



The effect of protein and lipid source in organic feed for (organic) rainbow trout on sensory quality

Hyldig, Grethe; Green-Petersen, Ditte; Jacobsen, Charlotte; Baron, Caroline Pascale; Jokumsen, Alfred; Lund, Ivar; Nielsen, Henrik Hauch

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WEFTA 2011

Seafood for the Modern Consumer
Abstracts



41st WEFTA Meeting, 27–30 September 2011, Gothenburg, Sweden



CHALMERS

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27-30 September 2011
Seafood for the Modern Consumer

Edited by

Lillie Cavonius

Linnea Eriksson

Ingrid Undeland

Marie-Louise Wennerhag

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WELCOME ADDRESS

It is a great honour and pleasure to welcome you all to the 41st WEFTA meeting in Gothenburg, Sweden. In 1980 and in 1993, the WEFTA-family was also brought here, but this is the first time that Chalmers University of Technology has the honour to be hosts. We are certainly very proud of this.

The theme of the 41st WEFTA meeting - "*Seafood for the modern consumer*" - implies a special focus on meeting modern seafood consumer demands for sustainability, appealing presentation and packaging, convenience, all natural ingredients, high sensorial quality, healthiness, safety and trust. Indeed these multiple demands constitute a huge challenge for seafood scientists, authorities and industry. The contents of this year's WEFTA program reflect though, that both the research community and industry are well equipped to meet this challenge. During the meeting we will for example learn more about the possibilities with algae, farmed mollusks and seafood rest raw materials as sustainable food and feed sources. Life cycle assessment also comes in as an important tool for evaluating environmental performance in the product supply chain. The latest seafood consumer trends will further be revealed, as well as several novel seafood product concepts. We will get a taste of what the mega-trend "nano" can offer the seafood sector, and information about a whole range of imaging techniques suitable for seafood. In addition, a novel fishy "app" will be demonstrated. With focus on retaining quality, an impressive series of plant- and fish-based extracts will be highlighted, but also the important impact of water and salt. Allergy development, diabetes and *in vitro* antioxidant effects in response to seafood-derived compounds are examples of what will be discussed in the nutrition and safety session. We will also be introduced to systems biology as an approach to study the effects of seafood on metabolic activities in different tissues. A glimpse of HACCP 2011 will finally be given, as well as insights into how safe your sushi is. In short, we foresee a mix of topics well suited for the conscious consumer of 2011.

This year we aimed at only having one plenary session, something which turned out to be a main challenge when so many interesting papers were received. Opening up for posters became a necessity to fit everything in. We are very grateful for the advice from the scientific committee on division of the presentations. In the final program, 37 long presentations, 37 short presentations and 37 posters are scheduled. In addition to this, we are proud to have five very interesting keynote speakers coming both from industry and academia.

I would like to express my sincere and personal thanks to the rest of the WEFTA 2011 organization team, the editors of this book of abstracts, all of the authors, participants, chair persons and WEFTA national representatives. I shall also use this opportunity to thank our sponsors for their support to this conference.

On behalf of the whole organizing team, I hereby wish you a very fruitful WEFTA meeting, enjoyable social activities and a pleasant stay in a hopefully sunny Gothenburg.

Ingrid Undeland

Chair WEFTA Meeting 2011



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A glimpse of Gothenburg and the meeting venue



Quality Hotel 11



Quality Hotel 11



Gothenburg City hall



The Gothenburg Wheel



The Poseidon statue and Avenyn



Gothenburg harbour with the "lip stick" building and the ship Barken Viking



The Älvsborg Bridge



Eriksberg and the old shipyard



The Opera house



The Poseidon statue

The harbour area



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Past and present challenges of fish technologists – where will new tools and methods lead us in the future?

Torger Borresen

Technical University of Denmark, National Food Institute, Denmark
torgr@food.dtu.dk



Over time the challenges of fish technologists have changed with the general technological development, and specifically the technological development within the fish processing industry. Most of the core WEFTA institutes were founded when the canning technology was introduced for long term storage of fish. This soon changed upon the introduction of chilling and freezing. The production of frozen fish fingers have been said to be the most significant landmark within the fishing industry. Methods of preservation have been further refined up to our days with the introduction of superchilling, modified atmosphere packaging, etc. allowing new product varieties to enter the market. But with the new research tools developed over the same time, it has been possible to go back and make new studies of ‘old’ processing methods, like salting, drying, marinating, etc. for understanding biochemical and microbiological principles, and their consequences for quality and safety of the processed products. This has led to development of new convenience products, ready to cook meals etc. with a much more consistent quality than previously.

However, some of the ‘old’ problems persist. In a recent report from Taylor & Francis,

publishing the Journal of Aquatic Food Product Technology it was surprising to see that among the papers downloaded the most, thus being the most ‘popular’ articles, were subjects like DMA, TMA and TMAO in by-products, *Listeria* in cooked and peeled shrimps, edible films based on gelatine and chitosan, and new minced products based on surimi! A quick analysis of the articles showed that due to new analytical techniques and new processing methods these ‘old’ problems could now bring new results that deserved publishing.

So with even better tools and methods where will that lead us in the future? Without doubt the developments within information and communication technologies, where operational components are being smaller and able to perform better, it will be easier to monitor processing operations and detect toxins and other components much faster. Further, nanotechnology brings a future where we have only seen the mere beginning. This will be exemplified, together with other new tools available for the fish technologists in the future.

Session 1:
Towards sustainable sources of seafood
- aquaculture and rest raw materials

Session 2:
Product development and product communication
within the seafood sector

Session 3:
Innovative process and detection technologies for
seafood

Session 4:
Retaining high quality through the seafood processing
chain

Session 5:
Nutritional and safety aspects of seafood consumption

Keynote: Microalgae – a novel resource in food and feed production

Rene H. Wijffels

Wageningen University, Wageningen, The Netherlands

*rene.wijffels@wur.nl, twitter @ReneWijffels



Microalgal biomass contains compounds that are of interest for feed and food ingredients. Compounds of interest include proteins, starch, carotenoids, edible oil and ω -3 fatty acids. An advantage over land crops is that microalgae have a high productivity and can be grown on the basis of seawater.

Presently there is a lot of attention for microalgae for production of biodiesel and less for the use for food and feed production. However, it remains unlikely that the process will be developed for biodiesel as the only end product. In order to develop a more sustainable and economically feasible process all biomass components (e.g. proteins, lipids, carbohydrates) should be used and therefore biorefinery of microalgae is very important for the selective separation and use of the functional biomass components. If biorefinery of microalgae is applied, lipids should be fractionated into lipids for biodiesel, lipids as a feedstock for the

chemical industry and ω -3 fatty acids, proteins and carbohydrates for food, feed and bulk chemicals and the oxygen produced should as well be recovered. If in addition production of algae is done on residual nutrient feedstocks and CO₂, production of microalgae at large scale against low production costs the biorefinery approach for micro algae will be feasible.

The opportunities and bottlenecks of microalgal production and refinery will be discussed. We believe the bottlenecks need to be resolved and a multidisciplinary approach in which systems biology, metabolic modelling, strain development, photobioreactor design and operation, scale-up, biorefinery, integrated production chain and the whole system design (including logistics) should be addressed. Microalgae will be produced for food and fuel. New business models will have to be developed in which fuel and food industry should collaborate.

Inclusion of the microalga *Isochrysis* aff. *galbana* in organic diets of European sea bass juveniles: effects on growth, feed utilisation and fillet composition

***Bianca Maria Poli*^{1,*}, *S. Venturini*², *F. Tulli*³, *G. Chini Zittelli*², *E. Tibaldi*³, *G. Giorgi*¹, *L. Rodolfi*², and *M.R. Tredici*²**

¹Florence University, Dept. Agriculture Biotechnology, Firenze, Italy

²CNR, Istituto per lo Studio degli Ecosistemi, Sesto Fiorentino (FI), Italy

³Udine University, Dept. Animal Science, Udine, Italy

*biancamaria.poli@unifi.it

The interest towards several marine microalgal species as sustainable dietary sources alternative to fish meal /oil and able in maintaining fish performances and health benefits of their flesh consumption, has good grounds. In fact microalgae can represent a potential alternative ingredient to supplement the diet of high-value fish species or in organic-based aquaculture systems, due to their high content of good-quality protein, bio-active compounds, antioxidants, vitamins, minerals and long chain n-3 and n-6 PUFAs (ARA, EPA, DHA). Oil extracted from microalgae has earlier been proven to successfully replace marine fish oil in diets for sea bream. Microalgae produced in controlled conditions using innovative photobioreactor technologies can supply products of constant and suitable quality to be included in aquafeed at relatively moderate prices. The marine microalga *Isochrysis* aff. *galbana* (T-ISO), showed high protein quantity and quality, lipids rich in LC-PUFA, DHA particularly, and a good vitamin's pattern, therefore could have the potential to become a sustainable alternative dietary source and contribute to meet the increasing demand for fish meal/oil that is a critical limiting factor for the future expansion of aquaculture activities.

A study was carried out to evaluate growth response, feed utilization and quality traits of the edible portion of sea bass (*Dicentrarchus labrax*) fed organic diets including dried *Isochrysis* aff. *galbana* (T-ISO). Three test isoproteic (50% DM) and isolipidic (18% DM) diets were compared. All preparations were formulated using "organic" ingredients. *Isochrysis* aff. *galbana* was incorporated to replace 10% (T-ISO10) and 20% (T-ISO20) protein supplied as dried fish trimmings which were the major protein source in the control diet (ORG). All diets were offered to apparent satiety to 9 groups of 21 European sea bass juveniles (100 g Initial Body Weight) under controlled environmental conditions (temperature 20°C; salinity 28 psu; light-dark cycle 12L:12D) according to a completely random design. Feeding the test diets over 70 days resulted in similar fish growth and feed utilisation. Apparent digestibility coefficients (ADCs) were measured in vivo using acid insoluble ash as indigestible marker. The effects of the dietary algae inclusion on the fillet composition were also considered.

The effect of protein and lipid source in organic feed for (organic) rainbow trout on sensory quality

Grethe Hyldig^{1,*}, D. Green-Petersen¹, C. Jacobsen¹, C. Baron¹, A. Jokumsen², I. Lund², and H. Hauch Nielsen¹

¹DTU Food – National Food Institute, Kgs. Lyngby, Denmark

²DTU Aqua – National Institute of Aquatic Resources, Kgs. Lyngby, Denmark

*grhy@food.dtu.dk

The aim of this work was to study which effects protein and lipid source in feed for organic rainbow trout (*Oncorhynchus mykiss*) may have on the sensory quality of the final product after up to 14 days of storage in ice. The protein sources used in the experiment were fishmeal and a mixture of vegetable protein. While the lipid sources were fish, linseed, sunflower, rapeseed and grape seed oil. After slaughtering all fish were frozen (-40°C) until the sensory experiment was performed, for which the trout were thawed and stored for 3, 5, 7 and 14 days in ice respectively. The sensory experiment included objective sensory profiling, of samples which were heat treated in a convention oven at 100°C until the core temperature was 70°C. The sensory panel consisted of 11 assessors which all were tested and trained. The sensory analysis included descriptors related to the odour, appearance, flavour and texture.

After 3 days of storage in ice an impact of lipid source is seen. Inclusion of linseed oil resulted in a sensory profile comparable to the use of fish

oil in the feed. While some of the other vegetable oils, especially grape seed oil results in a sensory profile rather different from the trout that had fish oil. However, this difference observed after 3 days of storage did not appear after a longer storage time, and consequently no differences in the sensory characteristics is observed after the 5 days of storage in ice. Nevertheless after 7 days in ice some differences are appearing again. Here the trout which have had rapeseed and grape seed oil in the feed has a more neutral flavour and odour compared to the other ones. After 14 days of storage the protein source had an effect, and the trout which received fishmeal in the feed were more tainted. Therefore, it is seen that the shelf-life is increased by feeding the fish with vegetable protein compared to fish meal. The conclusion of the experiment therefore was that both dietary vegetable protein and lipid sources can influence on sensory characteristics of trout stored in ice.

The Norway lobster (*Nephrops norvegicus*) condition and quality as foodstuff depending on diet

Susanne P. Eriksson^{1,*}, K. Nordström, H. Styf¹, L. Svanberg¹, K. Ringdahl², J. Lövgren², and B. Hernroth

¹Department of Marine Ecology-Kristineberg, University of Gothenburg, Fiskebäckskil, Sweden

²Institute of Marine Research, Swedish Board of Fisheries, Lysekil, Sweden

*susanne.eriksson@marecol.gu.se

A major source of subsidies to marine systems origin from fishing, as unwanted catch is routinely thrown back to the sea, so-called discard. The majority of discarded animals are dead or moribund when re-entering the sea floor and may thus be utilized by scavengers such as the commercially important benthic Norway lobster, *Nephrops norvegicus*. We've studied how a unilateral diet of commonly discarded animals from different Phyla affects the Norway lobster's general condition and quality as foodstuff. Lobsters were kept individually in the lab for three months during winter, and fed biweekly on a unilateral diet made from different animal Phyla: mussels (*Mollusca*), shrimp (*Arthropoda*), lean fish (*Chordata*), fat fish (*Chordata*), brittlestars (*Echinodermata*) and polychaete worms (*Annelida*), or a mixed diet

made from them all. Food intake in the lab differed between the different diet groups and was 6 times higher for the lobsters given worms compared to those presented with fat fish. To compare the quality of the lobsters as foodstuff, the amount of dry weight abdominal muscle tissue was analysed, as well as the amount of hepatopancreas (lobster paste) and its lipid content and fatty acid composition. The general condition and the quality of the lobsters as foodstuff were not related to the quantity of food eaten, but rather to the quality. The "fat fish" group had for instance double the concentration of total omega-3 in the hepatopancreas, 107 mg/g compared to the "worm" group of 54 mg/g, as well as a 33% larger hepatopancreas.

Effects of feed, feeding regime and growth rate on flesh quality, collagen cross-links and plasma hormones in farmed Atlantic salmon

Chris André Johnsen^{1,*}, Ø. Hagen¹, M. Adler², E. Jönsson³, P. Kling³, R. Bickerdike², C. Solberg¹, B. T. Björnsson³, and E. Åsgard Bendiksen²

¹Faculty of Biosciences and Aquaculture, University of Nordland, Norway

²BioMar, Trondheim, Norway

³Department of Zoophysiology, University of Gothenburg, Gothenburg, Sweden

*chris.andre.johnsen@uin.no

Maintaining control of the harvest quality of the edible product is becoming increasingly important for successful fish farming operations. The present experiment was designed to investigate interrelations, and possible conflicts, between high rearing intensity and maintaining desired quality of the end product, and to explore the mechanism underpinning quantitative and qualitative aspects of Atlantic salmon farming. Two size-matched (~2.4 kg) salmon groups with high or low fillet fat content were created by feeding isonitrogenous feeds with high or low energy content from ~1 kg. From ~2.4 kg until harvest (~4.0 kg) both the HE and LE groups were fed to satiation (Sat; 68 days) or restrictive (Res; 103 d), using the same feed as during the pre-conditioning period. Satiation feeding induced higher energy intake, whereas increased digestible feed energy lowered the feed intake without compromising growth. Although feed energy level or feeding regime did not cause poor flesh quality, restrictive feeding gave a firmer texture due to increased cross-link concentration ($r^2 = 0.28$). Liquid loss (~1.7% vs. ~3.2%) was also improved by restrictive feeding.

LE diet combined with restrictive feeding gave the lowest fillet fat content (~12.7% vs. HE-Sat; 14.5%), but satiated fed salmon had higher intensity of red colour than those fed restricted ration. The incidence of gaping was low across feed treatments and not influenced by the applied feed or feeding regime. Individual growth rates (i.e. TGC₃) correlated positively to fat deposition ($r^2 = 0.38$), but less evident to texture ($r^2 = 0.16$). A multiple linear regression analysis revealed PYD as the only factor influencing fillet firmness significantly, while TGC₃, pre-rigor muscle pH, fillet fat, alkaline soluble and insoluble HYP, and plasma leptin and ghrelin had no significant effect on fillet firmness. The increase in plasma leptin levels following long-term LE diet suggest that leptin is linked to energy balance in salmon, but may not act as an adiposity signal. From the present experiment it is concluded that both feed energy and feeding regime can be used to manage important quality attributes of Atlantic salmon, and that cross-linking of collagen fibres is an important factor to maintain desired fillet firmness.

Effect of genetic background and sex on Atlantic salmon muscle collagen properties and the relationship with muscle texture

Helena M. Moreno^{1,*}, ***M. C. Gómez-Guillén***¹, ***M. Pilar Montero***¹, ***C. Jacq***², ***T. Mørkøre***², and ***A. J. Borderías***¹

¹Institute of Food Science, Technology and Nutrition (CSIC), Products Department, Madrid. Spain

²Nofima, Ås, Norway

*hmoreno@ictan.csic.es

Salmon belonging to different families reared together on Norwegian farms throughout their whole life in the same conditions were classified into different groups depending on their sex (M: Male; Fe: female), genetic background (WP: fish hatched using eggs and sperm from wild parents; FP: fish hatched using eggs and sperm from farm parents) and muscle texture (L: low; H: high). The aim was to determine salmon muscle collagen properties and how they relate to different muscle mechanical properties. To carry out the study, the following different analyses were performed: mechanical properties, gaping degree, collagen solubilisation pattern, collagen amino acid composition, differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR). Results indicated that muscle texture properties were not linked to sex or the salmon's genetic background. Gaping appeared more frequently in WP salmon than in FP and especially in salmon with low muscle texture. The collagen solubilisation pattern is quite similar in all salmon and is not correlated to muscle texture, genetic background or sex. Amino acids, mainly involved in collagen triple helix conformation and stabilization (Pro, Hyp,

Gly, Lys and Hlys), are more abundant in FP salmon than in WP suggesting a better collagen structure conformation. The connective tissue denaturation enthalpy temperature is significantly higher in male salmon both in WP and FP but is not connected to salmon muscle mechanical properties. By FTIR it was observed that WP salmon, especially males, showed a higher ratio of collagen triple helix preservation compared with their female counterparts indicating that WP male salmon reveal a better triple helix preservation and lower random coil presence. In contrast, this ratio was quite similar in farmed salmon with the exception of FFeH which corresponded to a collagen with a lower triple helix presence.

To summarize, it could be stated that similar collagen pattern distribution, amino acid composition and collagen structure stability do not correlate to salmon muscle mechanical properties. Neither do sex and genetic background seem to be involved in salmon muscle mechanical properties but sex is related to changes in collagen molecular properties. Gaping defect is related to salmon muscle with less texture and genetic background.

