation. Process parameters were optimized by varying the NaCl concentration (0.2 – 1%), the electric field strength (2 – 5 V), and the electrode position in the membrane cell. To verify the virucidal effect phage inactivation experiments were conducted. Anolyte produced from a 0.2% NaCl solution at an applied voltage of 2 V reduced the number of phages up to 5-log units after a 15 minute residence time. Furthermore, a microbiological challenge test was designed to verify the inactivation potential of the anolyte. Therefore, anolyte was applied in a filtration rig contaminated with bacteriophages and containing impurities from the filtration process. A phage reduction of about 2-log units occurred when anolyte produced from a 1% NaCl solution at 5 V was tested. In conclusion, it was found that a reliable inactivation of phages by electro-activated water is possible.

Titanium dioxide nanoparticles activate IL8 related inflammatory pathways in human colonic epithelial Caco-2 cells

Kristin Krüger, François Cossais, Martin Klempt
Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel, Germany

Nanosized titanium dioxide (TiO_2) particles are widely used as food additive or coating material in products of the food and pharmaceutical industry. Studies on various cell lines have shown that TiO_2 nanoparticles (NPs) induced inflammatory response and cytotoxicity. But the influence of TiO_2 NPs exposure on inflammatory pathways in intestinal epithelial cells has not been investigated so far. This study demonstrates that TiO_2 NPs with a particle size of 5 nm and 10 nm cause an activation of inflammatory pathways in the human colon adenocarcinoma cell line Caco-2. The expression of ICAM1, CCL20, COX2 and IL8 show a transient increase after NPs exposure measured by quantitative PCR, whereas larger particles (490 nm) fail to stimulate mRNA expression of these genes. Further, using nuclear factor (NF)-κB reporter gene assays, we show that NP-induced IL8 mRNA expression occurs, in part, through activation of NF-κB and p38 mitogen-activated protein kinase (MAPK) pathways. Furthermore, TiO_2 NPs did not affect Caco-2 enterocyte differentiation. We confirm that exposition of TiO_2 NPs can induce inflammation in the gut, but further work is needed to decipher the pathways involved in this inflammatory response.

Poster abstracts

Poster 1

Release of bioactive peptides - characterization of two cell-envelope associated proteinases

Bo Li, Torben Kliche, Knut J. Heller, Diana Meske, Wilhelm Bockelmann
Department of Microbiology and Biotechnology, Max Rubner-Institut, Kiel, Germany

Lactic acid bacteria (LAB) are nutritionally fastidious microorganisms, which are extensively used in fermentation processes. Due to several amino acid auxotrophies, LAB depend on efficient cell-envelope proteases with caseinolytic activity. Subsequently, released peptides are predominantly translocated into the cytoplasm. Some of the remaining peptides in the medium may exert bioactive activities like ACE-inhibition, anti-inflammatory effect and/or antimicrobial activity.

In our study, we detected proteolytically active lactobacilli and lactococci by means of an enzyme assay, where casein was labeled with a fluorescence dye (FITC) and soluble fluorescence was released through proteolyse of casein by the cell-bound proteases. Furthermore, skim milk was inoculated with those strains, which revealed highest proteolytic activities for hydrolysis of casein. The hydrolyates showed different HPLC-chromatography patterns and were found to exert different ACE-inhibitory effects.

Highest proteolytic activity was found in strain 92202, followed by strain 92059. For species identification purposes, we performed 16S rDNA sequencing and used bioinformatical tools (ARB) for species-tree construction. Nucleotide sequence analyses together with physiological sugar fermentation tests suggested that strain 92059 is homolog to *Lactobacillus delbrueckii* subsp. *bulgaricus* and 92202 is homolog to *Lactobacillus delbrueckii* subsp. *lactis*, respectively. Presence of proteinase gene in both strains was confirmed by Southern blot assay. Proteinase genes from both strains were amplified via PCR with primers designed on the basis of published sequences of *Lactobacillus delbrueckii* and cloned into pSMART vector for sequencing of the cloned genes. Expression of the genes are being optimized in *E. coli* using the expression vector pBad/gIII. When enzyme activities can be detected, the genes will be expressed in *Lactococcus lactis* and purified enzymes will be used to degrade milk protein for release of bioactive peptides.

Poster 2

Pilot study to compare bioavailability of quercetin from whey protein coated and uncoated liposomes in whey permeate drink

Monika Frenzel[1], Beate Schulze[1], Kerstin Harnack[1], Matthias Laudes[2] & **Anja Steffen-Heins**[1]

[1] Institute of Human Nutrition and Food Science, Kiel University, Kiel, Germany
[2] Medical Dept. I, University Medical Center Schleswig Holstein, Campus Kiel, Germany

Quercetin is a widely occurring flavonoid with health promoting properties like antioxidant activity, lowering of blood pressure, anticancer action or anti atherosclerotic properties.

These qualities make quercetin an interesting candidate as bioactive compound in functional foods. However, its bitterness and low water solubility makes its incorporation into foods difficult and is accompanied by low bioavailability in the human body. Carrier systems like liposomes can overcome these drawbacks. Since liposomes are sensitive to their environment, a biopolymer coating can be applied to provide better stability in the food matrix and acidic stomach conditions as well as to achieve better sensory characteristics.

To evaluate the impact of application of liposomes and whey protein coated liposomes as quercetin carrier on bioavailability, a randomized, double blind and diet controlled cross over study was conducted. Coated and quercetin loaded (formulation a) as well as uncoated and quercetin loaded liposomes (formulation b) were prepared and incorporated in a flavored and sweetened whey permeate drink. A coated quercetin free liposome drink (formulation c) was prepared to serve as control. Hard gelatin capsules were filled with a corresponding amount of quercetin or pure maltodextrin whereas the latter served as placebo. At three different study days the probands ingested 100 mg of quercetin from either drink formulation a) and a placebo capsule, formulation b) and a placebo capsule or formulation c) and a quercetin capsule. Furthermore, the probands received a standardized polyphenol-free diet and had to keep their physical activity to a minimum. Before each study day a wash out period was implemented.

Plasma levels of quercetin were followed over 24 hours after quercetin application. Pharmacokinetic parameters were calculated and compared between the different application forms.

This project is part of the Agro Cluster *Food Chain Plus (FoCus)* funded by the Federal Ministry of Education and Research (BMBF).

Poster 3

Efficiency of different NF-membranes for the enrichment of milk oligosaccharides

Karina Altmann, Ingrid Clawin-Rädecker, Katja Zerge, Wolfgang Hoffmann, Peter Chr. Lorenzen

Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel, Germany

Human milk oligosaccharides (MOS) stimulate the immune system, inhibit the adhesion of pathogens on the epithelial surface and exhibit potential prebiotic activity [1-4]. In comparison with human milk (5-8 gL^{-1}), the amount of MOS in cow's milk is low (0.03-0.06 gL^{-1}). For this reason, it is currently not possible to use bovine MOS as functional ingredients in food production [4].

The aim of the present work was to compare the efficiency of different nanofiltration (NF) - membranes for the enrichment of MOS.

Initially, bovine milk was skimmed and ultrafiltered (NMWCO= 5 kDalton). Lactose in the UF permeates was hydrolysed by action of β-galactosidases. Subsequently, the NF experiments were carried out in a laboratory device (ÄKTA-Crossflow). Flat sheet NF membranes of different composition (composite, cellulose acetate, polyethersulfone) were applied. The MOS composition of the NF retentates was determined by high pH-anion exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) and parallel online electrospray ion-trap mass spectrometry (IT-MS).