Quality of portion-size meagre (*Argyrosomus regius*) cultured in earth ponds

A. Gonçalves¹, M. de Sá¹, É. Fabiane Furlan¹, T. Gama Pereira¹, C. Cardoso¹, N. Bandarra¹, P. Pousão-Ferreira², L. Ribeiro², and Maria Leonor Nunes^{1,*}

¹Upgrading of Fishery and Aquaculture Products Research Unit, National Institute of Biological Resources – INRB, I.P./L-IPIMAR, Lisboa, Portugal

²Aquaculture Research Station, National Institute of Biological Resources – INRB, I.P./L-IPIMAR, Olhão, Portugal

*mlnunes@ipimar.pt

Meagre (*Argyrosomus regius*) is an emergent species in Mediterranean aquaculture, having a production around 4784 tons in 2008. Southern France, Italy, Spain and Portugal are the most important markets for this species. Few studies have been published about the quality of farmed meagre and a higher knowledge in this field is needed to improve the quality features of this species. At present producers are trying to differentiate between smaller fish (600 g to 1 kg), which are sold whole fresh and larger fish (1 to 3–5 kg) mostly presented sliced or filleted and smoked. Thus, the purpose of the present work was to evaluate the quality of portion-size meagre (600 – 1000 g) cultured in earth ponds at INRB I.P./L-IPIMAR Aquaculture Research Station, Olhão (Portugal). The fish was slaughtered in ice:water slurry. Several fish were used to: i) evaluate the sensory acceptance; ii) characterize the sensory properties, comple-

mented by the instrumental texture and colour determination of the fillets; iii) developed the Quality Index Method and define the shelf life in ice; iv) evaluate the nutritional value of fish flesh. The results evidences that fresh and cooked fish was very well accepted by the large majority of the sensory panel, composed by twenty people. Regarding particular sensory attributes, 60 % of the panel like moderately to very much the flavour of cooked meagre whereas the firmness was the less appreciated attribute. Mean values for hardness and chewiness were 4.8 ± 1.3 and 0.8 ± 0.2 N, respectively (n = 28 raw fillets). The chemical results evidence a low fat content (in the range 1 - 2 %) for the meagre flesh. Results of fatty acids composition and freshness evolution are still in progress

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The role of feed (ingredients) in the development of flavours in oysters (*Crassostrea gigas*) cultured in land based systems

***Jasper van Houcke*^{1,*}, *J.B. Luten*², *A.C. Smaal*³, and *J.P.H. Linssen*²**

¹HZ University of Applied Sciences, Delta Academy, Vlissingen, The Netherlands

²Wageningen University, Department of Product Design and Quality Wageningen, The Netherlands

³Wageningen University, Department of Aquaculture and Fisheries, Wageningen, The Netherlands

*j.van.houcke@hz.nl

The Pacific oyster (*Crassostrea gigas*) and the native European flat oyster (*Ostrea edulis*) are the two species cultured in the Netherlands (Eastern Scheldt, Lake Grevelingen and Wadden Sea). There is a distinct difference in flavour (taste and smell) between both species according to experts, although scientific data are lacking. There is nowadays a growing interest in land-based culturing of shellfish in the Netherlands. This implies that the knowledge on the role of feed and ingredients (type (algae, artificial feed), composition) in the cultivation of the oysters become very important and in particular on the development of flavour of the final harvested product.

French studies have shown that the odour of Pacific oysters in France originates mainly from the oxidation of polyunsaturated fatty acids (PUFAs). The biochemical composition and flavours of oysters can significantly differ depending on the algae species and their composition.

Numerous studies have shown the important taste compounds in seafood, such as, free amino acids, 5'-nucleotides and bromophenols, but the

data on these compounds in fresh raw oysters are limited.

The overall objective of this project is to investigate the role of feed (ingredients) in the development of flavours in Pacific cupped oysters during land-based production. The sub-objectives are:

- To determine the flavour profiles by sensory and instrumental methods of Pacific cup oyster and the flat oyster from various culturing sites in the Netherlands (Lake Grevelingen, the Eastern Scheldt and the Waddensea)
- To identify the relation between the flavour profile (components) and the sensory characteristics of both oyster species
- To study the role of algae and artificial feed on the sensory and flavour profiles of Pacific cupped oyster in land based culturing situation
- To develop a feeding strategy to deliver tailor made land based cultured Pacific oysters with appealing flavour for the consumers.

Estonian farmed rainbow trout competitiveness in red fish market

***Loreida Timberg**, K. Koppel, and R. Kuldjärv**

TFTAK, Competence Centre of Food and Fermentation Technologies, Estonia

*loreida@tftak.eu

Salmon and rainbow trout, both red flesh fish species, are very popular among Estonian fish consumers. Annually approximately 15.000 tons of red fish is consumed in Estonia. Only about 700 tons of red fish comes from Estonian aquaculture, and most of it is imported from abroad, e.g. Norway, Finland and Denmark. Estonian aquaculture sector is growing fast to meet the demand of red fish, but quality of different farms rainbow trout is fluctuating.

The objective of this study was to compare Salmon and rainbow trout aquacultured in different countries using different technologies (seawater, freshwater, flow-through, and recirculation). Descriptive sensory profiling, chemical and physical analysis, and a central location consumer acceptance test were carried out. Four fish samples – Norwegian seawater aquaculture salmon, Finnish seawater rainbow trout, Estonian freshwater rainbow trout (flow-through technology) and Estonian freshwater rainbow trout (recirculation technology) were studied.

According to proximate composition analysis Norwegian salmon and Estonian flow-through rainbow trout had similar water and lipid contents and Finnish rainbow trout and Estonian recirculation rainbow trout also had similar values.

The two types of Estonian trout carried musty/earthy flavor and aroma; Finnish trout and Estonian recirculation trout were more intense in flesh color. 130 consumers clustered into three sections: the first cluster (29% of consumers) loved all four fish samples. The second and largest cluster (45% of consumers) preferred Finnish rainbow trout, Norwegian salmon and Estonian flow-through rainbow trout. The third cluster (26% of consumers) liked only seawater aqua cultured fish: Norwegian salmon and Finnish rainbow trout. According to these results Estonian freshwater aquaculture technologies should be modified in order to produce rainbow trout with more acceptable flavor properties.

High quality fish oil and proteins from herring rest raw material: influence by process conditions

Ana Karina Carvajal^{1,2,*}, R. Slizyte¹, R. Mozuraityte¹, I. Storror¹, and M. Aursand^{1,2}

¹SINTEF Fisheries and Aquaculture, Trondheim, Norway

²The Norwegian University of Science and Technology, Trondheim, Norway

*anakarina.carvajal@sintef.no

The herring industry in Norway generated in 2010 more than 350 000 tons of rest raw materials. The major part of this rest raw materials are used for production of silage intended for animal and fish feed - products which are considered as low priced and low quality products. To obtain high quality oil and proteins, that are suitable for human consumption, the quality of the raw material is important. During storage or transport endogenous enzymes can lead to degradation of proteins and lipids in the rest raw material. In order to avoid quality loss by enzymatic and oxidation processes it is important to process rest raw materials shortly after slaughtering of the fish.

The objective of this work has been to produce high quality oil and proteins from ultra fresh herring rest raw material. In order to evaluate how extraction method and extraction temperature affects the quality of the oil and proteins, two different processes were tested: i) thermal treatment, and ii) enzymatic hydrolysis with commercial proteases. A mobile pilot plant (SINTEF Mobile SeaLab) designed to produce fish oil and protein fraction was used for production of herring oil and protein fractions. The mobile plant was positioned close to the factory of Grøntvedt Pelagic (Uthaug, Norway), enabling the use of ultra fresh rest, less than one hour old, rest raw material straight from the filleting line.

The total lipid content, lipid distribution, and fatty acid composition of the oil, stick water, and sediment fraction has been determined. Oxidative quality and stability of the produced herring oils was evaluated. The produced herring oil from fresh rest raw material had a low oxidation status and high oxidative stability, and was of better quality compared to conventional oil or oil from silage production.

Enzymatic hydrolysis can be used as a processing method for recovering protein and oil from valuable fish biomass which is not directly used for human consumption. Fish protein hydrolysates (FPH) have documented functional, bioactive and antioxidative properties. However the hydrolysis process often creates bitter taste in the product and this often prevents successful application of FPH in the food industry. The possible sources of bitter taste may be the composition of starting material, but processing method and conditions are also a key factor for changes of sensory properties. It was observed that hydrolysis time as well as process temperature has significant influence on sensory properties of FPH. Both bitter taste and colour of final FPH powder were affected by process conditions. FPH also show antioxidative properties in model system and antioxidative properties also depended on process conditions during production of herring protein fraction.

Life Cycle Assessment (LCA) focused on carbon footprint applied to seafood products - a basis for consumer choices. Case study comparing MSC certified Alaska pollock caught by pelagic trawls with cod caught by long-lines

Veronica Sund

SIK, The Swedish Institute for Food and Biotechnology, Gothenburg, Sweden
vs@sik.se

Life Cycle Assessment is a method for evaluating environmental performance in the supply chain of products. The ISO-standardized method is frequently used by the industry as a basis for environmental improvements in their production and to communicate to consumers. Various environmental impacts may be studied in an LCA, carbon footprint, i.e. greenhouse gas emissions, is one example. In this study the carbon footprint of the supply chain of two fish products are studied. A brief introduction to LCA methodology will be given at the beginning of the presentation.

There is an ongoing debate whether it is sustainable to eat fish caught on the other side of the globe – that thus require long-distance transport. Carbon footprint studies of fish products of different origin representing alternative dinner choices for people can demonstrate the magnitude of long-distance boat transports of seafood products in relation to other life cycle activities. The status of the fished stock is indirectly reflected in a carbon footprint study, since energy efficiency decreases in overfished stocks.

In the present study climate burden of cod caught in a Norwegian *long-line fishery* in the

Barents Sea was compared with that of Alaska pollock landed in the US MSC-certified (Marine Stewardship Council) *pelagic trawl fishery* in Bering Sea, using LCA-methodology. The fisheries were also evaluated regarding stock situation, by-catch and seafloor impact. Fillets from these fisheries are processed to breaded pre-fried products in Kungshamn, Sweden and sold in Sweden. This study focused on the fishery, transport and processing of two seafood products.

The cod product had three times the climate impact of the Alaska pollock product, and a large part of the burden was assigned to the refrigerant leakage in cod fishery, which constituted almost a third of the burden exercised by the fishery. Transports contribute with 22 % to the studied life cycle emissions of the Alaska pollock product.

Conclusions are that it is more important how than where the fish has been caught and that transport mode is more important than transport distance. Both products are efficient regarding the aspects studied, but can be further improved.

Life Cycle Assessment (LCA) of using underutilized marine raw materials processed by the pH-shift method to replace fish mince in seafood products

Friederike Ziegler¹, V. Sund¹, and I. Undeland²

¹SIK, The Swedish Institute for Food and Biotechnology, Gothenburg, Sweden

²Chalmers University of Technology, Department of Chemical and Biological Engineering - Food Science, Gothenburg, Sweden

*fz@sik.se

Life Cycle Assessment is a methodology standardized by ISO that today is used widely both by industry and research for environmental assessment of products. It follows the product throughout the supply chain and can comprise single or multiple types of environmental impacts such as e.g. the carbon footprint (greenhouse gas emissions), eutrophication, acidification, depending on the goal of the study. Economic aspects such as production costs are not normally part of LCA, but there is a related framework termed called LCC (Life Cycle Costing). When LCA and LCC are done together, a third term is sometimes used; eco-efficiency analysis.

Here we used LCA complemented by LCC to quantify the carbon footprint and production costs of isolating proteins from underutilized marine resources using the pH shift method. Underutilized resources from the sea can be species that today are mainly used for feed production as well as filleting by-products. One application of such protein isolates is to replace “virgin” marine raw material such as fish mince in seafood products of fish finger or fish ball type.

Resource use was quantified along the entire chain from fishing over transportation and processing to the final product protein isolate. The raw materials used for pH-processing were whole herring, (in Sweden today only 50% is used as food) and white fish filleting by-products. One of the possible applications of the protein isolate was then evaluated by comparing a conventional marine raw material used in seafood products (fish mince) with an isolate-based marine raw material.

Preliminary results of these experiments are promising. The main part of environmental impact of seafood products takes place in the fishery and therefore, even though the isolate is quite heavily processed, this does not have to be a disadvantage from a carbon footprint perspective. Differences between using different raw materials and processing technologies will be demonstrated. Practical and methodological challenges related to the application of LCA methodology in this type of development project will be discussed.

Surimi seafood from byproducts containing omega-3 fatty acids

Margrét Geirsdóttir¹, P. Hamaguchi¹, Ó. Þ. Hilmarsson¹, I. Klonowzki¹, and H. G. Kristinsson^{1,2}

¹Matis, Biotechnology & Biomolecular division, Icelandic Food Research, Reykjavík, Iceland

²Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL, USA

*mg@matís.is

The positive effect of omega-3 fatty acids and fish consumption to preclude cardiovascular disease is well acknowledged. At the same time consumers find it difficult to include seafood products into their diet. Surimi is an example of consumer friendly seafood that can be used to import omega-3 fatty acids.

The aim of this project was to develop a novel high value and high quality seafood product – a surimi with added omega-3 fatty acids. Furthermore the aim was to use cod frame mince that is a low value and underutilized by-product as a raw material for the production.

By using the alkaline aided pH-shift method a protein isolate was processed from cod frame mince. Products were made by addition of different amounts of omega-3 oil (0, 1, 2 and 3 %) to the protein isolate at pH 7.2 along with sodium tripolyphosphate, sodium carbonate and sodium chloride and stuffed into casing (2 cm diameter). Products were cooked for 30 minutes in a water bath at 90°C, cooled in ice water and vacuum packed. Analyses were made after 2, 7, 14 and 21 days of cold storage at 2-4°C and

included gel quality (folding and puncture test), color, cook loss, rancidity development (TBARS, Peroxide value and rancidity odor) and aerobic microbial count (23°C).

Gel strength, white color and microbial stability of the products were positively affected with increased addition of omega-3 oil and furthermore gel strength was increased with storage. Higher peroxide values (PV) were measured as omega-3 level increased. The same was not found for TBARS that unlike PV correlated well with results from sensory analysis and did not change significantly until after 3 weeks of storage when high microbial count affected the judges in rancidity evaluation.

The results show that it's possible to make surimi from cod frame mince protein isolate with added omega oil which is white, with good gelling properties and good oxidative stability. This product can open up new opportunities for low value by-products like cod frame mince, and increase choices for consumers to get more omega-3 fatty acids conveniently into their diet.

Farmed sea bass and seabream by-products: A new opportunity for high quality heat-induced gel products

Carlos Cardoso^{1,*}, R. Mendes¹, P. Vaz-Pires², and M.L. Nunes¹

¹National Institute of Biological Resources, Department of Fisheries and Aquaculture Product Upgrading, Lisbon, Portugal

²University of Oporto, Abel Salazar Institute of Biomedical Sciences, Oporto, Portugal

*e-mail: ccardoso@ipimar.pt

Sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) are commonly farmed fish species, whose market for whole fish is showing signs of saturation. This creates a potential opportunity for processed products, such as heat-induced gel products, which can be produced from by-products of filleting, such as trimmings. These gel products may be value-added products, since the sensory attributes can be tailored to suit consumer preferences.

Therefore, the aim of this work was threefold: to explore the potential of by-products of farmed fish species in the production of heat-induced gel foods, to study the effect of species, and to understand the underlying protein phenomena.

Heat-induced (90 °C for 1 hour) gel products were prepared from minced fish by-products of both species. For each species, two batches were prepared: one with 0.5 % (w/w) microbial transglutaminase (MTGase) and other without this enzyme. Different quality parameters were assessed: texture, water holding capacity (WHC), and colour. Moreover, protein solubility in SDS+DTT and urea+DTT, electrophoresis, and scanning electronic microscopy (SEM) were

applied in order to achieve a deeper insight into the differences between the products.

This study showed that the quality of sea bass gel products was higher than that of products prepared from gilthead seabream. Whereas sea bass gels without MTGase presented a gel strength of 17.1 ± 1.4 N.mm, a hardness of 26.1 ± 0.5 N, and a rupture force of 70.3 ± 14.1 N, gels prepared from seabream under the same conditions had a significantly lower gel strength (13.6 ± 2.2 N.mm), hardness (12.3 ± 1.0 N), and rupture force (47.4 ± 7.3 N). WHC was also higher for sea bass gels. Similar differences in hardness and WHC were also observed in those gels containing MTGase. Concerning protein solubility, electrophoresis, and SEM studies, a higher level of protein aggregation was obtained in seabream gels, which may explain the poorer quality of these gels.

This work shows that proteins from sea bass by-products are more adequate for the production of gel foods than those from seabream, thereby yielding a quality level suitable for the preparation of gelled foods.

The Vendace Project – an applied project for sustainable development in the largest lake in Sweden - Vänern

Elén Faxö^{1,*}, C. Eklund-Jonsson¹, I. Undeland², J. Karlsson³, and L. Dominique³

¹Food & Health Concept Centre, Gothenburg, Sweden

²Chalmers University of Technology, Department of Chemical and Biological Engineering, Food Science, Gothenburg, Sweden

³University of Gothenburg, Department of Food and Nutrition and Sport Science, Gothenburg, Sweden

*elen.faxo@fhcc.se

The primary purpose of the project is to make better use of the Vendace (*Coregonus albula*). The fish is today discarded after separation of the caviar, a highly valued delicacy. By implementing appropriate techniques and tools to develop food products based on the fish instead of discarding it, we are working towards a sustainable development of the area of Vänern.

The project also focuses on optimizing the utilization of other fish species today discarded due to lack of production opportunities. The fish not fully used constitutes an environmental hazard by being dumped back into the lake and is considered a cost for the fishermen instead of a possible source of income. The outcomes of the activities planned in the project are expected to be; new regional foods and traditional foods with improved product quality. The project is performed in close cooperation of the fishermen active in lake Vänern and other stakeholders and will hence contribute to a sustainable future with regard to both human and environmental resources.

In order to build the trademark of the products from Vänern, communication of health benefits connected with consumption of the products could be one important tool. Analyses of the content of vitamin D and n-3 polyunsaturated fatty acids (n-3 PUFA) showed that 100g Vendace filets contain in average 12 μ g of vitamin D and 0,6 g of n-3 PUFA. A daily consumption of Vendace (125g) would therefore cover the recommended daily intake (RDI) of Vitamin D and approximately 30% of the n-3 PUFA (Omega 3) RDI.

A qualitative study regarding attitudes towards fish and Vendace products indicated that consumers prefer locally produced fish products that are high in fish meat and n-3 PUFA. Further arguments to strengthen the Vendace products attractiveness would be the connection to the well-known caviar and the projects contribution to sustainable development in lake Vänern.

Potential for line-mussel production in the Great Belt (Denmark)

Daniel Pleissner**, *K. Lundgreen*, *L. Böttiger*, and *H. U. Riisgård

Marine Biological Research Centre, University of Southern Denmark, Kerteminde, Denmark

*danielp@biology.sdu.dk

Bivalve aquaculture is of increasingly economic importance in Europe. In Limfjorden, Denmark, an extensive blue mussel (*Mytilus edulis*) fishery exploits the wild stocks, and during the last 10 years line-mussel farming has been introduced to increase the production. But eutrophication and seasonal oxygen depletion cause high mortality of mussels during late summer, especially in the central parts of Limfjorden. Within the MarBioShell project (2008-2012) we are evaluating the potential of the Great Belt region, between Kattegat and the Baltic Sea as a new cultivation site to cover the increasing demand for blue mussels.

In the present study, as a first step towards the evaluation of the potential for line-mussel production mussels were grown in suspended net-bags in the Great Belt and in Limfjorden, and special emphasis was laid on the importance of phytoplankton biomass (measured as chlorophyll *a* concentration) for the specific growth rate of different size classes of mussels.

In both Limfjorden and Great Belt the mean chlorophyll *a* concentration was around $3 \mu\text{g l}^{-1}$ between July and October 2010. The mean

salinity in the Great Belt was about 14 psu, which is approximately two times lower than the salinity in Limfjorden.

Laboratory feeding experiments have however revealed no influence of salinity on growth rate of *M. edulis* exposed to salinities between 15 and 25 psu. In Limfjorden and Great Belt the average weight specific growth rates of mussels of a size of 20 mm were 3.1 and 3.3 % d^{-1} , whereas 40 mm mussels had a growth rate of 2.2 and 2.0 % d^{-1} , respectively. Concentrations of the polyunsaturated fatty acids docosahexaenoic and eicosapentaenoic acids were 10 and 20 mg g^{-1} in mussels grown in Great Belt (7 Oct.) whereas 6 and 5 mg g^{-1} were measured in mussels grown in Limfjorden (21 Sept.), respectively.

Comparable specific growth rates, but preferably higher concentrations of polyunsaturated fatty acids in mussels grown in the Great Belt compared to Limfjorden indicate a promising potential for line-mussel cultivation in the Great Belt region where strong currents both provide a steady input of phytoplankton and eliminate the risk of oxygen depletion.

Poster 1.1

Mussel meal - A sustainable high protein component in organic feed

Odd Lindahl

The Swedish Royal Academy of Science, Kristineberg, Fiskebäckskil, Sweden
odd@kva.se

The blue mussel has a high content of the essential sulphur-rich amino acids methionine, cysteine and lysine, matching the content in fishmeal and can, to the extent shells are included in the feed, also provide calcium carbonate. Since mussels are at the second step of the marine food-chain, the use of mussels instead of fish for feed production is also of large ecological importance at a time when many fish-stocks are over-exploited on local/regional, and global scales.

Due to the plumage, hens and chicken have a greater need than any other food producing animals of sulphur-rich amino acids in their feed. Most feedstuffs used in poultry feed have a content below the requirements of the birds of some essential amino acids. The non-organic share in which fish meal constitutes a considerable part should, according to the original plan (EEG 2092/91 and 1294/2005) decrease to 0 % in 2012, when organic laying hens and chickens should only feed on organically approved feed-stuff components. It has been difficult to find organically produced feed-stuff containing enough of the essential

amino acids and this suggested regulation was changed in spring 2008 allowing for the continued use of fish meal. However, there is a growing resistance to the use of fish meal in feed. The shortage of good quality protein sources also jeopardizes animal health. At present there is an increasing interest in mussel meal and a huge potential for farming feed mussels or using second grade food mussels for the production of mussel meal.

To farm mussels for meal production is more expensive compared to catching fish and making fish meal. However, to farm feed mussels is much cheaper than high quality seafood mussels. Further, mussel farming can also be used for improving coastal water quality. In Sweden, a trial has been carried out where a mussel farming enterprise was paid for harvesting nitrogen. The extra income from this ecosystem service may bridge the gap in costs of using mussels instead of fish for producing valuable meal, and it can also make mussel meal more competitive on the market.

Poster 1.2

Growth and survival of juvenile freshwater crayfish (*Astacus leptodactylus* esch. 1823) fed artemia enriched with commercial emulsions

***Seval Bahadır Koca**, N. Ozgur Yigit, I. Diler, O. Uzunmehmetoglu, and B. Yazıcıoğlu**

Suleyman Demirel University, Egirdir Fisheries Faculty, Isparta, Turkey

*sevalkoca@sdu.edu.tr

A 30-day experiment was conducted to investigate the effects of enriching the fatty acid composition of Artemia through lipid emulsions containing highly unsaturated fatty acids (HUFA) and to study the impact of HUFA-enriched Artemia on growth and survival of juvenile freshwater crayfish *Astacus leptodactylus*. Juvenile crayfish was fed Artemia enriched with commercial emulsions (Redpeper and OlioW₃) as sources of lipid and an un-enriched control. The highest eicosapentaenoic

acid (EPA) level was found in Artemia enriched with OlioW₃ (3.17±0.00 %) and the highest docosahexaenoic acid (DHA) in Artemia enriched with Redpeper (3.56±0.15). Weight gain, specific growth and survival rates of juvenile crayfish increased with increased amount of EPA and DHA in dietary Artemia. The highest weight gain, specific growth rate and survival was obtained in the juvenile fed Artemia enriched OlioW₃ and Redpeper.

Poster 1.3

European whitefish tailored to benefit the whole value chain

Susanna Airaksinen^{1,*}, J-P. Suomela², M. Sandell³, J. Koskela¹, and M. Kankainen¹

¹Finnish Game and Fisheries Research Institute, Turku, Finland

²University of Turku, Department of Biochemistry and Food Chemistry, Turku, Finland

³Functional Foods Forum, Turku, Finland

*susanna.airaksinen@rktl.fi

This ongoing project aims to develop the quality of European whitefish as a food product in a targeted manner. A whole value chain approach is tested. Fat content and fat composition is modified by aquaculture practices in order to tailor them according to usage and as required by distinct value chain actors. The effects of varying feed fat content and quality are evaluated with respect to the i) productivity ii) processability, iii) product quality, iv) healthiness, v) sensory quality and vi) value chain economics of the fish. The main end products will be evaluated.

Commitment and cooperation in the value chain

enables transcendent development of raw material. Results will be utilized to advice and guide the practices to obtain profitable and high quality production. Knowledge based development, communication and marketing is facilitated. A collaboration network aims to facilitate the development of consumer products of high quality and healthiness.

Tasks include feed design, feed trial, product quality determination and usability, aquaculture practice predictions and economical value chain analysis. Project content and some preliminary results will be presented.

Poster 1.4

Analysis of the bioactivity of fish protein hydrolysates used as feed enrichment for Atlantic cod (*Gadus morhua*) larvae

***Hólfríður Sveinsdóttir*^{1,*}, *H. L. Heimisdóttir*^{1,2}, *J. Jóhannsdóttir*¹, *P. Y. Hamaguchi*¹, *A. B. Bergsson*¹, *H. G. Kristinsson*¹, and *S. Svavarsson*⁴**

¹Matís ohf. - Icelandic Food and Biotech R&D, Reykjavík, Iceland

²University of Akureyri, Akureyri, Iceland

³Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL, USA

⁴Iceprotein ehf., Saudárkrókur, Iceland

*holmfridur.sveinsdottir@matis.is

In several marine fish species, the addition of protein hydrolysates has been reported to have a positive effect on larval performance. Bioactive compounds of protein hydrolysates have been found to be well suited to support larval growth. The provision of a more digestible diet to the newly hatched fish should improve both their growth and survival since amino acids from dietary proteins are absorbed mainly as peptides or amino acids.

The objective of this study was to analyze and deliver bioactive compounds found in pollock (*Pollachius virens*) protein hydrolysates to improve the survival rate and growth of Atlantic cod larvae.

Peptide samples were prepared by enzymatically hydrolyzing minced Pollock fillets with a commercial enzyme mixture: Alcalase and Protamex. Properties of the bioactive compounds were analyzed by Oxygen Radical Absorbance Capacity (ORAC), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity together with cell-based immunomodulating assays. Intracellular antioxi-

dative activity of pollock protein hydrolysates was determined by a novel cellular antioxidant assay using HepG2 cells. A pollock protein hydrolysate was used for enrichment of the live feed offered to cod larvae from the onset of exogenous feeding and the effects of treatment on larval growth and selected innate immune parameters studied.

Results showed that pollock protein hydrolysates possess good antioxidant properties supported by chemical as well as cell based antioxidative assays. The intracellular antioxidative activity increased with increased concentration of the protein hydrolysates. Cell viability was not affected when cells were incubated for 48 h with different concentrations of protein hydrolysates.

This study presents antioxidant properties of protein hydrolysates originating from pollock assessed by chemical as well as cellular based methods. This result is an important step in analyzing bioactive properties of fish protein hydrolysates for the use as feed enrichment in marine fish aquaculture.

Poster 1.5

Antioxidant activity of hydrolysates from discarded crustaceans

***José María Vietes Fernández**, P. Ramos-Ariza, R. Gómez-Mariño, M. García-López, C.G. Sotelo, R.I. Pérez-Martín**

Seafood Biochemistry Group, Instituto de Investigaciones Marinas (IIM-CSIC). Vigo, Spain
*jmvieites@iim.csic.es

Fisheries discards represent a problem because of the evident waste of natural resources and their impact on the ecosystem. The study of alternative ways of use and valorization of both discarded species and the byproducts of target captured species is nowadays an active area of research. In this work, four species of discarded decapod crustaceans caught off the Galician-Northern Portugal coast and/or the Grand Sole were considered for production of protein hydrolysate, namely: the galatheid *Munida spp.* and the portunid crabs *Polybius henslowii*, *Liocarcinus depurator* and *Macropipus tuberculatus*. Furthermore, three of them belong to the group of dominant decapod crustaceans on the Galician coast.

400 g of homogenized crustacean were hydrolyzed for 1-2 hours in a water-thermostated 2 L-reactor with controlled temperature and pH. The reaction was stopped by heating at 90°C for 15 min. The mixture was allowed to cool and then consecutively sieved through 500 and 20

µm meshes. The hydrolysate was finally centrifuged at 16000 x g for 20 min to remove suspended particles. An aliquot of the hydrolysate was used for the determination of protein concentration and antioxidant activity, the remainder of the hydrolysate was freeze-dried and stored until use.

The analysis of the protein concentration in the hydrolysate has allowed us to determine the recovered protein that, depending on the sample, was found from 28% to 41%. Finally, we note that the data of antioxidant activity (ABTS, DPPH) expressed as percentage of inhibition for the different samples show values between 60% and 83%.

The hydrolysates analyzed from crustaceans currently considered as discards from fishing activity, show a high antioxidant activity together with the pigments that give them makes them interesting in the field of animal food, mainly in fish farming.

Poster 1.6

Feed based on vegetable materials changes the muscle proteome of the carnivore rainbow trout

***Flemming Jessen**, T. Wulff, J. Bach Mikkelsen, and H. Hauch Nielsen**

National Food Institute, Technical University of Denmark, Kgs. Lyngby, Denmark

*fjes@food.dtu.dk

Feed production for aquaculture of carnivore fish species relies heavily on protein and lipid from the limited resources of wild fish and other sea living organisms. Thus the development of alternative feeds replacing fish meal and oil with components of vegetable origin is important for a sustainable production of fish from aquaculture. However, such a change in feed will have an effect on the fish composition and metabolism and may also affect eating quality as well as different health and nutritional properties.

A proteomic approach was taken to compare the muscle protein profile of rainbow trout fed two different diets identical in protein and oil content, but with diet C based on fish meal and oil and diet V based on rapeseed oil and vegetable proteins. In addition to the proteomic

investigation the textural properties of the fish were analysed by sensory profiling. Protein expression profiles were achieved by 2-dimensional gel electrophoresis. The result showed that 40 spots were significantly ($p < 0.05$) different between the two groups. By multivariate data analysis (partial least squares regression) data from protein expression and sensory analyses were correlated revealing 46 spots which contributed significantly to texture. Eleven of these spots were also among the 40 spots significantly differing between the groups C and V. Protein spots of interest will be identified using tandem mass spectrometry.

It is concluded that the changed diet influenced protein expression in rainbow trout muscle and this change in protein expression was also reflected in the fish quality (textural properties).

Poster 1.7

Seafood consumption in Portugal - Why do Portuguese eat so much fish?

Cheila Almeida^{1,3,*}, S. Vaz²; and F. Ziegler¹

¹SIK, The Swedish Institute for Food and Biotechnology Gothenburg, Sweden

²CENSE, Centre for Environmental and Sustainability Research, Faculdade de Ciências de Tecnologia, Universidade Nova de Lisboa, Portugal

³Centro de Oceanografia, Faculdade de Ciências, Universidade de Lisboa, Portugal

*cpa@sik.se

Consumers are important actors towards sustainable seafood consumption since seafood consumption has direct consequences on overfishing and damage of marine ecosystems. Portuguese are the largest seafood consumers in the European Union and one of the largest in the world, with more than 50 Kg/per capita/year. We aggregate figures from different data sources in order to build a Portuguese seafood consumption scenario.

The per capita seafood consumption has been very high ever since the 1960ies having a slowdown only in 1970ies, to almost half of it, during a period of political and economic instability. Portuguese seafood consumption is diversified, since the fishery landings have almost 40 different seafood categories; including fish, cephalopods, crustaceans and bivalves. The sardine is the most important species in quantity, but when we look at the economic value, octopus presents the highest importance. Nevertheless the Portuguese seafood commercial balance has a huge deficit since almost 2/3 of the seafood consumption is based on imports.

The main imported product is salt and dried cod. Its consumption declined in the last years and now represents around 10% of seafood consumption. The cod has been in the Portuguese food habits since the XV century but it had had a higher importance during the 1930ies, when it was the main source of animal protein food. The historical facts demonstrate that the high importance of cod in Portuguese food tradition has political, economic and cultural reasons.

We argue that the sustainability of the Portuguese consumption is not finding more fish to food, neither to substitute it with meat (Portuguese meat consumption is also high). Instead it's about gaining more value from the seafood products that already are produced and better knowledge of their impacts along the supply chain. It's important to promote the consumption of less popular species and from further down in the marine food chain. And inform the consumers about the environmental consequences of their choices and provide products assessments to achieve to a seafood footprint.

Poster 1.8

Session 1:
Towards sustainable sources of seafood
– aquaculture and rest raw materials

Session 2:
Product development and product communication
within the seafood sector

Session 3:
Innovative process and detection technologies for
seafood

Session 4:
Retaining high quality through the seafood processing
chain

Session 5:
Nutritional and safety aspects of seafood consumption

Keynote: Consumer and market trends within the seafood sector; alignment of innovation process

Ole Kragh Malle

Royal Greenland A/S, Svenstrup J, Denmark
olma@royalgreenland.com



This presentation gives an insight into Royal Greenland's product development process and product communication in the seafood business. A definition of product development and an approach to product development are described together with the four phases in Royal Greenland's innovation model. Furthermore, an insight into the innovation development flow chart and its steps are presented.

The first step and starting point in Royal Greenland's product development process is an idea generating phase which in many cases is based on consumer trends within the food sector. These trends also have an effect on the technology within the food business besides the products themselves. Both trends have great influence on the idea generating phase. This along with mega food trends are presented combined with some examples.

Today, consumers are far more demanding and unpredictable than they once were. While they want more quality and value for less money, time, and effort, their consumption needs and desires change. This fact challenges the way manufacturers communicate and when it comes to seafood, health and sustainability are some of the hot unique selling points (USP's). In this matter the role of the packaging design and messages are presented along with examples from the Royal Greenland brand communication platform.

In the end the seafood trends which are influenced by the consumer trends are presented along with other challenges within the seafood sector.

The use of cooks, industry experts and consumers in developing new seafood products

Morten Heide* and ***M. Carlehøg***

Nofima, Tromsø, Norway

*morten.heide@nofima.no

Food product development usually focuses on the creation of an individual food product, such as a new type of smoked salmon or fish fingers. Few studies have focused on how new food products interact with other ingredients in a dish or how to make recipes in which the new product works well and is favourably evaluated.

This project aimed to develop recipes for a new peeled shrimp product intended for the Norwegian consumer segment. Cooks hold a special position in product development since they are expert evaluators of both product quality and use. Because of their special capabilities 3 cooks from different culinary backgrounds were hired to develop the recipes. All of the cooks received samples of the new peeled shrimp product and developed in total 9 recipes. Before consumer testing, the number of dishes needed to be reduced. In order to do this

an industry expert panel was used. The cooks who developed the recipes prepared all the dishes and presented them for the industry expert panel. After an elimination process 6 recipes were chosen for further consumer testing. Finally the recipes were tested on consumers using a central location test. In total 35 consumers were recruited and tested the 6 dishes. The dishes were prepared by professional sensory personnel, and presented to the consumers in a canteen setting. The recipes were rated for overall 'liking' on a 7-point scale. The results showed that 4 of the recipes were favourably evaluated by the consumers.

By using cooks, industry experts and consumers, this project developed 4 recipes for a new peeled shrimp product. The recipes are currently presented on the back of the product packaging and in recipe booklets in the marketplace.

Effect of meal composition on culinary quality of fish in a pasteurised MicVac ready-meal

E. Holtz¹, Hanna Rüdel^{2,}, O. Olofsson², A. Broberg¹, G. Hall¹, L. Ahrné¹*

¹SIK – The Swedish Institute for Food and Biotechnology, Göteborg, Sweden

²MicVac AB, Mölndal, Sweden

*hanna.rudel@micvac.se

MicVac is the modern solution for food producers aiming at satisfying today's demanding consumers looking for a fresh and tasty meal. The unique in-pack microwave cooking and pasteurisation method gives the final product a better taste, a higher quality and nutritional values at a longer shelf life - appreciated by brand-owners, retailers and consumers.

The MicVac concept consists of microwave pasteurization of freshly packed food ingredients to enable a shelf-stable ready meal of good nutritional quality and high product safety for up to approx. a month in chilled conditions! Limiting factor to extend the shelf life further is generally the acceptance level of sensory quality. These quality changes arise despite packaging in materials with low oxygen permeability. A meal prepared according to the MicVac concept generally implies that all ingredients (protein part, starch part, vegetables and sauce) are portioned in the tray, which is sealed, prior to pasteurisation. Therefore, interactions such as migration of fat or water between ingredients during processing and storage might take place. To gain true insight into how meal composition may affect the quality of fish in a ready-meal

during storage, this study has focused on assessing the quality changes caused by three different sauces.

Ready-meals containing salmon, pasta, carrots and bell peppers were prepared and pasteurised. The sauces tested were: water, a tomato based sauce, and a cream based sauce. The packages were stored in dark conditions at +8°C up to 8 weeks. During storage samples were taken for Culinary Profiling analysis using a trained sensory panel. The analysis aimed at detecting changes in appearance, smell, taste and texture in each meal component over time. Samples were also taken for measurement of texture, colour, water content and fat content. The results show that the sauce composition has an important effect on fish quality during storage. In conclusion, when designing a ready-meal, interactions between different components will affect the quality of individual components and of the culinary quality of the ready-meal as a whole – and thereby also the possibility to extend the shelf life further.

The result and insights from the study will be presented.

Development of flavored freeze-dried pink salmon (*Oncorhynchus gorbuscha*)

***Alexandra Oliveira*^{1,*}, *D. Nguyen*^{1,2}, *L. Gurer*¹, *Q. Fong*^{1,3}, *P. Bechtel*⁴, *B. Himmelbloom*¹,
and *C. Crapo*^{1,3}**

¹Fishery Industrial Technology Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Kodiak, AK, USA.