The highest retention of acidic oligosaccharides by nanofiltration (recovery= 84-88%) was achieved by a composite membrane with 500-1000 Dalton. NF membranes with a NMWCO of 150-300 Dalton revealed a recovery of about 72% of the acidic and neutral oligosaccharides. To estimate the enrichment, the MOS content in relation to total sugar in NF retentate was determined. The highest MOS content was achieved with a 200 Dalton cellulose acetate membrane (62%) and the 500-1000 Dalton composite membrane (57%). Further investigations will focus on potential bioactive properties of the NF retentates of MOS.

References:

[1] **Boehm, G.** *et al.*, Journal of Nutrition 137 (3) 2007: 847-849
[2] **Gopal, P.K.** *et al.*, British Journal of Nutrition 84 (1) 2000: 69-74
[3] **Martinez-Ferez, A.** *et al.*, International Dairy Journal 16 (2) 2006: 173-181
[4] **Mehra, R.** *et al.*, International Dairy Journal 16 (11) 2006: 1334-1340

Poster 4

Generation of anti-inflammatory peptides from β-casein on pilot plant scale

Karina Altmann, Adam Wutkowski, Ingrid Clawin-Rädecker, Martin Klempt, Hans Meisel, Peter Chr. Lorenzen

Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel, Germany

Milk provides a rich source of biologically active components. Growing attention is paid to bioactive peptides from milk proteins hidden within the sequence of native proteins, which can be released by enzymatic hydrolysis. By oral consumption of active peptides or liberation of these peptides during gastrointestinal digestion they may exert anti-inflammatory, antioxidative or antihypertensive activities [1-3].

The aim of the present work was to generate anti-inflammatory peptides from bovine β-casein by hydrolysis using different preparations of tryptic enzymes on pilot plant scale.

For preparation of the hydrolysates, proteolysis of a 5 % β-casein solution (w/w) was performed with tryptic enzymes for 3-4 h at 37°C under pH-stat conditions of 7,8. The total proteolysate was fractionated by ultrafiltration (NMWCO = 5 kDa). Freeze-dried peptide fractions were characterized by RP-HPLC. The molar mass and primary structure of peptides were identified by LC-MS and Proteome Discoverer 1.4. The anti-inflammatory activity of peptide fractions was examined by TNF-α mediated activation of the pro-inflammatory transcription factor NF-κ-B in HEK cells.

Peptide fractions > 5 kDa generated by a TPCK-treated trypsin preparation from pork pancreas showed a potential anti-inflammatory activity. In comparison to the control (HEK cells + TNF-α without hydrolysate), the addition of the hydrolysate resulted in a reduction of NF-κ-B activation in HEK cells over 50% with a value of 3,84 ± 0,32. The hydrolysate > 5 kDa produced by a food grade trypsin preparation from codfish showed almost the same NF-κ-B activation. Proteolysis with an enzyme preparation from pork pancreas, which exhibits trypsin activity as well as a small chymotrypsin activity, resulted in a lower anti-inflammatory activity. Peptides > 5 kDa generated with codfish enzymes, which have only a small trypsin but a high chymotrypsin activity, showed no anti-inflammatory activity with an NF-κ-B activation of 7,24 ± 1,82. The active components were identified as mainly hydrophobic peptides with molar masses between 2 and 7 kDa.

References:

[1] **Meisel, H.** *et al.*, Current Medicinal Chemistry 12 (16) 2005: 19005-1919
[2] **Mills, S.** *et al.,* International Dairy Journal 21 (6) 2011: 377-401
[3] **Korhonen, H.** *et al.*, International Dairy Journal 16 (9) 2006: 945-960

Poster 5

Tryptophan-containing dipeptides originating from food proteins are C-domain selective inhibitors of angiotensin converting enzyme

Steffi Rudolph, Diana Lunow, Thomas Henle
Institute of Food Chemistry, TU Dresden, Germany

The somatic isoform of Angiotensin-converting enzyme (ACE) contains two enzymatically active sites, the C- and N-domain, from which the C-domain is supposed to play a major role in blood pressure regulation and, therefore, is a promising pharmacological target in order to reduce blood pressure without side-effects. We report for the first time that tryptophan-containing dipeptides such as Ile-Trp or Val-Trp, originating from enzymatic hydrolysates of the food proteins, are selective and competitive inhibitors for the C-domain of ACE with a selectivity factor of 40 and 70, respectively. Structure-activity studies showed that an N-terminal aliphatic amino acid and a tryptophan moiety in the P2`position of the inhibiting peptides are favorable structures for C-domain inhibitory activity in bioactive dipeptides. In contrast, the lactotripeptides IPP and VPP, which were widely used as bioactive ingredients for hypotensive food formulations, showed a slight selectivity for the N-domain. All identified peptides are competitive inhibitors for the C and N-domain of ACE.

Hence, tryptophan containing dipeptides are interesting ingredients for functional food as a natural prevention for hypertension with reduced side effects due to its selective inhibition of the C-domain. This study may provide a new perspective to the search for bioactive peptides as ingredients for functional foods.

Kiel Food Science
Symposium 2014
Kiel ◆ Germany ◆ May 20-21 ◆ 2014

MRI
Max Rubner-Institut
Federal Research Institute
of Nutrition and Food

Poster 6

Probiotics and studies on enhancing their viability in fermented milk products

Zeynep Atamer[1], Firuze Ergin[2], Meike Samtlebe[1], E. Mine Comak Göcer[2], Ayse Asci Arslan[2], Jörg Hinrichs[1], Ahmet Kücükcetin[2]

[1] Institute of Food Science and Biotechnology, Department of Dairy Science and Technology, University of Hohenheim, Stuttgart, Germany
[2] Department of Food Engineering, Faculty of Engineering, Akdeniz University, Antalya, Turkey

Probiotic bacteria have become increasingly popular in the last years due to their nutritional value and positive health-promoting properties. They are mainly applied in manufacturing of fermented foods. In order for a fermented product to provide health benefits, it is proposed that the probiotic food should contain at least 10^6 viable probiotic bacteria per gram of product when consuming. However, large fluctuations and poor viability of the probiotic bacteria in yoghurt products during storage have been reported. The survival of probiotic bacteria in food processing, during storage until consuming and through gastric transit depends on a wide range of variables such as the strain, the fermentation conditions, and the food matrix. The physiological state of the probiotic bacteria being added to a product can also be a major factor affecting the overall culture viability. In this respect, the induction of a stress response, via exposure of the culture to a sub-lethal stress before use in food processing is proposed to be applied for enhancing the viability. A lactic acid bacterium has to survive a battery of environmental stresses including temperature, acid, bile, exposure and osmotic and oxidative stress, as well as food matrix stresses. Once the cells have survived these stresses, they can colonize and grow to enough numbers to elicit the beneficial effect to the host. In this study, applications of different stress pretreatments in the processing of fermented milk products on the viability and *in vitro* properties of probiotics were investigated. The survival of *Lactobacillus acidophilus*, the most commonly used strain in probiotic yoghurt, in different fermented milk products was studied.

Poster 7

PATHWAY-27: pivotal assessment of the effects of bioactives on health and wellbeing, from human genoma to food industry

Alice Arianna, Achim Bub, Bernhard Watzl

Department of Physiology and Biochemistry of Nutrition, Max Rubner-Institut, Karlsruhe, Germany

Every year European countries spend millions of euros on the treatment of conditions such as metabolic syndrome, heart disease, diet-related diseases and diabetes. Notably, diet can help greatly to reduce risk factors for these conditions, including elevated blood pressure, blood lipids and blood sugar. The PATHWAY-27 network (www.pathway27.eu), which includes 25 partners broadly distributed in the EU, including one Candidate Country (Turkey), has been established to address the potential of selected bioactive compounds in the prevention of the Metabolic Syndrome.