²Department of Aquatic Product Processing Technology, University of Nha Trang, Nha Trang City, Khanh Hoa, Vietnam.

³Marine Advisory Program, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Anchorage, AK, USA.

⁴United States Department of Agriculture, Agricultural Research Services, Seafood Laboratory, University of Alaska Fairbanks, Kodiak, AK, USA.

*acoliveira@alaska.edu

Meal, Ready-to-Eat (MRE) is a lightweight packaged, self-contained meal used traditionally in combat or field conditions by the military. Shelf-stable and nutritionally balanced, MRE's have gained popularity in segments of consumers seeking practicality and reliability in food rations used during outdoor activities such as camping, hiking, hunting and climbing. Seafood plays a small but valuable role in the world market for freeze dried (FD) foods, with shrimp being the most recognizable product. FD seafood products have unique flavor and desirable nutritional characteristics for inclusion in MRE's. The objective of this study was to develop FD flavored products from Alaskan salmon.

Fresh deep-skinned and boneless pink salmon fillets were frozen in blocks, diced into small cubes (~5-7 mm³), and vacuum packaged for short-term storage at -30 °C. Five test batches of salmon cubes (C; F1-F4) were flavored using a two step process of blanching, either at 60°C for 30 min (C; F1; F2) or at 95°C for 3 min (F3; F4), followed by a glazing step either at 30°C for 30 min (C; F1; F2) or at 0°C for 10 min (F3; F4). Solutions used for blanching and glazing

contained variable ratios of the following ingredients: water, salt, sugar, fish sauce, and onion, garlic and chili powder. After glazing fish cubes were FD (Virtis Freeze Drier 52ES), to produce four test products (F1-F4) and a control (C).

Products C, F1 and F2 were FD for 27 h, while F3 and F4 were FD for 11.5 h and 11 h, respectively. The chemical composition and microbial load of the products was determined using standard laboratory methods. A Consumer Acceptability Test was conducted, including 114 participants, to compare preference among products. Processing yields averaged 27% for low water activity (aw<0.20) products (C, F1 and F2), while processing yields for F3 (aw=0.49) and F4 (aw=0.67) were higher at 32% and 37%, respectively. Results from chemical, sensory and microbial analysis will be presented and discussed in relation to changes in processing parameters.

In conclusion, it was possible to produce flavored FD salmon products, varying in water activity levels, which show potential for inclusion in MRE's.

Innovative consumer-oriented product development of enriched seafood

Kolbrún Sveinsdóttir**, *R. Jónsdóttir*, *M. Geirsdóttir*, and *E. Martinsdóttir

Matís ohf, Reykjavík, Iceland

*kolbrun.sveinsdottir@matis.is

Seafood protein isolates are now finding their way into seafood products with good success due to very recent advances in processing technologies. Fish protein hydrolysates (FPH) have higher bioactivity than isolates which make them attractive as functional food ingredients. Certain polyphenols derived from seaweeds (phlorotannins) have very high bioactivities and have the potential to deliver health effects, as well as improve the stability of food product, e.g. against rancidity. Incorporating these bioactive ingredients into niche seafood products will therefore have a multitude of benefits for the consumers. Seaweed polyphenols may have several advantages in seafood products, including a more appropriate flavor profile and overall higher activity than plant polyphenols. These developments open the door to a large range of innovative seafood products enriched with FPH or bioactive ingredients from seaweed giving the consumers greater choices when seeking to enrich their diet with compounds with nutritional benefits. The product development of an Icelandic producer of seafood products aimed for targeted consumer groups is described. The aim was to increase their market share and reach

new consumer groups by developing ready to eat seafood meals enriched with bioactive compounds such as seaweed with defined bioactivity and hydrolysates to increase the protein content. Focus groups of consumer were used to get insight into consumer attitudes to enriched seafood products and sensory evaluation was used during the product development process. Prototypes, based on a popular traditional product already in the market, were developed containing seaweed extract (SEA) or hydrolysates (PROT). The two prototypes were compared to the traditional product (TRAD) in a central location consumer test (blind testing). The effect of information about ingredients and health effects on consumer liking were studied in a home-use consumer test. Information about product and health related attitudes were collected at the same time. In the central location test liking scores were similar for the SEA, PROT and TRAD sample groups. Ingredient and health effect information affected liking depending on the consumer attitudes. The results will be used for further product development and marketing of enriched seafood.

Innovative use of seafood in industrial households. Consumers' expectations

Marit Rødbotten^{1,}, A. Segtnan¹, A. Vorre Skuland¹, B.E. Olsen², P. Lea¹, J. Pickova³,
M. Åslī¹, T. Mørkøre¹, and J.T. Rosnes¹*

¹Nofima, Ås, Norway

²Gastronomisk Institutt, Stavanger, Norway

³Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

*marit.rodboten@nofima.no

This paper describes part of a project named "Innovative and attractive fish dishes" which Nofima has been responsible for and the work has been sponsored by the Research Council of Norway.

The study aimed at investigating barriers and possibilities for innovative use of seafood in industrial households.

Attractive seafood dishes for large scale production are in minority among the dishes served in the HoReCa segment. The currently served seafood dishes are characterised by low quality due to improper handling, processing, packaging or storage conditions leading to loss of sensory and nutritional value. There is a growing demand for more healthy and nutritious fish meals both from consumers and government. However, to meet this demand the good quality must be maintained through optimal handling and processing from raw material to served meal.

Three different methods were used to investigate the objectives of the study; First, barriers and possibilities were identified through focus group discussions with chefs (n=9) and consumers (n=13). Second: acceptance of a selection of innovative fish dishes using restructured fish and various vegetable stews was tested in an internet

survey (n=86). Third, consumer tests involving actual products were conducted in canteens in Stavanger (n=77) and Uppsala (n=93).

A primary finding was that both consumers and chefs agreed that fish was a very desirable dish. Consumers would like to eat fish out of home because they prefer not to make the fish dishes themselves, it is a healthy choice and it tastes good. Barriers for choosing fish in a lunch canteen were lack of variability, and bad odour, taste and texture. Consumers preferred natural fish meat structure (not minced fish meat) and that the dish should be composed with appropriate vegetables and sauce to provide a colourful and appetising presentation. Dishes with restructured fish were considered to be acceptable by consumers. Chefs indicated large problems associated with the present availability of fish material. Fresh fish was preferred to frozen fish materials.

There is a potential for serving a larger variety of fish dishes in the HoReCa segment. Both consumers and chefs are welcoming a greater variety of fish dishes. All three methods of consumer studies supported these findings. Fish that can be served as natural fish meat are most preferred which opens new possibilities for use of restructured fish meat.

Innovative and attractive fish dishes

Jan Thomas Rosnes^{1,*}, T. Mørkøre¹, M. Rødbotten¹, B.E. Olsen², M. Åsli¹, J. Pickova³, and J. Hansen¹

¹Nofima, Stavanger, Norway

²Gastronomisk Institutt, Stavanger, Norway

³Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

*thomas.rosnes@nofima.no

Attractive seafood dishes for large scale production are in minority among the dishes served in institutional households and industrial kitchens of the HoReCa segment. The seafood dishes present are characterised by low quality due to improper handling, processing, packaging, storage conditions or too tough regeneration of the dishes leading to loss of flavour, texture and nutritional contents. It is a growing demand for more healthy and nutritious fish meals both from consumers and government. In a project (InnovaFish) running from 2008-2010 researchers from Nofima and SLU worked together with the Norwegian Culinary Institute and 10 commercial companies to develop new fish products combined with vegetables for the HoReCa segment. Parts of the products were produced at the different companies and a concept was developed starting with raw fish material and ending with served meals in a canteen test.

The objective was to overcome some of the hurdles in the HoReCa market and to stimulate fish and vegetable consumption through development of innovative and attractive seafood dishes using novel processing- and packaging techniques.

Three different methods were used to investigate the consumer's preference for combinations of fish (e.g. shape, size) and vegetables. Based on results from the consumer tests, low-priced parts of the saithe (other than the loin) were restructured, portioned and stored frozen.

Fish raw material was combined with suitable vegetables and other ingredients in order to achieve attractive and novel fish dishes. Multi portion packaging material (gastronom, GN 1/2) designed to fit into HoReCa equipment was used for packaging and storage. Combinations of pre-processing and heat processing steps were defined in order to increase retention of healthy components in the treated raw materials. In the end of the project consumer tests with restructured fish and vegetables were conducted in canteens in Stavanger and Uppsala. These dishes were considered acceptable by the consumers, and the project has increasing the knowledge on production of fish meals for industrial kitchens and consumers and decision-makers perception of new fish dishes in an industrial household setting.

New ready to use seafood products with texture adaptations for elderly

***Irene Peral Díez** and R. Llorente Holgado**

Food Research Division, AZTI Tecnalia, Parque Tecnológico de Bizkaia, Bizkaia, Spain

*iperal@azti.es

Health conditions associated to ageing affect food swallowing, perception of sensory properties (taste and flavor) and make food cooking at home difficult. A daily personalized nutrition plays an important role in disease prevention and promotion of well-being in the elderly. Seafood is due to its nutritional and texture properties suitable for the development of texture modified diets focused on these consumers.

For the seafood processing industry the design of texture modified ready-to-eat products is a promising business opportunity.

The aim of this preliminary study is to analyze the feasibility of designing modified texture seafood prototypes based on traditional recipes and adapted to senior population mastication and swallowing requirements. Palatability and preparation methods are also considered.

The study covers: State of art of existing commercial references of texture adapted foods

(mainly fish), prepared food formulation techniques to modify and control food texture based on viscoelasticity modulating properties of hydrocolloids and their application in commercial references and finally a review of existing international texture scales to be applied onto these products.

As a result the implementation of hydrocolloids as texture ingredients in the formulation of different seafood products (from soup to pudding) targeted to elderly consumers has been designed. The feasibility of the industrialization of the process has also been considered.

There is a lack of commercial ready-to-eat texture modified seafood products to be included in the daily menu of older people or those consumers affected by chronic diseases and the ageing process. Improved sensory characteristics (flavor, texture and taste) and self-cooking might have a positive effect in psychological satisfaction and enhanced quality of life.

The past and future of seafood promotion in Turkey

*T. Tolon and Dilek Emiroglu**

Ege University Faculty of Fisheries, Aquaculture Department, Bornova, İzmir, Turkey

*dilek.emiroglu@ege.edu.tr

The consumer trend for healthy food consumption over the whole World is emphasizing the importance and value of the seafood products day by day. Reversely, the seafood consumption in rest of the Europe is still beyond the targets which determined by the food specialists. The major limiting factors for seafood consumption in developing countries of Europe as Turkey is known as the lack of organization in the seafood market, deviations from the seafood quality standards, hygiene in local seafood markets, product variety in the market but mainly, the consumption behavior of the consumer.

In the age of technology and communication, classic promotion and advertisement

methods have lost their impact on global and local consumer. Many seafood companies are now allocating an important share of investment on the new promotion methods compared to 2000s. Some globally spread seafood companies are trying to involve and get more share from the market pie in countries which has a huge consumer potential such as 73 million, Turkey.

In this study, the local change in promotional activities and market strategies which aim to increase the processed seafood consumption in Turkey has been reviewed over 10 years.

French consumers' impressions of fresh, frozen and thawed cod; Results from a focus-group study

Themistoklis Altintzoglou^{1,*}, M. Heide¹, and M. Carlehög²

¹Nofima, Tromsø, Norway

²Nofima Marin, Nofima, Tromsø, Norway

*themis.altintzoglou@nofima.no

Product labeling is influencing consumers on their choice of food. How supermarkets label thawed fish is not similar across countries. In England, this information is presented in small letters at the back of the package, while in France consumers see the word “thawed” on the front of the package, printed in large letters.

The aim of this study was to explore French consumers' attitudes and associations with fresh, frozen and thawed cod in order to advice the relevant industry on product development.

The method used in this study was focus group discussions. 16 consumers in two focus groups discussed about fresh, frozen and thawed fish. The discussions were then transcribed and the content was analyzed in order to draw conclusions.

In the beginning of the session, the participants discussed how they always aimed at preparing and consuming fresh fish which was bought from a fishmonger due to its perceived higher quality in comparison to frozen fish. Later in the discussions, consumers begun to admit that they

might occasionally resort to use their “back-up” frozen fish from their home freezer, in case they had no time to go to the fishmonger. Interestingly, after admitting the use of frozen fish, consumers discussed more openly about the fact that frozen fish was a convenient option that they use regularly. Finally, consumers discussed that freezing fish could benefit the end quality of the product, considering “how far fish has to travel” until it arrives at the retail store. French consumers were not positive about thawed products. They were worried that thawing fish could decrease the “nutrient content”, increase “microbial risks” and discussed that they would not know how to handle and store them.

This study showed that the image of frozen fish in France might be ambiguous. In focus group discussions consumers want to maintain an image of using the fresh product, as this is perceived as the best product. However, consumers admitted using frozen fish and came up with several positive attributes that can be used in seafood product development.

Development of new business concepts for the seafood sector

Jorunn Sofie Hansen^{1,*}, G. Håbesland², D. Skipnes¹, and A. Vorre Skuland¹

¹Nofima, Stavanger, Norway

²Brandgarden, Oslo Norway

*jorunn.sofie.hansen@nofima.no

Seafood has a strong position as a healthy food. The consumer demands exciting and sophisticated meal adventures, but at the same time they want convenient food with great quality and taste. The Norwegian consumer eats less fish and other seafood than the governmental recommendation, which is at least two fish meals a week. However, this trend is even more pronounced among children, adolescents and young adults. From a health perspective, the consumption of seafood needs to increase in these groups.

The challenge is to develop sophisticated product ideas, which make fish and seafood dishes a natural choice for these groups in different eating situations. The aim of this project was to develop new product concepts and business opportunities based on consumer insights which the seafood sector wishes to realise. The process started with thorough analyses: In addition to understanding the consumers' wishes and needs, we also wanted input from the grocery stores and store managers with regards to launching new products and identifying bottlenecks in this process.

Important innovation platforms found in our study were: a) Pure and fresh, b) Fish is celebration, c) I succeed! and d) Fish for children. These platforms have been the basis for developing our innovative portfolio, made up of nine business opportunities. These business opportunities have been described in depth, including consumer needs and strategic importance:

1. Fish Counter Inc.
2. Gourmet Minced Fish
3. Fish Soup Kit
4. Fish Sandwich
5. Grilled fish
6. Friday Fish Dinner
7. Grand Cru
8. The Treasure Chest
9. Arctic Adventure

A larger assortment of attractive fish dishes in the grocery stores can potentially increase the consumption of fish in the population and give added value to the seafood industry.

Foreign trade profile and trends of processed seafood products in Turkey

T. Tolo, H. Saygi, and Dilek Emiroglu*

Ege University Faculty of Fisheries, Aquaculture Department, Bornova, İzmir, Turkey

*dilek.emiroglu@ege.edu.tr

Seafood processing technology applications and consumer demands for processed seafood products is rapidly increasing in Turkey. Existing companies are widely applying processing techniques as fresh-chilled, frozen, canned, smoked and pickled for seafood. Periodical changes in the supplied quantities and high prizes of fresh seafood, reroute the consumer demands to domestic and foreign originated processed seafood.

54 thousand tons of fishery products have been exported to foreign countries and 73 thousand tons of fishery products were imported to meet

the 546 thousand tons of domestic consumption in 2009. The total production in Turkey was 623 thousand tons.

In this paper, the seafood processing companies, assessment of foreign trade and trends in consumer demand for processed seafood products were analyzed by time series analysis. Reviewed profile of the seafood processing sector would guide the foreign traders as well as domestic ones to re-organize their future marketing strategies for the brand new and developing processed seafood market in Turkey.

Poster 2.1

Overview of the fisheries sector in Turkey

Dilek Emiroglu* and D. Günay

Ege University Faculty of Fisheries, Aquaculture Department, Bornova, İzmir, Turkey

*dilek.emiroglu@ege.edu.tr

The agricultural sector, accounting for 8% of the national income and 31% of the employment, is an economic and social sector of Turkey. The fisheries sector, which is a sub-sector of agriculture, is on the 3rd place in terms of production. The total global fisheries production was recorded to be 163 million tons in 2009. Turkey was on the 33rd place regarding global production and on the 5th place among the EU countries with its nearly 623 thousand tons of fisheries production. The fisheries sector in Turkey consists of 3 sub-sectors; the aquaculture, capture fisheries and seafood sectors. 75% of the total production was obtained through capture fisheries, and the anchovy fishing had the largest share with 205 thousand tons of capture in 2009. The aquaculture sub-sector has shown a quit

significant increase throughout the years. What started in the 1970s with 1000 tons of trout and carp production has reached about 160 thousand tons today. Especially the production of gilthead seabream and seabass has an important share among the EU countries. The seafood sector has been developing in a very controlled and deliberate manner in recent years. Seafood has an important share among the exported products of Turkey and has an important contribution to the national economy in terms of return on foreign exchange.

In this study, the fisheries sector in Turkey is discussed in a comprehensive manner according to the years of production, consumption, import and export status, contribution to the economy and the employment status of the sector.

Poster 2.2

Development of innovative functional product from farmed species: Utilization of meagre (*Argyrosomus regius*) and dietary grape fibres

R. Mendes^{1,*}, Carlos Cardoso¹, B. Ribeiro^{1,2}, H. Silva¹, C. Serrano³, C. Ramos³, and P. Cameira dos Santos³

¹National Institute of Biological Resources, L-IPIMAR, Lisbon, Portugal

²Superior Institute of Agronomy, Tapada da Ajuda, Lisbon, Portugal

³National Institute of Biological Resources, L-INIA, Oeiras, Portugal

*rogerio@ipimar.pt

Meagre (*Argyrosomus regius*) is a species whose farming has gained importance over the past years. Rearing interest is mostly supported by its excellent biological characteristics and high rates of growth, feed conversion, and fertility. Meagre shows good marketing potential. However, small-sized meagre (600-1000 g) poses marketing difficulties since this size fish have a large head, large bones, little flesh, and are not very tasty. In order to strengthen the market prospective for these small sized fish, alternative products must be developed. In this context, the development of restructured fish products and the application of new food additives have been used as a way to create healthier seafood products and diversify commercial alternatives. However, in this case, new processed high added value meagre products depend on the gelling quality of meagre minces.

Therefore, the aim of this work was to develop a new functional product from meagre, thereby testing the potential of the incorporation of grape dietary fibre (GDF).

Sausages were prepared from minced meagre (with an average weight of 800 g) according to a formulation previously optimized, involving

mixing of starch (3.4 %, w/w), sodium caseinate (1.3 %, w/w), soya protein (1.1 %, w/w), salt (1.0 %, w/w), MTGase (0.5 %, w/w), and smoke aroma (0.1 %, w/w). Moreover, two batches were prepared: one with 3.9 % (w/w) inner pea DF (control) and another with partial replacement of inner pea DF by GDF (3.0 %, w/w). Different quality parameters were assessed: texture, water holding capacity (WHC), and colour. Furthermore, the antioxidant capacity of GDF was evaluated during refrigerated storage (2 ± 2 °C) of the products.

Firstly, this study showed that it was possible to achieve meagre sausages with appropriate texture in both batches. Meagre sausages with GDF presented a gel strength similar to control (25.6 vs 26.7 N.mm), however were softer (29.9 vs 49.1 N). WHC was higher for control products (63.6 vs 54.6 %). The GDF sausages were darker, redder and more yellow, thus presenting a closest resemblance with pork sausages.

Hence, this work demonstrated the feasibility of the incorporation of an antioxidant GDF.

Poster 2.3

Session 1:
Towards sustainable sources of seafood
– aquaculture and rest raw materials

Session 2:
Product development and product communication
within the seafood sector

Session 3:
**Innovative process and detection technologies for
seafood**

Session 4:
Retaining high quality through the seafood processing
chain

Session 5:
Nutritional and safety aspects of seafood consumption

Keynote: Potentials of nano-processing within the seafood sector

Amparo Lopez-Rubio

Novel Materials and Nanotechnology Group. Institute of Agrochemistry and Food Technology (IATA-CSIC). Valencia, Spain
amparo.lopez@iata



Recent years have witnessed a tremendous expansion of research and technology developments in the nanotechnology field resulting in significant application developments in the food and agricultural areas. Nano-processing refers to nano-scale fabrication, which in most technological areas aims towards the manufacture of improved quality products. Using nanotechnologies, devices that are lighter, faster, more reliable, efficient and safer can be produced. As a result, nano-processing can contribute to the development of sophisticated industrial products that perform better and are more advantageous in many other ways than any other devices manufactured so far. However, in the food area the main aim of nano-processing is

to improve the quality and safety of food as well as to impart novel functionalities to food products. Specifically, nanotechnologies have been applied to the development of nano-composites for food packaging materials and to the design of nanostructures for ingredient delivery. In this talk, the focus will be on describing the most outstanding developments in the nano-processing of foods and food-related materials and how these new technologies can be applied within the seafood sector. Examples of bioactive packaging systems and encapsulation structures containing functional ingredients will be provided, so as to show the amazing possibilities of nano-processing for food applications.

Separation of marine oil components by advanced membranes – Possibilities and advantages

Andrea Meniconi¹, A. T. Boam¹, and P. Skavaas²

¹Evonik Membrane Extraction Technology Ltd., Wembley, United Kingdom

²Due Miljø AS, Oslo, Norway

*andrea.meniconi@evonik.com

In recent decades there has been an increase in consumers' concerns over the quality and safety of many products and ingredients used in food, medicines and cosmetics. Consumers' preference has strongly moved to products originated from natural sources as opposed to synthetic ones. As a result of this market demand, the production of natural compounds is rapidly expanding as well as the interest in dedicated technologies. This certainly applies for marine oils also.

The natural compounds, e.g. marine oils are firstly extracted in bulk from natural material sources, e.g. fish, krill or a marine biomass like algae and then, to some extent, fractionated and/or purified through a series of separating operations. Current state-of-the-art technologies for separation involve the use of either distillation technology (short-path or conventional distillation), or conventional preparative liquid chromatography. These technologies require high capital expenditure to set up and have significant operating costs. In recent years, membrane technology, particularly organic solvent nanofiltration (OSN), has attracted attention as an alternative molecular separation technology. The main advantage of employing OSN for purification of natural extracts as marine or other natural oils is that by selecting suitable molecular weight cut-off (MWCO) membranes, this technology can be used to fractionate molecules of similar molecular weight (e.g. in the 200 to 1000 Da

range) at a much lower operating temperature compared to conventional processing operations.

In addition to the large savings in energy costs, natural compounds are often susceptible to thermal damage and thus the milder operating conditions of a membrane process can minimise nutritional losses from thermal degradation.

The property of the OSN technology, separation of molecules based on size/charge, makes the technology suitable for many oil refining applications e.g. free fatty acids removal and fractionation and concentration of DHA/EPA mainly found in marine oils.

A quite different system, still being an advanced membrane technology, has been developed and patented by SINTEF and the company DUE MILJØ to refine marine oils. The system uses a ceramic membrane - very much different from the polymeric OSN membranes. The system called PBT, reduces pollutants like PCB/Dioxin levels below EU regulations and reduces stearins, smell, colour and FFA-enzymes while keeping the natural vitamins, antioxidants and lipid profile. The system is different from dead end filtration as it is more precise in separation and improves yields significantly.

This presentation reports the successful development of OSN and other membrane based processes for natural compounds fractionation and added benefits in casu marine oils. A case study reporting the enrichment of high value antioxidants in natural oil will be presented.

Proteomic approach to monitor the oxidative status of proteins in seafood products

M. Pazos, L. Méndez, Rodrigo Maestre J.M. Gallardo and I. Medina*

Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Spain

*maestre@iim.csic.es

Oxidation is a major cause of protein deterioration in fish muscle contributing to undesirable textures. The elevated number of oxidative initiators and the high variety of proteins and amino acids target of oxidation, confers an extremely high complexity to protein oxidation. In fact, the identification of the mechanism involved in the protein oxidation of meat-based foods has been traditionally limited by methodology limitations. The implementation of recent advances in proteomics is suggested to add decisive information in the understanding and control of protein oxidation in fish muscle. To date, western-blotting directed to protein-DNPH derivatives is almost the only proteomic method applied to measure protein oxidation in fish muscle, however immunoblotting procedures have disadvantages such as high cost, elevated time consuming and possible protein loss during the protein transfer to the membrane.

The investigation was aimed to evaluate the viability of a Proteomic-based methodology that combines fluorescent-labeling of protein carbonyls, gel-electrophoresis and identification by tandem mass spectrometry (MS/MS), for monitoring protein oxidation in fish muscle and tissues.

Protein carbonyls were labeled with a fluorescent-marker. Proteins were separated on

gel-electrophoresis and fluorescence was visualized on the gels with a UV trans-illuminator. Spots of interest were identified by tandem mass spectrometry (MS/MS).

The proposed proteomic procedure was developed using isolated fish protein fractions subjected to metal-catalyzed oxidation (MCO). Diverse oxidative susceptibilities were detected for the different fish proteins. Results were validated in an array of chilled experiments on mackerel muscle. The combination of fluorescent-labeling and 2-D gel electrophoresis revealed an early carbonylation in some specific proteins in non-oxidised samples. Such carbonylation increased in high extend in oxidized samples, accompanied with the appearance of new oxidized protein spots. Oxidation significantly increased carbonylation of important structural fish proteins related with muscle texture, actin and myosin, but also produced an important increment of carbonylation in some sarcoplasmic proteins.

These results demonstrate that the proposed method is a good alternative to the costly and time-consuming immunoblotting procedures for monitoring protein oxidation in fish muscle.

Development of a PCR-Elisa system to authenticate the species of the *Thunnus* genus and *Katsuwonus pelamis*

Francisco J. Santaclara^{*}, R.I. Pérez and C.G. Sotelo

Seafood Biochemistry Group, Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Spain

^{*}fransantaclara@iim.csic.es

The family Scombridae contains 15 genera and 51 species. The genera *Thunnus*, *Katsuwonus*, *Scomber*, *Auxis*, *Sarda*, *Scomberomorus*, and *Euthynnus* are some of the most representative ones belonging to this family. The fact that these species are morphologically very similar and that the transformation processes, mainly canning, applied to them involves the removal of morphological characters making impossible the species identification using these characters in commercial products.

The use of genetic techniques for fish species identification becomes indispensable to carry out the authentication of processed fish. In this work, a PCR-ELISA method to authenticate products elaborated from the species belonging to the genus *Thunnus* and *Katsuwonus pelamis* was developed. This method is based on PCR-ELISA methodology, using the *cytochrome b* gene as molecular marker.

The developed method works in a sequential way: the first step is aimed for the determination of genus *Thunnus*; in a second step it is possible to assign the positive samples of the first step to the species *Thunnus thynnus*, *T. alalunga*, *T. albacares*, *T. obesus*, and verify if the negative samples to *Thunnus* genus belong to the species *Katsuwonus pelamis*.

The main advantage of this technique is that it can be applied to fresh, frozen or canned products, and that it only requires a conventional PCR system (thermocycler), since the results can be evaluated with a naked eye. Therefore the proposed methodology can be very useful in the normative control of products elaborated from Scombridae species, particularly in the authentication of any kind of products labeled as “tuna”.

Simple shelf life prediction models for wireless sensor networks in fish supply chains

Tómas Haflíðason*, ***G. Ólafsdóttir***, ***B. Margeirsson***, and ***S. Bogason***

Faculty of Industrial-, Mechanical Engineering and Computer Science, University of Iceland, Reykjavik, Iceland

*tomash@hi.is

Wireless sensor networks (WSN) offer the possibility to monitor temperature of fish products in real time during transport and send data to central location for storage and further analysis. The WSN are equipped with microprocessors with little computational capacity, but they can perform basic arithmetic calculations and trigger alerts directly from the product's location. Temperature during transport has the greatest impact on the quality, and shelf life of chilled perishables goods. One alert type of interest for supply chain actors is to give early warning alerts if shelf life has been reduced because of abusive temperature conditions during the transport. Shelf life models based on e.g. predictive microbiology have been widely studied, but most need computational capacity and memory, which is not available in the WSN systems. The aim of this paper is to identify if simple shelf life prediction models are applicable in WSN systems and if the accuracy is enough to trigger relevant alerts based on these models.

The studies involved mapping of logistics of cod supply chains and monitoring of ambient and product temperature during field testing of

WSN. Temperature data was collected from cod products packed in EPS (Expanded Polystyrene) boxes, during both a simulated trial and container transport by ship from Iceland to Europe. Samples were further stored under controlled conditions and sensory analysis was carried out to determine the end of shelf life. Simple models based on the accumulated time and temperature load were applied and their reliability to determine the end of shelf life was explored by comparing against sensory analysis and predictive microbial models.

The results demonstrate that the application of simple time and temperature models to predict the shelf life of the cod products could give reliable information on the shelf life of products. The prerequisite conditions are that the age of the raw material, pre-handling and processing conditions are known and the simple models require predetermined criteria for the shelf life of products. Simple shelf life models can therefore be useful to support early warnings in WSN systems and would be an added benefit for supply chain management compared to only temperature alerts.

Product adjusted packaging

Anlaug Ådland Hansen

Nofima, Ås, Norway
anlaug.hansen@nofima.no

Modified atmosphere packaging (MAP) has in several studies been proven to preserve quality of raw fish products because of the antibacterial effect of carbon dioxide. To achieve an optimal effect of MAP a sufficient partial pressure of CO₂ is needed, which is defined by the gas volume to product volume ratio (g/p ratio) and the CO₂ level in headspace. However, for real life products, compromises between preservation and product design very often results in less optimal packaging. Therefore it is important to evaluate packaging technologies in order to develop a more useful application without compromising quality and shelf life.

The aim of this study was to examine different packaging methods with lower gas volume to product volume ratio, or no gas volume, and still get a sufficient period of shelf life.

Farmed Atlantic cod (*Gadus morhua*) loins were packaged in expanded polystyrene (EPS) boxes with ice and transported by plane from north of Norway (Vesterålen) to a packaging test centre in Germany, with arrival the day after. A thermoformer machine was used for the test packaging. The packaging methods were: vacuum packaging, vacuum packaging + CO₂-emitter (lab-emitter made by Nofima), MAP with low g/p ratio, and MAP with low g/p ratio + CO₂-emitter (lab-emitter made by Nofima).

After consumer packaging, the samples were packaged in EPS boxes with ice, and transported by plane back to Norway for further chill storage and sampling.

Temperature was logged during transport and during further refrigerated storage.

Sampling was performed after 1, 7, 9, 13 and 15 days. Analyses that were performed were gas mixture of headspace, oxygen transmission rate of the used packages, microbiology (total bacterial count, H₂S bacteria, lactic acid bacteria), sensory analyses (11 odour- and appearance attributes), pH in muscle and liquid loss.

Preliminary results show that CO₂-emitter prolonged shelf life, both for the vacuum packaged samples and for the MAP samples, compared to those without a CO₂-emitter. Results showed that there were only small differences between total bacterial counts, whereas the sensory scores did differ. By use of pyrosequencing (454-technology) further testing at our lab will show what kind of bacteria that actually is present on raw cod products during storage, and how different preservation technologies effect growth of spoilage bacteria and sensory quality.

A conclusion is that when low headspace in package of raw fish is preferred, a CO₂-emitter can compensate for the lowered gas volume in MAP. In vacuum packages, an additional effect of CO₂ gas was achieved by use of CO₂-emitter inside such package, and still having a “vacuum look” of the package, which can be an attractive packaging method for future packaging of convenient raw fish products.

Modeling and simulation of salmon fish in superchilling technology

***Lilian Daniel Kaale**, T. M. Eikevik, K. Kolsaker**

Norwegian University of Science and Technology (NTNU), Dep. Energy and Process Engineering, Trondheim, Norway

*lilian.d.kaale@ntnu.no

The finite difference method was used to develop a model for analyzing the superchilling process of salmon fish. The objective of this study is to develop a one-dimensional model which can predict degree of superchilling of salmon fish at a given time. In order to predict the degree of superchilling, the thermo-physical properties of salmon fish are evaluated based on its composition and the properties of its components found in the salmon. Modeling and simulation of food processing are crucial aspects for the food industry. Modeling is the building of tools able to predict the future of the food product or process with good or enough accuracy depending on the purpose of model building while the purpose of simulation is to gain understanding of the process being simulated. The model used in this article is a simple finite difference method adapted to handle the nonlinear physical properties. Solving this kind of equations requires either sophisticated integration schemes, or finer resolution in space and time. Our model uses the latter approach. The model is implemented

in MATLAB and has been tried with the built-in ODE-solvers with various successes.

The model showed that, during the superchilling process, the temperature at the surface of the salmon fish decreased quickly to reach the freezing temperature. During the superchilling process the surface temperature dropped from +4 °C to about -19 °C, while the core temperature dropped to about +2 °C. The model also shows the superchilling storage period of the salmon fish and can predict the degree of superchilling after certain time of storage. At the surface, the degree of superchilling was about 50 % compared to that of core which was about 0.01 %, due to water-to-ice transition. The degree of superchilling increased from 9 to 24.4 % with increased superchilling process time from 2 to 6 minutes, respectively. The developed model allows for the evaluation of degree of superchilling during superchilling process of salmon fish. It can also be used to evaluate the enthalpies, and temperature profiles during superchilling of the salmon fish.

Poster 3.1

Operational performance of the desalting process of cod pieces: Pilot study

***Graziele Gustinelli Arantes de Carvalho**, L.S. Sant'Ana, P. Martin, and P. de Magalhães Padilha**

UNESP, Centro de Aquicultura (CAUNESP) and Departamento de Química e Bioquímica, São Paulo, Brazil

*grazielegustinelli@yahoo.com.br

Changes in consumer life-style have led the fishing industry to produce ready-to-eat products. Salted fish require desalting prior to cooking and in the traditional method this desalting is usually done by the consumers at their home for at least 24 hours. To meet the HACCP standards and the demands of modern consumers it is necessary to have more knowledge about the desalting process to commercialize desalting of fish with adequate quality and attend necessities of modern life. The purpose of this pilot study was to evaluate the operational performance of the desalting process of cod pieces subjected to desalting in controlled conditions. Fifteen dried pieces of salted cod used in the desalting experiments weighted 133.89 ± 39.27 g. The cod pieces were desalted for 48 hours in tap water and periodically analyzed at 0, 12, 24, 36 and 48 hours. The samples were weighted to determine weight changes and water uptake. The moisture changes were determined by oven drying for 16 hours at 105°C . For water activity we used an Aqualab 3T. Sodium was determined by flame

atomic absorption spectrometry. Substantial weight gain is seen in the cod pieces after 24 hours of soaking (221.54 ± 39.7 g). After this, weight continued to increase but in smaller quantities. Moisture increase from 49.62% to 82.91% (and stayed stable until the end of experiment). Total moisture variations decreased from -30.18% to -35.41%, and in the first 24 hours the moisture variations decreased quickly.. The water uptake is stronger at the beginning (0.63 L), it can be attributed to the surface salt crystal. After 12 hours water uptake is weaker, but the absorption was continued until the end of experiment. The initial water activity was 0.747 ± 0.003 and had a peak increase around 12 hours achieving 0.934 ± 0.0064 . At 48 hours this value was 0.972 ± 0.014 . This process is strongest in the first 24 hours, becoming slower afterward. Sodium at beginning was 58.22 ± 2.32 mg/g and decreased considerably in the first 24 hours. After this period of time, the slope diminished slightly until equilibrium was reached with 2.83 ± 1.73 mg/g at 48 hours.

Poster 3.2

Comparison of two mitochondrial DNA sequences for the identification of *Scomber* species

***Iciar Martínez*^{1,2,*}, *A. Velasco*¹, *R. Perez-Martin*¹, *E. Forås*³, *M. Yamashita*³, and *C. G. Sotelo*¹**

¹Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Spain

²Norwegian College of Fishery Science, University of Tromsø, Tromsø, Norway

³SINTEF Fisheries and Aquaculture, SINTEF Sealab, Trondheim, Norway

³National Research Institute of Fisheries Science, Yokohama, Japan

*iciarm@iim.csic.es

In countries where mackerel products are appreciated, consumers prefer and are willing to pay higher prices for their local raw species, giving rise to the opportunity for fraudulent mislabeling. Different DNA fragments have been targeted for species identification, often from the *mtDNA* due to its resilience and high copy number in most tissues. We use sequencing of a 464 bp (a 140 bp fragment for canned products) from the Kocher fragment of the *mt Cytb*. However, faster methods not requiring sequencing are usually preferred due to their simplicity, and a PCR-RFLP analysis of a 505 bp fragment using *Hae* III and *Hinf* I from the NADH dehydrogenase subunit 5 (*mt ND5*) has been proposed to differentiate *S. japonicus*, *S. australasicus* and *S. scombrus*. The present work is part of the research project TraCtrolMac dealing with the identification of traceability control mechanisms for mackerel.

Our objective was to identify the most suitable approach for species identification to verify the information accompanying mackerel products along the entire production chain.

We analyzed 142 samples including authentic processed and unprocessed reference samples and products of *S. scombrus*, *S. colias*, *S. japonicus* and *S. australasicus* purchased in Norway, Japan and Spain, by sequencing of the 464 bp fragment of the Kocher fragment and of the 505 bp *mt ND5* fragment. All samples clustered correctly according to their claimed origin except a Spanish product from Cabo Verde that contained *S. japonicus*. Three pieces of one of the Japanese products belonged to at least two different *S. australasicus* individuals.

Sequence analysis of any of these two loci allows the easy species identification of all the samples. PCR-RFLP of the *mt ND5* fragment however, would have misidentified all *S. colias* as *S. japonicus*, and would not have been able to identify as *Scomber* a few true *S. scombrus*. Sequencing, however, remains a demanding and time consuming task, and future work should focus in designing a fast method specific for all four species.