The general objective of the project is the exploitation of bioactive compounds as ingredients of foods that, within the common diet, could significantly benefit human health and wellbeing. Therefor three model compounds (docosahexaenoic acid - DHA, beta-glucan -BG, and anthocyanins -AC) and three model food matrices (bakery, dairy and egg products) will be used.

In four European study centres, one being the Max Rubner-Institut in Karlsruhe, several intervention studies will be conducted with the following aims: a) increased knowledge on bioavailability, activity, synergism and mechanisms of action of bioactive compounds when administered as integral parts of foods, b) establishing guidelines and best practice for intervention studies c) development and validation of innovative biomarkers.

Furthermore the development of new bioactive-enriched food with scientifically proofed positive effects on human health and improved formulation to guarantee acceptance of consumers shall increase the innovative potential and competitiveness of small and medium-sized enterprises.

Poster 8

Influence of selected exogenous factors on value and composition of nutritionally significant mushroom polysaccharides

Miriam Sari, Reinhard Hambitzer
Niederrhein University of Applied Sciences, Mönchengladbach, Germany

Basidiomycetes like *Grifola frondosa* (Maitake) or *Lentinula edodes* (Shiitake) have a long history in culinary and medicinal usage in Asia. Lately they have become the focus of international medicinal research. High bioactive polysaccharides like β-glucans own pharmacological functions such as antitumor activities and nonspecific immuno-stimulant effects as well as antiviral, anti-inflammatory and anti-diabetic properties. Especially hot-water-based extracts obtained from the fresh or dried mushrooms have been in focus for usage as medical treatments for example in cancer therapy as well as functional foods with general health benefits. The healing potential of β-Glucans is linked to their chemical structure, solubility and methods of isolation and further treatment.

Although studies have been carried out for decades, essential findings regarding the active principles or further developments for medication productions are still not given. Especially β-glucan-content in dependence of certain endogenous and exogenous factors like ripening stages, time of harvest, climate or different morphological parts of the fruiting body are still unknown.

Different experiments were carried out to determine the β-glucan-content in Shiitake mushrooms under certain conditions. An enzyme-based test kit was used for determination of quantity of the mushroom β-glucans. Aim of this study is to find out more about the influence of different factors to achieve substantial samples. First results indicate the impact of certain factors like temperature or humidity during the growth process of the mushrooms or time of storage after harvesting. Especially the storage conditions after harvesting seem to have a strong impact on the β-glucan-content. Further experiments will be carried out to investigate correlations between conditions and β-glucan-content in more detail.

Poster 9

Effect of glycomacropeptide on body weight, visceral fat and bone mineral content in ovariectomized rats

Katharina E. Scholz-Ahrens[1,2], Ulrich Kulozik[3], Corinna Thomä[3], Jürgen Schrezenmeir[2,4]

[1] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel, Germany
[2] Institute of Physiology and Biochemistry of Nutrition - Kiel, Max Rubner-Institut, Kiel, Germany
[3] Department of Food Process Engineering and Dairy Technology, TUM, Freising, Germany
[4] Johannes Gutenberg-University, Mainz, Germany

Introduction: Numerous bioactive peptides have been isolated from milk with potential beneficial health effects. Glycomacropeptide (GMP), a fragment of kappa-casein, had been shown to stimulate zinc absorption, an element that is involved in bone mineralization. Furthermore, GMP was found to be associated with satiety, indicating that GMP may be involved in energy metabolism. We postulated that GMP supplemented to a diet would prevent ovariectomy-induced bone loss in rats and could reduce weight gain and visceral fat content in this animal model for postmenopause.

Methods: Aged Fisher rats (n=80) were allocated to four groups. Animals of groups 1 and 2 were either sham operated (Sham) or ovariectomized (OVX). All animals got a semisynthetic diet with 160 g/kg diet protein from egg white and 7 g/kg calcium and 5 g/kg phosphorous for 16 weeks. Groups 3 and 4 (GMP-W and GMP-H) were supplemented with 10 g/kg of two different types of GMP. Calcium absorption and retention were assessed in repeated metabolic balances. Bone specimen, organs and tissue were taken at the end of the experiment. Calcium content in bones was analysed by AAS after ashing.

Results: OVX significantly reduced uterus weight, and ash and calcium content in femora, tibiae and lumbar vertebrae indicating that the animal model was appropriate. OVX increased body weight and tended to increase visceral fat mass after 16 weeks. Neither GMP-W nor GMP-H did significantly affect visceral fat mass, liver weight, or mineral content of bone.

The specific two GMPs tested here in this concentration did not beneficially affect bone mineral content (ash or calcium). Both GMPs did not affect energy metabolism in this regimen of pair-feeding.

Poster 10

Influence of fibre enriched biscuits on the postprandial glucose and insulin response

Jasmin Wonik, Jan Philipp Schuchardt, Katharina Möller, Inga Schneider, Andreas Hahn
Institute of Food Science and Human Nutrition, Leibniz University, Hannover, Germany

Introduction: fine bakery products, especially biscuits, are common snacks in Germany and often induce high blood glucose levels. The aim of this project was to develop fibre enriched biscuits with a low Glycemic Index (GI) and insulin response.

Subjects and Methods: to investigate the biscuits' GI and area under the curve for the postprandial insulin concentration ($iAUC_{Insulin}$), we conducted two short-term crossover studies. 24 and 26 healthy subjects, respectively, were served a portion of 50 g available carbohydrates of either biscuits or white bread (WB) as the reference food. In study 1 the test biscuits (Cranberry and Nut) mainly contained oat flakes and wholegrain flour in addition to resistant starch (RS). In study 2 only the Nut variant, enriched with Inulin instead of RS, was tested against WB in repeated measures. The total fibre content in the biscuits was app. 6 %. Venous blood samples were collected after 0, 15, 30, 45, 60, 90 and 120 min.

Results: the GI of the Cranberry and Nut biscuits in study 1 were 57.4 and 56.3 respectively. In study 2 the Nut biscuit induced a GI of 58.9. No significant differences in the $iAUC_{Insulin}$ were observed.

Discussion and Conclusion: these findings demonstrate that the fibre enriched biscuits can be classified as low-GI foods. Compared to GI of other biscuits, the GI of the Cranberry and Nut biscuits was considerably lower. The high fibre content and the various fibre sources may be responsible for the GI-lowering effect. Conclusively, this study showed that consumption of biscuits as snacks may not necessarily induce a high glycemic load.

Poster 11

A preliminary study of the viability of Atlantic salmon (*Salmo salar*) exudates analysis by nuclear magnetic resonance spectroscopy to evaluate fresh fish quality

Ana-Belén García-García[1], Rosa Escudero[1], Carlos Santos[1], M. Isabel Cambero[1], Antonio Herrera[2], David Castejón[2]

[1] Departamento de Nutrición, Bromatología y Tecnología de Alimentos, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain
[2] CAI-RMN, Universidad Complutense de Madrid, Madrid, Spain

Water is the main component of fish and can escape from muscle as purge or drip according to physical and biochemical factors after conversion of muscle to flesh. Drip loss can be exacerbated by cutting, heating, grinding, pressing or freeze-thawing. The loss of exudates from muscle is unavoidable due to some loss of moisture occurs because of the presence of water in a free form in muscle tissue. It is an easy to obtain substrate which manipulation does not mean loss or alteration of the source sample. The aim of the present study is to evaluate the feasibility of NMR techniques to analyse salmon exudates as a suitable matrix to obtain fresh fish composition and to monitor changes of most relevant components during storage. Portions of approximately 150 g of *S. salar,* coming from two different fish markets, were vacuum packaged and stored at 4ºC. Exudates were collected at different storage times (days 1, 7 and 13) and lyophilized for analysis by NMR. ^{1}H-NMR spectroscopy was carried out at 500.13 MHz using a Bruker AMX500 spectrometer 11.7 T. 18 mg of sample were placed into a 5 mm NMR tube, together with 295 µl of D_2O, 295 µl of phosphate buffer and 65 µl of a solution of TSP 10 mM in D_2O. Spectra revealed a large number of compounds (fatty acids, amino acids, carbohydrates, nucleotides). During storage, degradation of sugars and nucleotides or presence of TMA, as spoilage indicator, is shown. Profiling was similar to that obtained in previous studies from salmon muscle [1], so exudates could be used as an alternative analytical matrix. In conclusion, exudates analysis by NMR could be a non-invasive, easy and fast method for monitoring fish freshness.