Poster 3.3

Differencing sea bass fillets frozen in different conditions by impedance measured at frequencies from 1 to 100 MHz

T.r Jančić¹, H. Medić¹, N. Marušić¹, T. Petrak¹, I. Lacković², and Sanja Vidaček¹

¹Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

²Faculty of Electrical Engineering and Computing, University of Zagreb, Zagreb, Croatia

*svidacek@pbf.hr

In our earlier studies impedance of thawed fish fillets was measured at frequencies from 1 Hz to 1 MHz and results indicated that measurements at frequencies near 1 MHz have a potential to differentiate fillets with different freezing histories. However, as muscle tissue showed anisotropy even at these frequencies, the aim of this study was to evaluate if the measurements at frequencies up to 100 MHz could be better indicators of freezing history.

In this study, sea bass fillets were frozen at -20 °C, stored for 1 or 3 months with constant or fluctuated temperature during storage, and thawed overnight at +6 °C. The impedance properties, protein solubility, pH, water holding

capacity, lipid oxidation and color of the fillets were measured. The HP 4294A Precise LCR meter was used to measure impedance magnitude ($|Z|$) and phase (ϕ) at 200 frequencies from 100 Hz to 100 MHz. Needle-type multi-electrode array was used to interface the sample to the instrument.

Impedance measurements and especially phase measurements showed the potential of detecting the differences among the fillets at frequencies from 1 to 3 MHz and from 40 to 100 MHz. Compared to measurements at lower frequencies, anisotropy of muscle tissue was reduced. Further studies will focus on other fish species and different fishery products.

Poster 3.4

Sensory testing of seafood products in the frame of the DLG food quality contest

Jörg Oehlenschläger

DLG (German Agricultural Society), Buchholz i. d. N., Germany

J.Oehlenschlaeger@gmx.net

Each year more than 600 seafood based products from Europe and Japan are tested for their sensory quality by the DLG. The products are from the normal production line and cover the whole range of seafood products: QF fillets, fish fingers, smoked fish, marinades, canned products, salads, pizzas, snacks, finger food, convenience products and complete meals. The sensory tests are performed by assessors specially educated and trained for this type of sensory assessment. As a result of the assessment the products can win a Golden, Silver or Bronze medal which indicates to the

consumer that the product is free of sensory defects (Gold), has minimal sensory defects which cannot be recognised by an untrained consumer (Silver), or has minor sensory defects (Bronze). Products which do not fulfil the criteria will not be awarded.

The training of the sensory experts, the system of certification of the sensory qualification of the experts, the test itself, the preparation of the products prior to testing, the assessment forms used, the calculation of demerit points and the importance for the international markets participating in this test will be explained.

Endpoint temperature assessment in surimi through visible spectroscopy – dealing with different water levels

Svein Kristian Stormo^{1,*}, A. H. Sivertsen¹, K. Heia¹, and D. Skipnes²

¹Nofima, Tromsø, Norway

²Nofima, Stavanger, Norway

*svein.stormo@nofima.no

From a microbiological point of view, thermal processing is useful to preserve food and keep it safe. Assessing endpoint temperatures (EPT) enables effective temperature control throughout processing, but assessment methods have so far been labour-intensive or sensitive to water associated with the sample. The aim of this study was to develop a non-invasive method able to deal with the water levels of fresh samples. Visible spectroscopy measurements were combined with multivariate analysis for EPT prediction of surimi samples. No measures were carried out to control the water on the surface or the water content of the samples. In the range from 400 to 700 nm an apparent correlation

between temperature treatment and spectra intensities was observed. The spectral changes reflect the changes in relative scattering intensities caused by protein denaturation. A similar correlation was not observed for the near infrared (NIR) region. By excluding the NIR region, where water absorbs strongly, we present a model in range from 400 to 700 nm that show a prediction error less than 2 °C in the temperature range 46.6 to 74.4 °C. This model is robust ($r^2=0.96$) and covers the temperature range for mild surface pasteurization, thereby showing potential for use in the food processing industry.

Advanced imaging for automated inspection of 3D, color, surface scattering and subsurface scattering properties of fish and fish products

J.R. Mathiassen, Ekrem Misimi^{}, and S.O. Østvik*

Department of Processing Technology, SINTEF Fisheries and Aquaculture, Trondheim, Norway

^{*}ekrem.misimi@sintef.no

Imaging technology is used for automated inspection in systems for grading, sorting and processing of fish and fish products. Despite the desire to automate as many processes as possible, many inspection and quality grading tasks are still done using manual labor. Many such tasks have so far been difficult to automate cost-effectively, in part due to the challenge in objectively measuring all the parameters that human graders subjectively use in their complete visual characterization of the inspected product. Thus, there is a need for advanced imaging that can cost-effectively and objectively measure the visual characteristics that expert human quality graders use. Such an imaging system would also be useful for automated inspection of other food products.

The aim of this work is to develop an advanced imaging system, suitable for cost-effective automated inspection, which is capable of simultaneously imaging of 3D, color, surface scattering and subsurface scattering properties of fish, fish products and other food products.

A high-speed CMOS camera was installed over a conveyor belt, together with a unique illumination setup that enabled the simultaneous imaging of 3D, color, surface scattering and

subsurface scattering. The conveyor belt operated at a speed of 1000 mm/s, and images were acquired at a sufficiently high speed to enable a resolution of between 0.5-1.0 mm/pixel. Several species of fish were imaged, in order to demonstrate the feasibility of using the advanced imaging system for automated inspection.

The presented imaging system was capable of high-speed, high-resolution acquisition of 3D, color, surface scattering and subsurface scattering images. These images were able to quantify visual characteristics that are subjectively used by human experts. The application of the imaging system, to detect defects in herring and Atlantic salmon, demonstrated that most common defect types could be detected using a statistical classifier that combined information from 3D, color, surface scattering and subsurface scattering images.

An advanced imaging system has been developed, which is suitable for cost-effective automated inspection. The system is capable of simultaneously imaging of 3D, color, surface scattering and subsurface scattering properties of fish, fish products and other food products.

Using image analysis to monitor biological changes in consume fish

Bjørn Skovlund Dissing^{1,*}, S. Frosch², and M. Engelbrecht Nielsen¹

¹Dept. of Biology, Technical University of Denmark, National Food Institute, Division of Industrial Food Research, Biological Quality Group, Kgs. Lyngby, Denmark

²Technical University of Denmark, National Food Institute, Division of Industrial Food Research, Food Production Engineering Research Group, Kgs. Lyngby, Denmark

*bdi@food.dtu.dk

The quality of fish products is largely defined by the visual appearance of the products. Visual appearance includes measurable parameters such as color and texture. Fat content and distribution as well as deposition of carotenoid pigments such as astaxanthin in muscular and fat tissue are biological parameters with a huge impact on the color and texture of the fish muscle. Consumer-driven quality demands call for rapid methods for quantification of quality parameters such as fat and astaxanthin in the industry.

The spectral electromagnetic reflection properties of astaxanthin are well known and have in previous studies been shown to change as a function of astaxanthin concentration. This may be utilized to quantify the amount of astaxanthin contained in salmonid fishes by assessing a spectral measurement of the fillet.

Existing ways of assessing the amount of astaxanthin and fat in salmonid fishes is based on highly laborious chemical analysis. Trichromatic digital imaging and point-wise colorimetric or spectral measurement are also ways of estimating either the redness or the actual astaxanthin concentration of the fillet. These methods all have drawbacks of either

cumbersome testing or lack of spectral or spatial information.

The use of multispectral imaging to assess the variation in fat and astaxanthin concentration both between fish and within fish is investigated. Since reference values cannot be obtained corresponding to pixel-wise measurements, sub areas of the fillet are predicted and averaged in order to establish correlation to reference measurements. In the present experiment salmon fillets were sampled at different locations. Each sample consists of a biopsy which was cut in three layers in order to understand the depth distribution of astaxanthin as well as the spatial distribution. For each layer in each sample, chemical fat and astaxanthin determination was carried out as a reference value and a multispectral image was acquired to establish a correlation. A prediction model has been calibrated to predict the astaxanthin concentration on a pixel-wise level resulting in an astaxanthin-map of the fillet reflecting the intra fillet variation in astaxanthin concentration. The model is validated according to spatially varying samples across the fillet.

Use of low-field NMR to Estimate freezing storage time and quality changes in hake (*Merluccius merluccius*, L.)

***I. Sánchez-Alonso*¹, *I. Martínez*^{2,3}, *J. Sánchez-Valencia*⁴, and *Mercedes Careche*^{4,*}**

¹Instituto de la Estructura de la Materia (IEM-CSIC), Madrid, Spain

²Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Spain

³Norwegian College of Fishery Science, University of Tromsø, Tromsø, Norway

⁴Instituto de Ciencia y Tecnología de los Alimentos y Nutrición (ICTAN-CSIC), Madrid, Spain

*mcareche@ictan.csic.es

Freezing storage is one of the most efficient means of increasing the shelf life of seafood but it alters the organoleptic properties of the product which becomes tougher and dryer, with lower water holding capacity (WHC), apparent viscosity (AV), extractability and solubility of myofibrillar proteins and increased shear resistance (SR). Current methods to predict frozen storage time of seafood are usually labour intensive and/or destructive. A fast method, with the potential to be used on-line would be highly desirable, and low field nuclear magnetic resonance (LF NMR) seemed a good candidate to fulfill this need because it is non-destructive and non-invasive, it requires only minimal or no sample preparation and offers the possibility to produce characteristic fingerprints for a given sample.

The aim of the present work was to establish whether LF NMR could be used to predict changes in the quality of frozen stored hake and/or estimate its shelf life.

Hake fillets stored at -10°C (temperature selected to shorten the experimental period) were regularly sampled for up to 21 weeks and WHC, AV, SR and T₂ relaxation measurements (LF NMR analyzer minispec mq 20) were measured.

LF NMR clearly detected three populations of water: water strongly bound to macromolecules (T_{2b}), trapped water (T₂₁) and free water (T₂₂). As storage time increased, and concomitant with an increase in the T₂₂ and a decrease in the T₂₁ water populations, the WHC and AV decreased and the SR increased, reflecting the characteristic loss of juiciness and tougher texture developed by hake during frozen storage. Two mathematical models were constructed: a simple regression using the biexponential analysis of the relaxation times (T₂₁, T₂₂) and amplitudes (A₂₁, A₂₂) and a partial least square regression (PLS) of CONTIN analysis of the same parameters. Both models seemed suitable to estimate the quality of the product.

Use of hyperspectral imaging for classifying fresh salmon (*Salmo salar* L.) according to the type of packaging used during storage

***Izumi Sone*^{1,*}, *R.L. Olsen*², *A.H. Sivertsen*¹, *G. Eilertsen*¹, and *K. Heia*¹**

¹Nofima, Tromsø, Norway

²Norwegian College of Fishery Science, Tromsø, Norway

*izumi.sone@nofima.no

Use of hyperspectral imaging for investigating spectroscopic changes during storage of fresh salmon in different atmosphere conditions and for classifying samples according to the type of packaging was evaluated. Loin samples of pre-rigor filleted Atlantic salmon were stored at 4°C in plastic overwrap, modified atmosphere with 60 % CO₂/40 % N₂ or in 90 % soft vacuum and analysed by hyperspectral imaging for up to 16 days. K nearest neighbour classifier was used for the classification. The results showed that

spectral variations were observed between the different atmosphere packaging and mostly in the visible region of the spectrum. Spectroscopic changes captured by hyperspectral imaging can be used to classify samples according to the type of atmosphere packaging and the successful classification (88.3 ± 4.2 %) was largely dependent of spectral characteristics in the visible region, possibly due to changes in the state of heme-proteins during storage.

Automated image analysis as a tool to quantify gaping and morphological features in smoked salmon slices

Grigory V. Merkin^{1,*}, L.H. Stien², K. Pittman¹, and R. Nortvedt¹

¹Dept. of Biology, University of Bergen, High Technology Centre, Bergen, Norway

²Institute of Marine Research, Austevoll Research Station, Storebø, Norway

*grigory.merkin@bio.uib.no

Gaping in fish fillets refers to the appearance of slits (gaps) either in the connective tissue between muscle segments or in the axial muscle between individual muscle fibres. Automated image analysis is a modern and efficient approach for non-subjective measuring of quality traits in fish fillets.

The aims of this study were (1) to develop fully automatic image analysis methods for evaluating gaping and morphological characteristics in smoked salmon slices (2) to compare the automatic image analysis method to visual gaping evaluation.

Market size Atlantic salmon (*Salmo salar*) (n=135) were sampled in September 2010 from Nofima Marine research station at Averøy, Norway. The fish were then gutted and transported on ice for one week, before machine filleting, smoking and machine slicing at Marine Harvest Kritsen fish processing factory (Marine Harvest®, France). A total of 308 fillet slices were evaluated in the current study.

The fillet slices were carefully placed on a horizontal base in a photobox (SeaSide AS, Norway) and photographed directly from above with digital camera (BASLER A102fc ®, Basler

Vision Technologies, Germany). The fully automatic image analysis method was developed in Matlab (The Math-Works Inc, Massachusetts, USA) using the MATLAB® Image Processing Toolbox and has six main steps: (1) pre-processing, (2) segmentation of slices from the background, (3) identification of gaps in the slices, (4) identification of gaps in the border of the slices, (5) quantification of the gaps in accordance with Andersen scale (Andersen et al., 1994) and (6) description of slice morphology.

Pictures of sliced fillets were inspected visually by one assessor to quantify number and size of gaps in slices.

The results obtained by the image analysis in gaping evaluation were compared with those from visual analysis and showed a good correlation ($r=0.73$, $p\text{-value}<0.05$). Stronger correlation ($r=0.78$, $p\text{-value}<0.05$) was observed between the area of gaps in the border and a morphological characteristic (the ratio of the object's perimeter and the convex hull perimeter) in slices. This indicates that these image analysis methods might become useful in salmon product processing.

Fresh fish on your iPhone

Joop Luten

Nofima, Tromsø, Norway
joop.luten@nofima.no

The sensory evaluation of fish freshness as part of the quality concept has been on the seafood research agenda since the fifties. There is an acceptance that sensory methods are the most effective for measuring freshness of fish. The basics behind the sensory methodologies have not changed until now. Due to international cooperation the various methods have been standardized and efforts have been made to define freshness.

The initiation of the Quality Index Method (QIM) for determination of fish freshness followed by a number of national and European collaborative projects has positioned QIM as a leading reference method. QIM has become the universal language of fish

freshness. QIM fish freshness evaluation schemes for at least 34 fish species are now available. However, a broad implementation of QIM by the stakeholders in the seafood chain was hampered by the lack of an easy tool to carry out the freshness assessment. The recent development of an App for the iPhone is aimed at bringing about an improvement in this situation.

In this presentation the concept, development and operation of the QIM App 'How fresh is your fish?' for the iPhone will be explained. The feedback on the iPhone App launch as well as future ideas for further development of the App will be presented.

Session 1:
Towards sustainable sources of seafood
– aquaculture and rest raw materials

Session 2:
Product development and product communication
within the seafood sector

Session 3: Innovative process and detection
technologies for seafood

Session 4:
Retaining high quality through the seafood processing
chain

Session 5:
Nutritional and safety aspects of seafood consumption

Keynote: Why water is a key to seafood quality

Tyre C. Lanier and C.D. Stevenson

North Carolina State University, USA
tyre@ncsu.edu



Lean fish meat is comprised of 75-80% water, as are most meat gel products derived from seafoods. The phenomenon by which this water is held within meat is central to quality problems encountered in processing as affecting yield (e.g. water losses during refrigerated holding, after freezing/thawing or cooking, and from drying), sensory aspects (e.g. juiciness, texture, visible purge) and food safety (effectiveness of solute preservatives, water activity). The prevailing theory for explaining the water-holding properties of meat is that water is held within the microstructure of meat primarily by capillary forces. Water-holding is thus related to the calculated height (h) of water maintained in a narrow capillary tubes as predicted by the Jurin rule. The primary predictive parameters in this

model are pore radius, surface tension of the liquid phase, and contact angle at the interface of the liquid with the pore inner wall.

In considering capillary holding of water in intact meat and in meat-derived protein gels, most attention has typically been given to pore size. However, contact angle, which is affected by surface charge and degree of hydrophilicity/hydrophobicity, and solvent surface tension, which is affected by soluble proteins, salts, and preservatives, both can also considerably impact water-holding properties of meat and gels. There is now considerable evidence for long range ordering of water originating at charged surfaces, which has implications for explaining the Hofmeister series effects on water holding, as well as capillarity in general.

Methemoglobin in oxidation of marine lipids

Vera Kristinova^{1,2,*}, L. Skrabalova³, I. Størro², T. Rustad¹

¹Norwegian University of Science and Technology (NTNU), Trondheim, Norway

²SINTEF Fisheries and aquaculture AS, Trondheim Norway

³Brno University of Technology, Brno, Czech Republic

*vera.kristinova@sintef.no

Marine omega-3 lipids (EPA and DHA) have been shown to have numerous positive health effects. To add nutritional value and increase the healthiness of the food, food industry wants to incorporate marine lipids into a range of products, including pates, sausages and meat minces. However, the high susceptibility of marine lipids to oxidation leading to rancidity constitutes a large production and marketing barrier, and may also pose a health risk for consumers. To be able to resolve the industrial and food safety issues associated with lipid oxidation it is important to understand the basic factors and mechanisms underlying the oxidative processes.

Hemoglobin, a protein with an embedded heme-group containing iron, is one of the major prooxidants in meat and meat products, where the predominant form is met-hemoglobin containing Fe³⁺. The oxidation of liposomes made from marine phospholipids catalyzed by met-hemoglobin has been studied in relation to one of the key environmental conditions, pH, and the content of several natural identical antioxidants.

Measurement of the consumption of the dissolved oxygen, one of the essential substrates for lipid oxidation, has been used to follow the oxidation. This method is relatively fast and

allows studying both the reaction kinetics, and the effects of added compounds and environmental conditions. In addition, the oxidative stability was measured by determining primary and secondary oxidation products (PV and TBARS).

The results indicate that methemoglobin mediated oxidation is first order kinetics with respect to the methemoglobin concentration and that the magnitude of methemoglobin action is not dependent upon the dissolved oxygen concentration in the system, but is limited by lipid hydroperoxides. A series of antioxidants (alpha- and delta-tocopherol, astaxanthin, ascorbyl palmitate, ascorbic and caffeic acid), showed concentration dependent antioxidant effects. Darkness, heat treatment and decreasing pH in the solution inhibited the pro-prooxidant activity of methemoglobin, while low concentrations of hydrogen peroxide showed the opposite effects. Dissolution of methemoglobin resulted in partial iron leakage, thus for an effective lipid oxidation defense with the use of antioxidants, simultaneous low molecular weight (free) iron and methemoglobin catalyzed oxidation must be considered, suggesting the use of a chelator plus a radical scavenger.

Effects of the anti-caking agent potassium ferrocyanide ($K_4[Fe(CN)_6]$) on lipid oxidation of salted cod (*Gadus morhua*) during salting, storage and rehydration

***Minh Van Nguyen*^{1,2,*}, *K.A. Thorarinsdottir*², *G. Thorkelsson*^{1,2},
A. Gudmundsdottir^{1,3}, *S. Arason*^{1,2}, and *A. Bjarki Bergsson*²**

¹Faculty of Food Science and Nutrition, University of Iceland, Reykjavik, Iceland

²Matís ohf./Icelandic Food and Biotech R&D, Reykjavik, Iceland

³Science Institute, University of Iceland, Reykjavik, Iceland

*mvn2@hi.is (M.V. Nguyen)

Potassium ferrocyanide (CN) is a non-physiological iron compound and has been used for many years as an anti-caking agent in salt for human consumption. However, CN is believed to promote lipid oxidation in food systems. To our knowledge, information from effects of CN on lipid oxidation of fish and salted fish products has not been found.

The CN was already present in salt used (10 ppm), but the present study was carried out to investigate the effects of increasing levels of CN (additional 2.5, 7.5 and 100 ppm in salt) on lipid oxidation of salted cod during salting, storage and rehydration.

Lipid oxidation was determined by the measurements of lipid hydrolysis, primary and secondary lipid oxidation products as well as by changes in fatty acid composition and evaluation of fluorescence compounds. The colour of salted cod flesh was also measured.

The results showed that free fatty acid (FFA), hydroperoxides (PV), thiobarbituric acid-reactive substances (TBARS) and fluorescence compounds (δF_{or} and δF_{aq}) significantly increased during the salting process. With storage times, FFA, δF_{or} and δF_{aq} increased whereas PV and TBARS remained stable during

the first 4 months and tended to decrease during the last 2 months storage. After rehydration, a significant decrease in PV, TBARS and δF_{or} was observed. An increase in CN concentration accelerated lipid oxidation of the salted cod as observed by increases in PV and TBARS as well as in the development of δF_{or} and δF_{aq} . A yellow discolouration (higher b^* value) of salted cod was associated with higher levels of oxidation derivatives. High correlation between PV, TBARS and FFA as well as between FFA and δF_{or} was found. The results of principal component analysis showed that TBARS, b^* value and δF_{or} were strongest indicators of lipid oxidation with salting and storage time.

In conclusion, both CN concentration and processing time (processing stages and storage time) significantly affected the lipid oxidation progress. Rehydration process could sweep away partial lipid oxidation products, resulting in decreased PV, TBARS and δF_{or} value. The results of fluorescence compounds confirmed that fluorescence measurement is a promising technique for accessing the further lipid oxidation products (tertiary products).

Antioxidant potential of water hyacinth (*Eichornia crassipes*): *In vitro* antioxidant activity and phenolic composition

Alagarsamy Surendraraj^{1,*}, *K.H.S. Farvin*², and *R. Anandan*³

¹Institute of Food and Dairy Technology, Tanuvas, Alamathi, Chennai, India.

²Division of Industrial Food Research, DTU Food, Technical University of Denmark, Kgs., Lyngby, Denmark

³Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Matsyapuri (PO), Cochin, India

*asurendraraj@rediffmail.com

The aims of the present study were (a) to extract and quantify the main phenolic acids and tocopherols from the petiole, leaf and flowers of *Eichornia crassipes*, (b) to evaluate the antioxidant capacity of the extracts in four *in vitro* systems (DPPH radical scavenging ability, iron chelating activity, reducing power and prevention of oxidation in a liposome model system) and its effectiveness in retarding lipid peroxidation in fish oil by accelerated stability test. Significant differences were observed in total and individual phenolic content and antioxidant activities of extracts from the various parts of *E. crassipes*. Out of the 11 phenolic acids analysed, ethanolic extracts contained high

amounts gallic, protocatechuic, gentisic and p-hydroxybenzoic acid, whereas, water extracts contained less amounts of varied number of phenolic acids. Ethanolic extracts of flower, which contained the highest total phenolic content, were found to have high DPPH radical scavenging activity and reducing power. Ethanolic extracts of leaf were found to have high Fe²⁺ chelating activity and inhibited lipid peroxidation in liposomes and fish oil. Our results demonstrate that *E. crassipes*, an underutilized aquatic weed, could be a potential natural antioxidant source for food, feed and pharmaceutical applications.

Effect of the Maillard reaction on antioxidant activity and functional properties of a sugar – shrimp hydrolysate system

Claire Sabourin¹, N. Decourcelle^{1,2,*}, Y. Grohens³, T. Benvegnu⁴, T. Aubr⁵, and F. Guérard¹

¹Université de Bretagne Occidentale, Laboratoire des Sciences de l'Environnement marin LEMAR - UMR 6539 CNRS/IRD/UBO - Institut Universitaire Européen de la Mer, Plouzané, France

²Université de Bretagne Occidentale, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne (LUBEM, EA 3882), IFR148 ScInBioS, IUT de Quimper, Bâtiment G, Quimper, France

³Université de Bretagne Sud, Laboratoire d'Ingénierie des Matériaux de Bretagne, Equipe E2PIC, , Lorient Cedex, France

⁴Ecole Nationale Supérieure de Chimie de Rennes, Equipe COS (Chimie Organique et Supramoléculaire) – UMR 6226, Campus de Beaulieu, Rennes Cedex, France

⁵Université de Bretagne Occidentale, Laboratoire de Rhéologie, Brest Cedex, France

* nicolas.decourcelle@univ-brest.fr

The modification of proteins or peptides through the Maillard reaction (MR) has an impact both on their technofunctional and biological properties. Within this context, this work showed the effects of MR on the emulsifying and antiradical properties of a shrimp hydrolysate (SH). The MR was conducted in dry (50°C; relative humidity = 75 %; 48 h) and mild conditions with a mix of SH (62,5 % (w/w)) and one of the following sugars (37,5% (w/w)): Xylose (X), Glucose (G), Fructooligosaccharide (FOS) or Dextran (D), respectively.

The reactivity of sugars was quantified by the loss of lysyl residues after MR (OPA assay). Reduction of creaming was performed, for emulsifying properties, during an ageing period of 240 min. The emulsions tested had a 50/50 (w/w) oil/water ratio and contained 0.05 % (w/w) of the glyconjugates prepared in distilled water without pH adjustment. The antiradical activity was determined using the reducing power assay and the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.

Loss of available amino groups expressed in terms of lysyl residues were 53 %, 32 %, 20 % and 11 % for SH incubated with Xylose, Glucose, FOS and Dextran, respectively, suggesting a higher reactivity of xylose and glucose compared to FOS and dextran in MR. These data were confirmed from size exclusion chromatograms using Superdex columns (Amersham). In emulsions, the mixture SH-X decreased creaming by 35 % ($p < 0.05$) compared to SH. Furthermore, SH-X and SH-G showed an increase of 23 % and 17.5 % of the reducing power compared to native SH. This increase was comparable to the one determined with the DPPH assay.

From these results, it can be assumed that emulsifying properties were affected by the Maillard reaction. The combination of the improvements of antiradical properties and emulsifying properties can lead to the development of new bifunctional ingredients.

Determination of quality and shelf life of rainbow trout (*Oncorhynchus mykiss*) coated with crab (*Potamon potamios*, Olivier 1804) chitosan during storage

A. Günlü¹, Sengül Bilgin^{1*}, Y. Bolat¹, L. İzci¹, S. Bahadır Koca¹, and S. Çetinkaya²

¹University of Süleyman Demirel, Eğirdir Fisheries Faculty Isparta, Turkey

²Ministry of Agriculture, Eğirdir Fisheries Research Institute Isparta, Turkey

*sengulb@gmail.com

The effects of chitosan coating (1%, w/v) on quality, shelf-life, changes in cholesterol and fatty acid contents of fresh and smoked rainbow trout (*Oncorhynchus mykiss*) fillets during storage (30 days) at $+4\pm 1^\circ\text{C}$ were investigated. Freshwater crab (*Potamon potamios*) was used for recovery of chitin and chitosan in this research. Fresh Rainbow trout fillets were coated with freshwater crab chitosan and commercial chitosan by using two different methods. The control group and treated fish samples were analyzed periodically (after 0, 5, 10, 15, 20, 25 and 30 days) for microbiological (TMA, TPA,

coliform bacteria, yeast-mould counts), chemical (pH, TBARS, TVB-N, fat, fatty acid, moisture and cholesterol), and sensory characteristics.

The results indicated that the effect of chitosan coating on fish samples was retention of good quality characteristics and extended shelf life during storage, which was supported by the results of microbiological, chemical and sensory evaluation analyses. In almost all groups the cholesterol value increased during storage. Total SFA, MUFA and PUFA contents did not change during storage.

Extended shelf life of industrially rehydrated salt cured cod by preservation with citric acid and potassium sorbate baths

B. Berlyanto Sedayu¹, H. Herland^{2,*}, C. Van Keer¹, and Heidi A. Nilsen²

¹KaHo Sint-Lieven, School of Engineering, Gebr. Gent, Belgium

²Nofima, Tromsø, Norway

*hilde.herland@nofima.no

Salt-cured cod and dried salt-cured cod (klipfish) are important products made from fresh cod. Salt-curing preserves the fish, hence increases the storage stability of the fish. Before the fish is consumed, it must be rehydrated. This may be a time consuming task and a dish made with these products must be planned ahead. Storage stability of rehydrated salt-cured cod has traditionally been rather short, but recent work has shown that by using new preservation techniques as well as new packaging methods it is possible to prolong the shelf-life of rehydrated cod. In this work we have studied effects of an industrial rehydration technique in combination with preservative baths and different packaging methods on quality of rehydrated cod.

Salt-cured cod pieces were rehydrated in water for 48 h with water changes every 6 hours. The pieces were injected with water at the 3rd water change using an industrial injector. After rehydration, the fish was treated with either citric acid (CA) or a successive treatment of citric acid and potassium sorbate (PS), for 15

minutes (CA) and 60 s (PS) respectively. The samples were either packed in vacuum or modified atmosphere (MAP), and stored cold.

The preservative treatments did not appear to have an effect on the quality of the fish. The use of the organic acids did have an effect on the pH of the samples, but this did not appear to alter the sensory quality. The preservation using both CA and PS did however influence the microbial development in the samples during storage; there were significantly less psychrotrophic bacteria in these samples than the untreated or treated with only CA samples. The samples treated with CA and PS also had significantly lower levels of total volatile base nitrogen (TVB-N). The sensory results correlated with the microbial and TVB-N results.

The results show that a CA bath is not sufficient to extend the shelf-life of industrially rehydrated cod, while using both CA and PS increases the shelf-life from 7 days to 21 days, regardless of packaging method.

Poster 4.1

Lipid oxidation and textural properties of refrigerated fish emulsion sausages as influenced by tannic acid and kiam wood extract

S. Maqsood¹, Soottawat Benjakul^{1,}, and A. Khansaheb Balange²*

¹Department of Food Science, Faculty of Food and Agriculture, United Arab Emirates University, Al-Ain, UAE

²Department of Fish Processing Technology and Microbiology, College of Fisheries, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Maharashtra, India

*soottawat.b@psu.ac.th

Fortification of marine fish oil rich in n-3 PUFA to food products could be an alternative means to improve its fat quality. However, marine fish oil is susceptible to lipid oxidation thereby negatively affecting quality and the nutritional value of fish products. To retard such a quality loss, antioxidants have been used to decrease lipid oxidation. The use of natural antioxidants is emerging as an effective means for controlling lipid oxidation and limiting its deleterious consequences. The research was undertaken to study the effect of tannic acid (0.02 and 0.04%) and ethanolic kiam wood extract (EKWE) (0.04 and 0.08%) on lipid oxidation and textural properties of fish emulsion sausages during 20 days of refrigerated storage. Control samples (C) had the highest peroxide value (PV) and thiobarbituric acid-reactive substances (TBARS) value up to day 16 and 8 of storage, respectively. With the addition of tannic acid and EKWE both PV and TBARS values in the sausages were retarded effectively, compared to the control ($P < 0.05$), especially when the tannic acid and EKWE at higher concentration were used. At the same concentration EKWE showed lower ability

to retard lipid oxidation in comparison with tannic acid. Tannic acid at both concentrations (0.02 and 0.04%) was also effective in retarding the formation of fishy odour in the samples throughout the storage compared to the control and EKWE treated samples ($P < 0.05$). Both tannic acid and EKWE had no detrimental effect on the sensory attributes of sausages. However EKWE treated sample had lower L^* and higher a^* and ΔE^* values, compared to the control samples ($P < 0.05$). After 20 days of storage the sample with added 0.04% tannic acid had higher hardness, gumminess and chewiness, compared with the others ($P < 0.05$). Samples added with 0.04% tannic acid also displayed more compact structure with no visible voids. Furthermore oil droplets with smaller size were dispersed more uniformly compared to the other samples. Thus tannic acid (0.02 and 0.04%) and EKWE (0.08%) were effective in retarding lipid oxidation and fishy odour development as well as at maintaining textural properties of fish emulsion sausages during the refrigerated storage of 20 days.

Poster 4.2

Effects of olive leaf extracts on fish fillets

Can Altinelataman^{1,*}, U. Çelik¹, E. Misimi², T. Dinçer¹, E. Burcu Şen¹, and A. Erdem¹

¹Ege University, Faculty of Fisheries, İzmir, Turkey

²SINTEF Fisheries and Aquaculture AS, Trondheim, Norway

*can.altinelataman@ege.edu.tr

In this study, the visual, textural, chemical, microbiological and sensorial effects of olive leaf extract on refrigerated sea bass fillets were examined during a 9 days period. Olive leaf extract were chosen due to their availability in the region and low-cost. Olive leaves were obtained from wild trees in the coastline zone on west of İzmir and sea bass from fish farm in Çeşme. Fresh olive leaves were dried at 40°C for 48 hours. 40 gr. dried leaves were boiled in 2 liters distilled water for 15 min. and extracts cooled down to room temperature. Fish were gutted, cleaned and filleted. Fillets were kept in extract for 20 min. and stored at 4°C. In addition, image color analysis and surface texture image analysis of fillets were used to evaluate the effect on the visual aspects of treatment with olive leaf extract. Texture profile analysis was used to evaluate adhesiveness and chewiness.

No significant difference in colour (L, a, b, Hue, Chroma) was found at the end of storage (day 9) between the samples of C (C: Control group; P: Processed samples with olive leaf extract) and P

as analyzes with image analysis. Several statistical texture descriptors based on average were calculated.

Adhesiveness of group C (at day 0) was significantly higher than group P, while the chewiness of group C was significantly reduced for 50% during 9 days. The sensorial analysis shows no significant difference between the groups. Microbiological analysis shows (according to TAMB counts) that neither groups did exceed the acceptable limit (< 6 log CFU/g) at the end of storage.

TVB-N values of group C for days 2,5,7,9 were significantly higher than the group P, while TBA values for C for days 5,7,9 were also significantly higher than for group P.

Since olive leaf extract has shown protective effect on TVB-N and TBA values, and no negative effects for visual and sensorial aspects, it can be effectively used for keeping quality parameters of fish fillets.

Poster 4.3

The effects of natural antioxidant extracts (thymus, green tea, sage and laurel) on the fatty acid content of fish burgers made from minced chub mackerel (*Scomber japonicus*)

Y. Özogul¹, E. Balıkcı¹, A. Şimşek¹, Deniz Ayas^{2,*}, M. Kenar¹, Ç. Kaçar¹, H. Yazgan¹, and S. Gökdoğan¹

¹Cukurova University, Fisheries Faculty, Adana, Turkey

²Mersin University, Faculty of Fisheries, Mersin, Turkey

*ayasdeniz@mersin.edu.tr

The effects of the use of natural antioxidant extracts (thymus, green tea, sage and laurel) on the fatty acid profiles of chub mackerel burgers stored at -18°C were investigated. In this project, fresh chub mackerel (*Scomber japonicus*) were used to prepare fish burgers. In addition to the ingredients for burger, natural antioxidants obtained from thymus, green tea, sage and laurel were added in fish burgers at different levels (0.3% and 0.6%) and burgers were stored at -18°C for 9 months. Fatty acid composition measurements of the fish burgers were carried out every month during a storage of 9 months. Lipid content was measured by the method of Bligh and Dyer (1959). Methyl esters were prepared by transmethylation using 2M KOH in methanol and *n*-heptane according to the method as described by Ichibara et al. (1996).

The most abundant fatty acids in fish burgers were myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1 n-9) vaccenic acid (C18:1n7), linoleic acid (C18:2n-6), cis-5,8,11,14,17-eicosapentaenoic acid (EPA, C20:5n-3) and cis-4, 7,10,13,16,19-docosahexaenoic acid (DHA, C22:6 n-3). The levels of SFA and MUFA fluctuated during the storage period in all groups. The levels of PUFA during the entire storage period were found to be 37.89-41.23% for the control; 38.04-43.24% for thymus; 39.51-43.05% for laurel; 33.93-41.36% for sage and 39.01-43.15% for green tea extract. The results showed preservative effects on PUFA for all extracts except for sage extract.

Poster 4.4

The effects of natural antioxidant extracts (thymus, green tea, sage and laurel) on the biogenic amine contents of fish burgers made from minced chub mackerel (*Scomber japonicus*)

Y. Ozogul¹, Y. Uçar¹, E. Kuley¹, F. Ozogul¹, E. Balıkcı¹, and Deniz Ayas^{2,*}

¹Cukurova University, Fisheries Faculty, Adana, Turkey

²Mersin University, Fisheries Faculty, Mersin, Turkey

*ayasdeniz@mersin.edu.tr

The effects of the use of antioxidant technology on the biogenic amines formation of chub mackerel burgers stored at -18°C were investigated. Chub mackerels (*Scomber japonicus*) were purchased from a local fish retailer (average weight 100-150g). Fish were headed, gutted and filleted. Fillets were then minced with a meat mixer. The fish meat was divided into nine groups. These are; the control with no plant extract, T1 with 0.3% thymus extract, T2 with 0.6% thymus extract, G1 with 0.3% green tea extract, G2 with 0.6% green tea extract, S1 with 0.3% sage extract and S2 with 0.6% sage extract, L1 with 0.3% laurel extract and L2 with 0.6% laurel extract. Fish burgers were prepared according to the method of Tokur et al. (2004) with minor modification. The mackerel burger mean weight was 50 g and there were 100 fish burgers in each group, composed of 87.8 % minced mackerel meat, 6% corn flour,

4% wheat flour, 0.2% garlic powder, 1.2% salt, 0.6% sugar and 0.2% onion powder. Plant extracts and other ingredients were added to each group and mixed well by a domestic mixer. The control and treated groups were packaged in cling film and stored at -18°C for 9 months. Eleven biogenic amines were investigated namely, histamine, putrescine, cadaverine, spermidine, spermine, tryptamine, tyramine, 2-phenylethylamine, agmatine, dopamine, and histamine. As storage time progressed all amines were dominant except for histamine and 2-phenylethylamine. Dominant amine levels also fluctuated during the storage period. Biogenic amine production developed at a slower rate in the fish burgers treated with natural antioxidant extracts. Natural antioxidant extracts were found to be effective in controlling the growth of bacteria and thus, biogenic amine formation.