[1] Castejón et al (2010). Magnetic Resonance in Chemistry, 48, 693-703.

Acknowledgements: UCM-BSCH GROUP 920276; AGL2010-19158 and CTQ2010-14936 PROJECTS.

Kiel Food Science
Symposium 2014
Kiel ◆ Germany ◆ May 20-21 ◆ 2014

MRI
Max Rubner-Institut
Federal Research Institute
of Nutrition and Food

Poster 12

Use of ^{1}HRMAS-NMR for monitoring "Manchego cheese type" ripening

Ana-Belén García-García[1], Karen Cruz-Díaz[1], Rosa Escudero[1], David Castejón[2], M. Dolores Romero de Ávila[1], M. Isabel Cambero[1]

[1] Departamento de Nutrición, Bromatología y Tecnología de Alimentos, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain
[2] CAI-RMN, Universidad Complutense de Madrid, Madrid, Spain

Manchego cheese type is a highly valuable Spanish product whose consumption has been increased worldwide on a progressive way. Made from ewe´s milk, it is a high fat content product that undergoes an enzymatic coagulation and a compression process of the curd. It has a firm and compact consistency, buttery texture and distinctive aroma and taste. Different flavours are developed depending on the ageing time so it is possible to acquire different versions ready for consumption. The aim of this study is to confirm the potential and optimize ^{1}H-HRMAS-NMR methodology for monitoring manchego cheese ripening, manufactured according to traditional procedure. ^{1}H-HRMAS-NMR analyses [1,2] were performed with a Bruker AMX 500 11.7T spinning rate spectrometer (resonance frequency 500,13MHz) equipped with HRMAS probe. 8-10 mg of cheese (without previous manipulation) were placed in a 50 µl zirconium oxide rotor with 20 µl of D_2O and TSP at 0.1 mM at 4ºC. The rotational speed was optimized to 6000 Hz. Samples were analyzed from early manufacturing times (2, 9 and 30 days) to the usual times of marketing and consumption (90 and 180 days). Results reported in this paper correspond to the first experiments achieved in order to study the whole ripening process of Manchego cheese type. Significant differences were detected in free amino acids and carbohydrates regions related to characteristic proteolysis and glycolysis processes. No changes in CLA content were detected. Integration of different signals of the spectra was performed, in order to know the intensity of the detected compounds and to set ratios to obtain useful equations to monitor the ripening process. Best settings were achieved with aromatic amino acid signals (x): time ripening= 1.27 ln(x) + 1.11(R^2=0.95)

[1] Castejón et al (2010). Magnetic Resonance in Chemistry, 48, 693-703.
[2] Laetitia Shintu et al (2004). Magnetic Resonance in Chemistry, 42, 396–401

Acknowledgements: UCM-BSCH GROUP 920276; AGL2010-19158 PROJECT.

Poster 13

Coffee – What else?
Characterization and authenticity assessment of coffee by high resolution LC-MS

Aiko Barsch, Verena Tellström, Klaus Meyer, Sven Brand, Michael Becker
Bruker Daltonik GmbH, Bremen, Germany

Globally, coffee represents one of the most important beverages. Analytically, it constitutes a very complex mixture of small molecules which differ in composition and quantities based on the different coffee cultivars, cultivation regions, and processing procedures.

Metabolomics studies have gained major importance in food analysis. We analyzed 13 different types of coffee capsule extracts, assigned by their manufacturer to different intensity categories, using a benchtop QTOF-MS instrument. The first task was to correlate high resolution LC-MS data to the coffee manufacturer's description via a non-targeted metabolomics approach.

Metabolic profiles of the samples could be statistically differentiated by PCA and PLS in accordance to their assigned coffee strength. Compounds responsible for sample grouping and differentiation were tentatively identified based on accurate mass and isotopic pattern information in MS and MS/MS spectra. Subsequent in-silico fragmentation generated single candidate structures. This tentative identification saved future analysis time and the cost of purchasing multiple reference materials to confirm the identity of the target compounds.

Some coffee types can be highly prized which increases the importance of quality control and authenticity assessment. The statistical models were used to classify coffee samples from the same vendor extracted on a different coffee machine. In a blind experiment, seven out of eight coffee samples were assigned to the correct coffee type based on the PCA model. A corresponding PLS model predicted the correct coffee intensity.

In summary, this proof of concept untargeted metabolomics experiment enabled differentiation of coffee types based on their assigned flavour intensity and to readily identify target compounds responsible for the differentiation. The established model was successfully applied to classify coffee samples in a blind experiment.

Poster 14

First results of the differentiation of conventionally and organically produced goat cheese by stable isotope and omega-3 fatty acid analysis

Brigitte Fahrenhorst-Reißner, Franz X. Martin
Chemisches und Veterinäruntersuchungsamt Münsterland-Emscher-Lippe, Münster, Germany

After comprehensive investigations of organically produced cow cheese the question was whether cheese of conventionally and organically produced goat milk can also be distinguished by the same parameters as cow milk and whether clear differentiation limits can be detected.

Until now 24 samples of conventionally produced goat cheese and 7 samples of organic goat cheese were tested. Two samples of organic cheese and two samples of conventionally manufactured cheese were artisan cheese from 4 German farms. The other samples were produced in 14 different cheese dairies of Germany, France and The Netherlands.

The analyzed samples of conventionally produced goat cheese contained amounts of α-linolenic acid (C18:3ω3) up to 0.599 g/100g fat. The stable isotope ratios of carbon ($^{13}C/^{12}C$), the $\delta^{13}C$ values, ranged between -24.8 and -27.7 ‰ m/m and were substantially lower than the values known for conventionally produced cow milk and cow cheese. As it could be expected, these results indicate that goats are fed with lower amounts of maize than cows. By examining the samples of organic goat cheese the amounts of α-linolenic acid ranged from 0.723 to 0.985 g/100g fat and $\delta^{13}C$ values were ascertained from -28.8 to -31.9 ‰ m/m.

On the basis of our current investigations conventionally produced goat cheese showed a slightly elevated maximum amount of α-linolenic acid, while the lowest limit of $\delta^{13}C$ values was substantially lower compared to cheese made of cow milk. According to these first results the parameters α-linolenic acid and the stable isotope ratio of carbon enable to distinguish conventionally and organically produced goat cheese. The number of samples is however still too small to create definite limitations. Further investigations will follow.

Kiel Food Science
Symposium 2014
Kiel ◆ Germany ◆ May 20-21 ◆ 2014

MRI
Max Rubner-Institut
Federal Research Institute
of Nutrition and Food

Poster 15

Organic fish products – authentication by stable isotope analysis

Joachim Molkentin[1], Ines Lehmann[2], Ute Ostermeyer[2]

[1] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel, Germany
[2] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Hamburg, Germany

Dwindling natural resources increase the importance of aquaculture in the production of edible fish. The potential risk for conventional aquaculture products being wrongly labelled as organic must be encountered by an improved traceability of edible fish, particularly at the retail level. In a BÖLN funded project (German Federal Programme on Organic Farming and Sustainable Agriculture, project no. 08OE026) the applicability of C and N stable isotope ratio analysis to discriminating between organically and conventionally farmed fish as well as wild-caught fish of selected carnivorous species was evaluated.

Samples of salmon, brown trout and pangasius originating from different farms or sea areas were collected over a period of 18 months, partly comprising processed products. After extraction of lipids from the fillet, defatted dry matter (DDM) and lipids (LIP) were subjected separately to stable carbon isotope ($\delta^{13}C$) analysis. In addition, stable nitrogen isotopes ($\delta^{15}N$) were analysed in DDM. Analyses were performed using a Thermo Scientific EA-IRMS system (Flash EA 1112, ConFlo III, DELTAplus XL).

Salmon, brown trout and pangasius from organic aquaculture showed higher $\delta^{15}N$ than the respective conventionally farmed fish, which allowed to identify the different husbandry largely. Whereas differentiation by $\delta^{15}N$ was complete for salmon and pangasius, the authentication of organic brown trout could be accomplished by combining $\delta^{15}N$ and $\delta^{13}C$ of DDM. Wild salmon could be distinguished from conventional by $\delta^{15}N$ and from organic by $\delta^{13}C_{LIP}$. Hence, the combination of $\delta^{15}N$ and $\delta^{13}C_{LIP}$ allowed the simultaneous identification and differentiation of all three origins, irrespective of processing. Samples of wild brown trout or pangasius were not included in this work.

The food chain level of animal prey and the percentage of plant versus animal material in the feed are important factors determining the isotopic signature of fish, thus allowing the authentication of organic fish from selected species.

Poster 16

Organic fishery products – authentication by carotenoid analysis

Ute Ostermeyer[1], Ines Lehmann[1], Joachim Molkentin[2]

[1] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Hamburg, Germany

[2] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel, Germany

Due to the increasing demand and the higher price for organically produced fishery products, there is a potential risk of false declaration of conventional products as organic goods. The applicability of carotenoid analysis to the confirmation of correct labelling of shrimp and salmonid products even on the retail level was investigated.

Samples of salmon, brown trout, and shrimps, primarily *P. monodon* and *L. vannamei,* from several organic and conventional farms and from free-living stocks had been repeatedly bought on the German market. In all samples the content of astaxanthin and canthaxanthin as well as the ratio of the configurational isomers of free astaxanthin were analysed by HPLC.

In conventionally reared salmons the *meso*-form was always predominant, which indicated the use of synthetic astaxanthin in their feed. The conventionally farmed salmon could be clearly distinguished from wild salmon. In all products of wild salmon the SS-isomer outweighed the RR-isomer, but no *meso*-form of astaxanthin could be detected. On the other hand, the tissue of organic salmon showed a very inconsistent distribution of the isomers. When the feed was supplemented with the yeast *Phaffia rhodozyma* or the bacterium *Paracoccus carotinifaciens*, organic salmon differed significantly from conventionally farmed as well as from free-living salmons. But in the case of feeding a shrimp shell supplement, it was not possible to distinguish organically from conventionally farmed salmon. Moreover, in some organic samples distributions of isomers occurred, which could also be interpreted as wild salmon.

The ratio of configurational isomers of free astaxanthin in shrimp flesh was not suitable for their differentiation, because shrimps have obviously the ability to change the structure of carotenoids taken up from their feeding stuffs.

The work was funded by the German Federal Ministry of Food, Agriculture and Consumer Protection (BÖLN project No. 08OE026 “BioFiDi”).

Poster 17

Organic fishery products – authentication by fatty acid analysis

Ines Lehmann[1], Joachim Molkentin[2], Ute Ostermeyer[1]

[1] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Hamburg, Germany

[2] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel, Germany

Aquaculture is important for the production of edible fish because of limited natural resources. Especially organically farmed fish and shrimps gain in importance even on the German market. These products are more expensive than conventional products and have to be controlled for correct labelling. Established analytical methods for fatty acids were evaluated for the authentication of fishery products.

Samples of brown trout, pangasius, gilthead sea bream, processed salmon and shrimps of different origin obtained from several retail stores and aquaculture plants were examined. In order to differentiate between three kinds of production, organic and conventional as well as wild, the fatty acid composition was determined after extraction of lipids from the fish fillet or shrimp tissue.

21 different fatty acids in the range of 14:0 to 22:6n-3 were analysed. For processed salmon a differentiation between the conventionally and organically farmed as well as wild caught individuals was feasible using linoleic acid. Besides other fatty acids the omega-3 fatty acids DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) were always found in lower contents in the conventional than in the organic or wild samples. For brown trout and pangasius combinations of several fatty acids allowed the distinction between conventional and organic farming. As distinct from wild gilthead sea bream the farmed animals cannot be differentiated between the two culture forms. The fatty acid composition of shrimps, with the exception of the species *Litopennaeus vannamei,* did not allow an extensive authentication of organic products.

Because of the potentially high variation in feed composition, it is not practicable to establish fixed limits for the examined parameters. But the suitability particularly of fatty acids analysis for determining the kind of production was basically demonstrated.

The work was funded by the German Federal Ministry of Food, Agriculture and Consumer Protection (BÖLN project No. 08OE026 “BioFiDi”).

Poster 18

Applying population genetics to identify the catch area of marine fish

Behrmann, Konstanze[1], Hartmut Rehbein[1], Markus Fischer[2]

[1] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Hamburg, Germany

[2] Hamburg University, Germany

Marine fishes divide up into different populations, which are separated by geographic areas and/or live cycles. Mating occurs more preferably between individuals of one population. Over many generations genetic drift leads to differentiation. The stronger the barriers between populations, and the longer the populations are isolated from each other, the more genetically distant the populations are.

On the German market the origin of the majority of fishery products is labelled more detailed than required by EU legislation (Commission Regulation (EC) No 2065/2001 and Council Regulation (EC) No 1005/2008). Subareas refer to populations or management areas as reported in ICES-reviews. Consumer-guides of NGOs like WWF and Greenpeace enjoy great popularity.

PCR-based DNA analytical methods of population genetics might be the best way to fight against fraud within the supply chains of marine and fresh-water fishes, as well as deception of consumers. DNA is still available in highly processed products, even when damaged due to low pH-values, severe heat treatment or enzymatic processes, but analytical methods are on the level of scientific investigation and not suitable for routine analysis. Furthermore, population genetic research is mostly concentrated on a few species and many species are underexplored [1,2].

Before population genetic can be applied for authentication purposes, biological data have to be collected and cleared regarding distribution and migration routes.

Population genetics is always based on statistical analysis, because variations of molecular markers (mitochondrial or nuclear DNA) between populations of one species may be small. Large sample sizes and a sufficient number of markers are needed for reliable analyses. The required number of markers and individuals depends on the kind of genetic markers chosen and the genetic distance between populations.

An overview will be given of the methods, requirements, chances and limitations of population genetic for authentication purposes of marine fishes.

[1] Martinsohn, J.T. (2011), European Commission – Joint Research Centre, Report EUR 24394 EN
[2] Reiss H. et al. (2009), Fish and Fisheries, 10(4), 361-395

Poster 19

LABELFISH – "The Atlantic network on genetic control of fish and seafood labelling and traceability"

Kristina Kappel[1], Belgees Boufana[2], Domingo Calvo Dopico[3], Andrew Griffiths[2], Marc Jérôme[4], Julie Maguire[5], Stefano Mariani[2], Rogério Mendes[6], Jorge Perez-Bouzada[7], Ricardo Pérez-Martín[8], Ute Schröder[1], Marc Shorten[5], Helena Silva[6], Amaya Velasco[8], Véronique Verrez-Bagnis[4], Carmen G. Sotelo[8]

[1] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Hamburg, Germany
[2] University of Salford, UK
[3] University of La Coruña, Spain
[4] Ifremer, France
[5] Indigo Rock Marine Research Station, Ireland
[6] Portuguese Institute for the Sea and Atmosphere, Portugal
[7] Xenotechs, Spain
[8] Instituto de Investigaciones Marinas CSIC, Spain

Globalization has led to a rapid increase in the variety of fish species found on European markets, which makes it difficult for control bodies to verify the labelling of fishery products. Nevertheless, species authentication is indispensable to protect consumers against fraudulent substitution and regional companies against distortion of competition.
Substantial efforts have been undertaken during the last years to develop DNA-based systems for the identification of fish species in fish food. However, there are no standardized methods harmonized across the European states.

LABELFISH is a project funded by the Atlantic Area Programme and includes participants from six countries in Europe (France, Germany, Ireland, Portugal, Spain and UK), which are characterized by an intense economic and social relationship with marine resources. LABELFISH aims to analyse the current situation of fish food labelling and traceability in six European countries, to propose and harmonize European standard genetic methods for fish species authentication and to create a permanent network for authentication of fish and seafood products.

Results concerning the mislabelling rate of cod and tuna samples purchased in markets of the different European countries will be presented. Moreover it will be shown that sole (*Solea solea*) is often found to be substituted by less valuable fish species in German restaurants. In this context, different aspects of fishery product labelling and surveillance will be discussed.

Poster 20

Anisakid nematode infestation in beaked redfish (*Sebastes mentella*) from three areas of the North Atlantic

Regina Klapper[1,2], Thomas Kuhn[2], Horst Karl[1], Sven Klimpel[2]

[1] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Hamburg, Germany

[2] Institute for Ecology, Evolution and Diversity, Goethe-University; Biodiversity and Climate Research Centre; Senckenberg Gesellschaft für Naturforschung, Frankfurt/Main, Germany

In North Atlantic waters, redfish (*Sebastes* spp.) comprise two highly commercially utilized species. One species is the beaked redfish *Sebastes mentella* that is found mainly in the Northwest Atlantic, Irminger, Norwegian, and Barents Sea, as well as in the Northeast Atlantic.

Naturally occurring nematodes have a complex life-cycle in which they parasitize invertebrate and vertebrate hosts. Humans can be accidental hosts by eating raw or undercooked fish that contain nematodes, mainly of the genus *Anisakis*. Consumption of these larvae can cause zoonotic infections with clinical symptoms. Therefore, a study was conducted in order to assess and compare the infestation of nematode parasites in the internal organs and flesh of redfish *S. mentella* from three areas of the North Atlantic.

A total of 300 *S. mentella* were sampled in the three areas Tampen (Northern North Sea), Bear Island (Northeast Atlantic), and East Greenland (Irminger Sea). Intestines and fillets were analysed using the UV-Press method (Karl and Leinemann, 1993). Identification to the genus level was carried out morphologically. For identification to the species level, the rDNA region comprising the ITS-1, 5.8S, ITS-2 and flanking sequences (ITS+) of various nematodes was sequenced (Zhu et al. 2000, Kuhn et al. 2011).

Differences between the three areas could be detected by a lower infestation of nematodes in redfish from Greenland. Prevalences of fish were 94% in fish from Tampen, 92% in Bear Island, and 75% in Greenland. Also the intensity of infection was significantly lower in samples from Greenland. Of those nematodes found in the flesh, 92-97% were localized in the belly flaps.

The infestation level of anisakid nematodes in the flesh of beaked redfish was relatively low (mean abundance= 3.76), and may even be lowered by removal of the belly flaps. The study will further give indications on the final host distribution.

Poster 21

Dioxins and PCBs in eggs and egg products: a statistical evaluation of data received by an analytical service provider

Olaf Paepke, Nina Lohmann, Frank Neugebauer, Felix Focke
Eurofins GfA Lab Service, Hamburg, Germany

Findings of elevated levels of polychlorinated dibenzo-p-dioxins and –furans (PCDDs/PCDFs) and polychlorinated biphenyls (PCBs) in eggs above the EU-maximum levels for food raised attention in the public in 2012. For this reason a statistical evaluation of data received within the routine analysis of eggs and egg products on behalf of companies from the food sector was performed in order to gain an overview of levels of dioxins and PCBs in these types of food products.

Analyses of 2,3,7,8-substituted congeners of PCDDs/PCDFs, dioxin-like PCBs (DL-PCBs) and non-dioxin-like-PCBs (NDL-PCBs) were performed on samples of eggs and egg products like whole egg, egg yolk or albumin from various, mainly European sources in 2008 to 2012 according to the analytical method of Eurofins GfA Lab Service [1]. More than 3000 different data sets were statistically evaluated. Results thereof including quantiles and rates of results above EU-action levels [2] and EU-maximum levels [3] will be presented taking into account levels of dioxins and PCBs, origin of products, time of production and – where possible – also information on QA/QC-systems of the pertaining food producing company. A brief overview on results is given in the following table:

Parameter	Action level [2]	Maximum level [3]	Number of data sets	Rate below action level	Rate below maximum value
Sum of dioxins WHO-PCDD/F-TEQ(2005)) *	1,75	2,5	3431	96,9%	98,7%
Sum of DL-PCBs (WHO-PCB-TEQ(2005)) *	1,75	N/A	2961	98,3%	N/A
Sum of dioxins and DL-PCBs (WHO-PCDD/F-PCB-TEQ(2005)) *	N/A	5,5	2935	N/A	99,0%
Sum of NDL-PCBs (ICES – 6) **	N/A	40	782	N/A	98,4%

* in pg/g fat ** in ng/g fat N/A = not available

References:

[1] Neugebauer F, Paepke O, Opel M, Arkudas R. (2011); *Organohalogen Compounds* 73: 1223
[2] Commission Recommendation 2013/711/EU
[3] Commission Regulation (EC) No 1881/2006, amended by Commission Regulation (EU) No 1259/2011

Poster 22

Automatic identification of unknown and unexpected chemical residues and contaminants in food samples using accurate mass LC-MS/MS screening techniques

Jianru Stahl-Zeng[1], Lauryn Bailey[2], André Schreiber[3], David Cox[3], Jeff Rivera[3]

[1] AB SCIEX, Darmstadt, Germany
[2] AB SCIEX, Framingham, MA, USA
[3] AB SCIEX, Concord, ON, Canada

Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) is a powerful analytical tool for the analysis of polar, semi-volatile, and thermally labile compounds of a wide molecular weight range, such as pesticides, veterinary drugs, mycotoxins and other food residues. Mass analyzers based on triple quadrupole technology operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results and are therefore well established for multi-target screening and quantitation of food contaminants.

However, the use of triple quadrupole based mass analyzers is limited to targeted screening and quantitation. But there is an increasing demand for retrospective and non-targeted data analysis. High resolution and accurate mass instruments are capable of performing targeted and non-targeted screening in a single LC-MS/MS run.

Here, a generic QuEChERS procedure was used to extract residues and contaminants from fruit and vegetable samples. Extracts were subsequently analyzed by LC-MS/MS using an AB SCIEX TripleTOF® system operated in high resolution accurate mass MS and MS/MS mode.

Full scan MS and MS/MS data was explored to identify known-unknowns using non-targeted data processing tools. Sample-control-comparison was successfully used to find unexpected contaminants. Identification was based on MS and MS/MS information, including formula finding, ChemSpider searching, and automatic MS/MS fragment ion interpretation. This challenging data processing workflow was automated and allows easy result review and reporting in the latest revision of TripleTOF® software.

Poster 23

The use of Microflow UHPLC in veterinary drug residue analysis

Harald Möller-Santner[1], Stephen Lock[2], Jianru Stahl-Zeng[1]

[1] AB SCIEX, Darmstadt, Germany
[2] AB SCIEX, Warrington, UK

Traditionally in veterinary drug residue screening of food samples, samples are extracted and analysed by LC/MS/MS usually at LC flow rates which are in excess of 500 µl/min and in combination with high pressures with smaller particle size HPLC columns to maintain sharp peaks and fast chromatography. These flow rates produce fast speeds and excellent peak shapes and results, but have a draw back in that they require higher volumes of organic solvent. The consumption of HPLC organic solvents, such as acetonitrile and methanol, is a growing cost of analysis and its disposal has an environmental impact. Therefore, ways to reduce solvent consumption in pesticide residue testing will be beneficial to the environment and reduce running costs of a testing lab.

Here we present new data using microflow LC, running below 40 µL/min, in combination with a LC-MS/MS method developed on an AB SCIEX QTRAP® system utilizing the *Scheduled* MRM™ algorithm. Initially, this approach has been applied to a screen of veterinary residues including sulphonamides to show its applicability to food analysis and data presented compare Micro LC with traditional LC flow rates.

Poster 24

Vitamin B complex detection in infant formula by LC/MS/MS

Harald Möller-Santner[1], Stephen Lock[2], Jianru Stahl-Zeng[1]
[1] AB SCIEX, Darmstadt, Germany
[2] AB SCIEX, Warrington, UK

Vitamin B complex is a group of water-soluble vitamins that play important roles in cell metabolism. Absence of individual vitamins in a diet can lead to several conditions including depression and high blood pressure, so they are often added to foods especially infant formula. Vitamin B is a complex mixture of different compounds each structurally different. Traditionally, individual methods have been used to screen for each vitamin B so one method that is capable to screen for several vitamin B compounds in a single analysis would be beneficial.

Here we present some new data acquired by LC/MS/MS with a screening method which contains all the major forms of Vitamin B. The required detection limits vary greatly between each vitamin B and range from low parts per billion to low parts per million levels.

The method has therefore been developed to detect all the vitamins in the required ranges and has meant that some transitions had to be detuned to maintain their linear response and enable one simple extraction for all vitamins. LC/MS/MS uses reverse phase chromatography and positive mode electrospray ionisation and meet the requirements of all the limits of detection. The mass spectrometry methods utilises *Scheduled* MRM™ and a small particle size HPLC column. NIST reference material was extracted and then simply diluted and analysed by LC/MS/MS to show the applicability of this method to routine sample analysis.

Kiel Food Science
Symposium 2014
Kiel ◆ Germany ◆ May 20-21 ◆ 2014

MRI
Max Rubner-Institut
Federal Research Institute
of Nutrition and Food

Poster 25

Membrane separation technology to eliminate bacteriophages in whey

Meike Samtlebe[1], Natalia Wagner[2], Erik Brinks[2], Horst Neve[2], Knut J. Heller[2], Jörg Hinrichs[1], Zeynep Atamer[1]

[1] Institute of Food Science and Biotechnology, Department of Dairy Science and Technology, University of Hohenheim, Stuttgart, Germany
[2] Department of Microbiology and Biotechnology, Max Rubner-Institut, Kiel, Germany

Cheese whey that incurs during production of cheese can be transformed into diverse native whey protein supplements and directly used to optimize processes and to develop new products in the dairy industry, which has economical and environmental advantages. However, cheese whey may contain bacteriophages in numbers which limit its applications. Phages are still responsible for most fermentation failures in the dairy industry. In previous studies, heat inactivation of phages has been studied in detail, and it was shown that most phages survive pasteurization [1]. In principle, a reliable thermal inactivation of heat resistant phages is possible in whey, but such a harsh heat treatment leads to a significant denaturation of whey proteins. Therefore, the aim of this work was to establish a non-thermal technology for phage reduction in whey, in order to guarantee a high percentage of native whey proteins.

A cross-flow membrane filtration process was designed and recently set up to separate whey proteins from whey-derived phages while keeping the proteins in their native form. The performance of the filtration process was characterized in terms of phage retention, total protein permeation as well as permeation of the major whey proteins. At first, the effect of pore size of the membrane on the removing efficiency of phages was investigated. Experiments conducted with in dairies widely distributed *Lactococcus lactis* phages P008 have confirmed that a reduction of 4.4-log units is possible using a 100 kDa membrane. The value for the protein permeation amounted to 32%. Furthermore, the effects of bacteriophage morphology and the formation of a surface layer on the retention of phages were analyzed in detail. First results of this cooperative project will be presented and discussed.

[1] Atamer, Neve, Heller & Hinrichs (2012): thermal resistance of bacteriophages in the dairy industry. *In*: Bacteriophages in dairy processing (Quiberoni & Reinheimer, eds), pp. 195-214, Nova Publishers, Hauppauge, NY, USA.

Kiel Food Science
Symposium 2014
Kiel ◆ Germany ◆ May 20-21 ◆ 2014

MRI
Max Rubner-Institut
Federal Research Institute
of Nutrition and Food

Poster 26

Assessment of the thermal stability of *Lactococcus lactis* bacteriophages in raw milk tested in a pilot plant pasteurizer

Natalia Wagner[1 (*)], Philipp Hammer[2 (*)], Horst Neve[1], Knut J. Heller[1]

[1] Department of Microbiology and Biotechnology, Max Rubner-Institut, Kiel, Germany
[2] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel, Germany
[(*)] Authors of equal contribution

Bacteriophages frequently cause fermentation problems in dairies and phage infection of lactic acid starter bacteria may result in serious delays of fermentation batches. It has previously been shown that *Lactococcus lactis* phages may exhibit a remarkably high thermal stability under laboratory conditions (i.e., in 1.5-ml stainless-steel test tubes in a water bath), and the most resistant phages were still detectable in skim milk after heating for 5 min at 95°C and 97°C, respectively [1,2].

The aim of this study was to investigate the thermal inactivation of lactococcal phages suspended in high titers in raw milk (10^7 phages/ml) using a pilot plant pasteurizer (sample volume: 30 l, continuous flow principle) described earlier [3]. Phages of two phage species with highest thermal stability (small isometric-headed phages P680 and P1532 of the 936 phage species [1], prolate-headed phage P635 of the c2 species [4]) were included, furthermore the heat-sensitive reference phage P008. The titer of the later phage decreased after heating for 25 sec at 75°C by 5 log units, whereas phages P635, P680, P1532 required temperatures of 80°C (6-log units reduction), of 95°C (5-log units reduction) and of 97.5°C (4-log units reduction), for significant inactivation (≥ 99,99% after 25 sec heat treatments). Thus, thermal inactivation of phages in the pilot plant pasteurizer is notably more efficient than treatments under laboratory conditions used for screening of phages with high thermal resistance. However, even under pilot plant conditions, high temperature & short time pasteurization (HTST) is not a hurdle for temperature-insensitive lactococcal phages. D- and z-values for thermal inactivation will be presented.

[1] Atamer, Dietrich, Müller-Merbach, Neve, Heller (2009) *Int. Dairy J.* **19**:228-235 (2009).
[2] Atamer, Neve, Heller, Hinrichs (2012) *In*: Bacteriophages in dairy processing (Quiberoni & Reinheimer, eds), pp. 195-214, Nova Publishers, Hauppauge, NY, USA.
[3] Peng, Hummerjohann, Stephan, Hammer (2012) *J. Dairy Sci.* **96**:3543-3546.
[4] Marvig, Aideh, Neve, Heller, Knøchel, Vogensen (2011) *Int. Dairy J.* **21**:556-560.

Poster 27

Regulatory elements in the genetic switch region of the genome of the temperate *Streptococcus thermophilus* bacteriophage TP-J34 from a yoghurt starter strain

Sabrina Koberg[1], Mazhar D. A. Mohamed[2], Katharina Faulhaber[3], Horst Neve[1], Knut J. Heller[1]

[1] Department of Microbiology and Biotechnology, Max Rubner-Institut, Kiel, Germany
[2] Agricultural Microbiology Department, Faculty of Agriculture, Sohag University, Sohag, Egypt
[3] Department of Microbiology and Infection Medicine, Tübingen University, Tübingen, Germany

Within the lysogeny module of the genome of the temperate *Streptococcus thermophilus* phage TP-J34, all but four adjacent open reading frames (orfs) are transcribed in one direction [1]. They are separated from the potential lytic cycle-promoting genes *cro* and *ant* by a genetic switch region, which contains the divergently oriented promoters P_1 and P_2 and several predicted operator sites. The orfs putatively code for the repressor Crh, a metalloproteinase Orf3, a superinfection exclusion (sie) mediating lipoprotein Ltp [2], and the integrase. Genes *crh*, *orf3* and *ltp* are transcribed as one polycistronic mRNA starting from promoter P_2. RT-PCR and Northern blot experiments suggested own promoters for *ltp* and *int* (confirmed with 5`RACE PCR obtaining the 5`end of transcripts). The repressor gene *crh* (essential for the establishment of lysogenization by suppressing lytic genes) was overproduced by heterologous expression in *E. coli* to perform electrophoretic mobility shift assays. Three operator sites in the intergenic regions between *crh* and *cro* (O_{1A}, O_2, O_3) and one between *cro* and *ant* (O_{1B}), respectively, were confirmed by competition assays with synthetic oligonucleotides. Glutaraldehyde was used as cross-linking reagent for Crh oligomerization (i.e., formation of dimers, tetramers and higher complexes). Knock-out experiments with *orf3* gene revealed a key role in induction of the lytic cycle. Studies on the interaction between Crh and Orf3 indicated that Orf3 prevents binding of the Crh repressor to its operator sites. Cro, the putative repressor of lysogenic genes, only bound to operator O_3 (probably resulting in repression of lysogenic promoter P_2). This would explain its antagonistic role.

[1] Sun, Goehler, Heller, Neve (2006) *Virology* **350**, 146-57.
[2] Ali, Koberg, Heßner, Sun, Rabe, Back, Neve, Heller (2014) *Frontiers in Microbiology* **5** (no. 98), 1-23.

Poster 28

Inactivation of pathogenic bacteria in cherimoya pulp by high hydrostatic pressure treatments

Antonio Galvez, Julia Toledo, Rosario Lucas, Maria J. Grande, Rubén Pérez-Pulido
Department of Health Sciences, University of Jaen, Jaen, Spain

Cherimoya (*Annona cherimola*) is a tropical fruit with antioxidant and cytoprotective properties due to its content in phenolic compounds. These compounds may help to prevent diseases associated with oxidative stress, such as cancer, atherosclerosis and neurodegenerative diseases. The local production of cherimoya and its seasonal availability are limitations to a widespread consumption of this fruit. Cherimoya fruit pulp preparations with an extended shelf life could find new markets as functional foods. High hydrostatic pressure (HHP) is a non-thermal food processing technology that offers ideal performance for preservation of bio-active molecules in foods while at the same time may serve to inactivate pathogenic and spoilage bacteria, thus improving the food safety and product shelf life. In the present study, pulp obtained from cherimoya was challenged with three cocktails of pathogenic bacteria including *Escherichia coli, Salmonella enterica* and *Listeria monocytogenes* strains. Fruit pulps were treated at 600 MPa and stored under refrigeration for 30 days. The antimicrobial peptide enterocin AS-48 was added to samples challenged with listeriae as an additional protective barrier. The tested enterobacteria survived in the refrigerated cherimoya pulp for up to 30 days without significant loss of viability, while listerial counts decreased by 2 log cycles. Enterocin addition decreased the viability of listeriae by additional 1.5 to 2 log cycles during storage. Application of HHP treatments reduced viable cell counts in cherimoya pulp by ≥5 log cycles, and the surviving fraction decreased below detectable levels in the treated fruit pulp during further incubation, possibly due to sublethal cell damage. Enterocin AS-48 improved inactivation of sublethally injured listeriae during storage. These results suggest that HHP treatments (singly or in combination with enterocin AS-48) could improve the safety of non-thermally processed cherimoya fruit pulp during refrigeration storage.

This work was supported by project AGL2012-38708 (Spanish MINECO).

Kiel Food Science
Symposium 2014
Kiel ◆ Germany ◆ May 20-21 ◆ 2014

MRI
Max Rubner-Institut
Federal Research Institute
of Nutrition and Food

Poster 29

Modulation of fructose fermentation in fecal slurries obtained from obese subjects by *Anaerostipes caccae*, different *Lactobacilli* species, dietary electron acceptors and antibiotics

Fouad M. F. Elshaghabee[1], Wilhelm Bockelmann[1], Diana Meske[1], Hans-Georg Walte[2], Michael de Vrese[1], Jürgen Schrezenmeir[3], Knut J Heller[1]

[1] Department of Microbiology and Biotechnology, Max Rubner-Institut, Kiel, Germany
[2] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel, Germany
[3] Johannes Gutenberg-University, Mainz, Germany

Increased consumption of high fructose diets correlates well with increased obesity and non-alcoholic fatty liver disease (NAFLD). Ethanol produced by the intestinal microbiota has been discussed to be involved in development of NAFLD in animals and humans. In order to take a closer look at this problem, a simple fermentation model was established for evaluating the modulation of fructose fermentation of fecal slurries obtained from four obese healthy subjects by addition of *Anaerostipes caccae*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus fermentum*, *Weissella confusa*, citrate, pyruvate, vancomycin or neomycin. The results obtained generally reflected the anticipated metabolic activities. Mannitol, lactate, acetate and ethanol were the major metabolites of fructose fermentation by fecal slurries. Occurrence of mannitol in fermentations of fructose in slurries could be considered as an indicator for presence of heterofermentative lactobacilli. Butyrate was the major metabolite when *A. caccae* was inoculated with fecal slurries. Addition of *W. confusa* (mannitol-negative) resulted in increased ethanol amounts and alcohol dehydrogenase activity. No ethanol could be detected when fermentation media were supplemented with *L. acidophilus*, *L. bulgaricus*, *L. fermentum* (mannitol-positive), citrate, pyruvate, vancomycin or neomycin. Our results represent the first *in vitro* trial for modulation of metabolites of obese gut microorganisms by using two heterofermentative lactobacilli species differing in their abilities to produce mannitol from fructose fermentation, the latter resulting in reduced production of ethanol. We now plan to examine the effects of dietary pyruvate, citrate, *L. fermentum* and butyrate-producing bacteria like *A. caccae* in animal model. By this we want to see, whether combinations of dietary electron acceptors, *A. caccae* or *L. fermentum* will change the overall metabolic profiles in general and production of ethanol in particular, and thereby effect development of NAFLD.

Key words: *Weissella confusa*, *Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Anaerostipes caccae*, fructose fermentation, ethanol, pyruvate, vancomycin, neomycin, intestinal microbiota, metabonomics

Max Rubner-Institut
Federal Research Institute of Nutrition and Food

Address	Hermann-Weigmann-Straße 1, 24103 Kiel, Germany
Phone	+49 (0)431 609 2250
Fax	+49 (0)431 609 2300
E-Mail	kielfoodsci@mri.bund.de
Internet	www.mri.bund.de