Poster 4.5

Combined effect of packaging and chitosan on the shelf-life of fresh Mediterranean swordfish (fillet steaks) stored under refrigeration (4°C)

M. Tsiligianni¹, Ekaterini Papavergou², N. Soultos², T. Magra², and I.N. Savvaidis^{1,}*

¹Laboratory of Food Chemistry and Food Microbiology, Department of Chemistry, University of Ioannina, Ioannina, Greece

²Department of Hygiene and Technology of Food of Animal Origin, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

*isavvaid@uoi.gr

The aim of the present study was to examine the combined effect of chitosan (1 % v/w) and packaging (aerobic, vacuum) on the shelf-life of fresh Mediterranean swordfish (fillet steaks) stored under refrigeration (4 °C) for a period of 28 days. Treatments examined in the present study were the following: A-S (control samples, aerobic packaging, untreated), A-CH-S (chitosan treated; 1 % v/w, aerobic packaging), VP (vacuum packaging, untreated) and VP-CH-S (chitosan treated; 1 % v/w, vacuum packaging). Swordfish steaks were dipped into chitosan solution for 1.5 min and samples were immediately packaged and stored for 8 (A-S), 12 (A-CH-S), 17 (VP) and 28 (VP-CH-S) days under refrigeration. Quality and shelf-life of the samples, under the above mentioned treatments, were assessed by using both microbiological and sensory analyses. The bacteria that prevailed in swordfish samples were; *Pseudomonas* spp., H₂S-producing bacteria, lactic acid bacteria (LAB) and *Enterobacteriaceae* (to a lesser extent). The bacteria prevailed, irrespective of treatment and reached high numbers (6.6-8.0 log cfu/g) after 8 days of storage. Treatment VP-

CH-S resulted in significantly lower bacterial populations for all the species examined, as compared to the control (A-S) samples. With regard to determined chemical freshness indices, thiobarbituric acid (TBA) values were low in all swordfish samples. Treatments A-CH-S, VP and VP-CH-S produced lower total volatile basic nitrogen (TVBN) and trimethylamine nitrogen (TMAN) values as compared to the A-S samples after day 8 and until the end of the storage period. Treatments A-CH-S and VP-CH-S resulted in lower levels of the biogenic amines tyramine, putrescine and cadaverine as compared to the A-S and VP samples. Levels of histamine remained low throughout the storage period for all treatments. Spermidine and spermine contents did not present any significant changes during storage of all swordfish samples. Based primarily on sensory evaluation (odor and taste attributes), the use of A-CH-S, VP and VP-CH-S extended the shelf-life of fresh Mediterranean swordfish fillets by ca. 3, 8 and 12 days, respectively, as compared to the control sample.

Poster 4.6

Press juice from herring (*Clupea harengus*) as a glaze for frozen herring fillets

Lillie R Cavonius* and I. Undeland

Chalmers University of Technology, Department of Chemical and Biological Engineering, Food Science, Gothenburg, Sweden

*lillie.cavonius@chalmers.se

Herring muscle Press Juice (PJ) is an aqueous fraction derived from the centrifugation of herring mince. It has previously been shown to have antioxidative effect in several different systems, and thus has potential application as a glaze on frozen fish fillet, especially in species prone to oxidation, such as herring. The glazing in itself poses a physical barrier to oxygen and it is well established that glazing improves the oxidative stability of frozen fillets and blocks. The primary aim of this study was to compare the increase in lipid oxidation of herring fillets glazed with herring PJ or water during frozen storage. However, since it has been shown that the pH of a fish sample is very important for the development of lipid oxidation, especially when mediated by hemoglobin (Hb), we chose to also include a control group in which the fillets were glazed with a 50 mM phosphate buffer having the same pH as the PJ (6.5).

After eight weeks of frozen storage, all fillets had a significantly higher concentration of primary oxidation products (measured as peroxide value, PV) compared to the start value; the largest increase was observed in the water-glazed fillets, the smallest in the buffer-glazed fillets. The concentration of secondary oxidation products (measured as TBA reactive substances) also increased significantly over 8 weeks; the largest increase was observed in the PJ-glazed group, the smallest in the buffer-glazed group. Up to 8 weeks, the phosphate-buffer as a glaze was thus a superior oxidation protectant in frozen herring fillets. That the PJ-glaze did not perform as well as hoped was presumably due to oxidation of trace lipids present in the PJ. This will be investigated further.

Poster 4.7

Oxidative stability of frozen mackerel batches – A multivariate data analysis approach

M. Helbo Ekgreen, S. Frosch, and Caroline P. Baron*

National Food Institute, Technical University of Denmark, Kgs Lyngby Denmark

*carba@food.dtu.dk

Mackerel are usually caught in the autumn and often frozen either on board the fishing vessel or soon after landing. Due to the seasonality of the catching period, mackerel can be stored frozen for a long time period before entering the production chain. However, frozen storage of fatty fish such as mackerel can lead to a significant loss in fish quality primarily due to oxidation of the long chain omega-3 fatty acids. These quality changes results in significant loss for the fish processing industries and in fish with poor eating quality.

In order to investigate batch-to-batch variation due to different catching methods and different freezing procedures 6 batches of frozen mackerel were obtained from the local producer of caned mackerel. Fish were processed as soon as possible after landing i.e. headed, gutted and individually frozen at the industry. However, one of the catch was abused and stored on ice for 8 days before entering the production line.

Subsequently, samples from the catches were sent to our laboratory where they were stored frozen at -30 °C for a period of 12 months. At intervals of 6 weeks samples were taken and analysed for proximate analysis as well as for oxidative deterioration and texture changes. The aim was to investigate the correlation between the raw material history and the quality loss observed during frozen storage using relevant multivariate data analysis such as Principal Component Analysis (PCA) and Partial Least Square Analysis (PLS). Preliminary results showed that it was possible to differentiate between the different batches depending on their history and that some batches were more oxidised than others. Furthermore, based on the results from the data analysis, critical control points in the entire production chain will be identified and strategies to prevent quality loss proposed.

Poster 4.8

Quality changes during frozen storage of Atlantic salmon (*Salmo salar*) at -40°C and -20°C

A. Palihawadana, E. Sirnes, M. Bjornevik, Ørjan Hagen, and C. Solberg*

Faculty of Biosciences and Aquaculture, University of Nordland, Bodø, Norway

*Christel.Solberg@uin.no

Norway is the largest producer of Atlantic salmon in the world. In 2009 about 800 000 tons were produced. A lot of the Atlantic salmon from Norway is exported as frozen. In the industry fish are normally stored frozen at -20 °C, with a limited shelf storage time. When stored at -40°C shelf storage time will increase with several years. It is therefore of interest to study how different freeze storage temperatures influence the quality of salmon from harvest to consumer. In this experiment we investigated the influence of low in-freezing temperature (-40°C in 1 week) and thereafter freeze stored at either -20°C or -40°C.

A total of 80 farmed pre rigor salmon were slit in a left and a right fillet, vacuum packed in plastic bags and individually frozen at -40°C for one week to give the same starting point. Thereafter one fillet from each fish was either stored at -20°C or -40°C. Drip loss, colour, texture, and fish muscle histology were analyzed

after 6, 12, 18, 24, 30, 36, 42, 48 and 54 weeks of frozen storage. Proximate composition and fatty acid analysis were performed after 0 days, 6, 18, 30, 42 and 54 weeks of frozen storage. The drip loss increased after 30 weeks at -20° and the measured colour did not change and were rather similar at both temperatures. Texture measured as shear force were about 150 mJ at the start, and then gradually decreased to 70 mJ after 30 weeks, these changes were similar at both temperatures. Histological observation shows that the detachment of myofibre-myofibre and myofibre-myocomata increased during the storage time, more at -20°C than -40°C. The degradation of EPA and DHA was less than 0.5% after 54 weeks of storage.

Vacuum packing and freezing to -40°C before storage prevented degradation of the salmon during 1 year's storage at -20°C.

Poster 4.9

How raw composition influences the development of lipid oxidation in mackerel (*Scomber scombrus*) muscle

***Rodrigo Maestre**, M. Pazos, and I. Medina**

Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Spain

*maestre@iim.csic.es

Lipid oxidation is the major cause of deterioration of fish muscle, especially in pelagic fish species. It is due to its high amount of fat, mainly composed by PUFAs, that act as substrate of lipid oxidation. However, the kinetics of the oxidative reaction is highly influenced by the occurrence of other components of fish muscle, such as the existing lipids and substances with capacity to catalyze or inhibit the formation of free radicals. The different sources of iron and their concentrations, together with haemoglobins and intrinsic reductants have also demonstrated a relative impact on muscle lipid oxidation. The complex relations among these parameters and how they affect the susceptibility of fish muscle to develop lipid oxidation is still unclear.

The goal of this research was to study the relationship between the muscle composition of Atlantic mackerel (*Scomber scombrus*) and its susceptibility to develop lipid oxidation during chilled storage. Compositional variations of fish at different seasonal periods were investigated, and then, related with the onset of lipid hydroperoxides.

An array of compositional parameters was determined in mackerel muscle. The data matrix built was correlated with the onset of lipid oxidation during chilled storage. Pearson correlations tests and multiple regressions were performed to reveal the components having higher contribution to explain the susceptibility to lipid oxidation.

The model obtained by multiple regressions revealed that the content of PUFAs together with the concentration of iron, hemoglobin and ascorbic acid were the most significant parameters affecting the accumulation of lipid hydroperoxides. These results establish that the levels of endogenous pro-oxidants and antioxidants present in mackerel muscle, together with the substrate of lipid oxidation are relevant factors that have significant influence in the onset of lipid oxidation.

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Poster 4.10

Roughhead grenadier: from trap to dish

***Kari Lisbeth Fjørtoft**, B.T. Nystrand, S. Bakke, and J. Kennedy**

Møreforsking Marin, Ålesund, Norway

*kari@mfaa.no

Roughhead grenadier (*Macrorous berglax*) is a deep-sea fish distributed along the coast of Norway, towards Svalbard, around Great Britain, Faroe Islands, Iceland, Greenland, and on the east-coast of Canada. Findings have shown that grenadier is regarded as a high quality fish and appreciated for its mild shellfish taste and texture, as well as for having numerous preparation options. Compared to traditional commercial species, market surveys have shown that there is a potential for obtaining a price similar to that of cod (*Gadus morhua*) and that there is a demand for grenadier, particularly in the finer restaurant segment and consumers in and around the capital of Norway.

At present, landings are at a low level but do exist (mostly frozen). In Norway, grenadier is mainly caught as by-catch in a strictly regulated Greenland halibut (*Reinhardtius hippoglossoides*) fishery, and is usually discarded due to lack of market. In order to commercialize grenadier, it is important to have access to raw material on a regular basis. It is therefore

essential to develop catch strategies that are highly selective for the species, and exclude other species, like Greenland halibut.

Fishermen are reliant on further provision if they are to embark on dedicated grenadier fishing. On the other end of the value chain, buyers (e.g. restaurants) need assurance that the fish can be delivered on a regular basis, particularly if it is to be put on their menus. Traps are regarded as selective and gentle fishing gears, capable of catching high quality fish. Tailor-made traps might contribute to satisfy both fishermen and their buyers in this respect. Traps may also be used in combination with other fishing gears, which may be an important factor for the fishermen in order to obtain a profitable fishery. If the traps are found to be efficient, *i.e.* no by-catch of Greenland halibut, the fishery for this species might evolve towards a profitable, year-round activity supplying the market on a regular basis. A deep-sea fishing trap was therefore adjusted and tested, aiming at a selective grenadier fishery.

Poster 4.11

Myosin denaturation in salmon meat distributed in chilled or frozen form

Kunihiko Konno*, *W. Thavaraj, and Y. Konno*

Faculty of Fisheries Sciences, Hokkaido University, Hokkaido, Japan

*konno@fish.hokudai.ac.jp

Salmon is one of the most popularly accepted fish species in the world. Some of them are wild and others are cultured. They are distributed in chilled or frozen form. Scientific studies of the quality of the meat are important. Based on the idea that a major component of the meat is myosin and that its susceptibility towards denaturation determines the quality, we proposed techniques to evaluate the quality of frozen Surimi. These are ATPase activity, salt solubility, monomeric myosin contents and chymotryptic digestion products. The same idea was applied to evaluate the quality of salmon meat or fillets. We also studied whether the stability of myosin from several species of salmon is the same or not.

Thermal stability of salmon myosin in myofibrils (Mf) (0.1 M NaCl, pH 7.5) was studied by measuring Ca-ATPase inactivation rate. Samples used were from Norway, Chile, Australia (cultured Atlantic salmon), Canada (wild sockeye salmon), and Japan (wild chum salmon). There was little difference in the

stability among salmon species showing a 50% ATPase inactivation in 20 min at 35 °C. Myosin stability in meat was further studied. Heating chum salmon meat at 35 °C damaged myosin to a similar extent as that obtained with homogenized meat Mf. Storage of the meat in refrigerator (5 °C) for a week did practically not cause myosin denaturation. Myosin denaturation upon freezing of the meat was also studied at -10, -20, and -40 °C. Myosin denaturation progressed remarkably at -10 °C showing roughly a 50% inactivation of ATPase in 3 weeks but only a slight decrease at -40 °C. Loss of salt solubility and myosin aggregation preceded the ATPase inactivation. Myosin denaturation in commercially available imported salmon meats at the supermarket in Japan was also analyzed. Decreased salt solubility, loss of monomeric myosin, and severe degradation of the subfragment-1 region of myosin upon chymotryptic digestion analysis were characteristic changes detected.

Poster 4.12

Comparing the activity of polyphenol oxidase enzyme in different parts of deep water pink shrimp (*Parapenaeus longirostris*) by using L-dopa substrate

Sukran Cakli^{1,*}, T. Dincer¹, A. Cadun¹, H. Rehbein², S. Tolasa¹, E.B.Y. Sen¹, O.A. Erdem¹, N. Demirtas¹, S. Dogan³, C. Kocak⁴, and A. Tokac¹

¹Ege University, Faculty of Fisheries, Department of Fishery and Fish Processing Technology, İzmir, Turkey

²Max Rubner-Institute Department of Safety and Quality of Milk and Fish Products

³Balıkesir University, Faculty of Science and Literature, Department of Biology Balıkesir, Turkey

⁴Ege University, Faculty of Fisheries, Department of Hydrobiology, İzmir, Turkey

*sukran.cakli@ege.edu.tr

Pink shrimp was caught monthly from three different parts of the Aegean Region for a year (except during the fishing forbidden period). The shrimp was caught by trawl and transported by algarna fishing vessels. After being caught, gender was determined and the samples were separated. Sampling of female and male shrimp was done from regions where melanosis occurs. For each gender, sampling was made as follows: carapace, abdomen, cephalothoraxes', pleopods, pereopod, uropod and telson. Analysis of all samples was done in triplicate. The analyses were done to determine the initial condition of the shrimps. The rapid implementation of flake ice and dry ice during transportation was expected to decrease the development and occurrence of black spot.

In this study the activity of polyphenol oxidase (PPO) enzyme was determined in commercial shrimp (*Parapenaeus longirostris*), by using L-DOPA as substrate. The activity was measured in samples from specific parts of the shrimp, in shrimp from different regions, and subjected to different time periods of exportation and processing. Also the differences in melanosis formation were determined. Enzyme activity was evaluated so that the effect of different capture regions, time of catch and gender on melanosis development and spread could be determined. The effect of gender on spreading of melanosis was investigated.

The results of this study contain the data of project number 109O210 which was sponsored by TÜBİTAK.

Poster 4.13

Quality changes during salt curing of codfish (*Gadus morhua*)

***Helena Oliveira*^{1,2,3,*}, *A. Gonçalves*², *S. Pedro*², *M.L. Nunes*², *P. Vaz-Pires*¹, *R. Costa*³**

¹ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, Largo do Prof. Abel Salazar, Porto, Portugal

²INRB,I.P./IPIMAR, Lisboa, Portugal

³ESAC – CERNAS, Escola Superior Agrária de Coimbra, Coimbra, Portugal

*helaoliveira@gmail.com

Salting, an ancient procedure to get a self-stable product, is at the present time mostly used to promote important sensorial changes that make such products very valued by consumers.

The focus of this work was to assess quality changes in codfish (*Gadus morhua*) during salt curing at temperatures between 3 and 12 °C and relative humidity lower than 60 % by chemical, sensorial, microbiological and physical analysis. The evaluation was regularly made over the curing process (6 months) on a batch of 250 kg, each fresh fish having a weight between 2 to 3 kg. During curing, the water content decreased from 58.37 to 50.60%, 54.11%, 53.72% and 44.27%, at 3°, 6°, 9° and 12 °C, respectively and protein content ranged from approximately 19.5 to 21.70%, 25.00%, 21.89% and 27.59% at 3°, 6°, 9° and 12 °C, respectively. Also, the salt content ranged from 19.96 to 21.92%, 20.72%,

21.58% and 24.51% at 3°, 6°, 9° and 12 °C, respectively. TVB-N levels increased during curing although the final values were lower than 19 mg/100g. In what concerns the content of free amino acids nitrogen a relevant increase (about 60 %) was observed. According to sensorial analysis, odors and colors indicating some degradation were never found. The microbial results showed that proteolytic bacteria were the most resistant throughout all the process. In general, curing makes the fish whiter. The results allow concluding that in cod cured at temperatures up to 12 °C the quality was not affected.

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Poster 4.14

Effects of different extraction conditions on the phenolic compounds of extracts from *Sargassum angustifolium*; in microwave-assisted extraction

Aria Babakhani Lashkan^{1,*}, M.Rezaei¹, K. Rezaei², and S.J. Seifabadi¹

¹Tarbiat Modares University, Noor, Iran

²University of Tehran, Tehran, Iran

*aria_babakhani@yahoo.com

Microwave-assisted extraction (MAE) is a new method for extraction of bio-compounds from natural resources. These procedures have many advantages including; consuming shorter time, needing less solvent and energy. In this study, Microwave-assisted extraction was applied for extraction of algal extracts from brown algal species (*Sargassum angustifolium*) of Persian Gulf. Each of the experimental parameters (solvent, sample to solvent ratio and extraction time) was applied at three levels according to Taguchi's statistical design. Extraction condition consists of three solvents (ethanol, methanol and water), three rates of algae to solvent (1/5, 1/10 and 1/20) and three extraction times and powers

of microwave (10min*90W, 20min*180W and 30 min*270W). After extraction procedure, total phenol contents of extracts are determined. Water extracts (72.99 TAEQ) of *Sargassum angustifolium* have highly phenolic compounds in comparison to other extracts (methanol; 11.25 TAEQ and ethanol; 5.16TAEQ). The results showed that microwaving has good effects on the water extraction as this solvent extract has high total phenol. The best ratio of sample to solvent for extraction of this alga is 1 to 20. Further the best condition for extraction of phenolic compound from *Sargassum angustifolium* is 30 min extraction time and a microwave power of 270 W.

Poster 4.15

How to upgrade sensory potential of salmon by-product hydrolysates used as high quality source of protein?

Christelle Kouakou^{1,2,*}, R. Baron², J.-P. Berge², L. Lethuaut¹, C. Prost¹, and M. Cardinal²

¹LUNAM University, ONIRIS, UMR 6144 GEPEA, Flavour Research Unit, Nantes, France

²Biotechnology and Marine Resources Department (BRM), Science and Technology Laboratory of Marine Biomass (STBM) IFREMER, Nantes, France

*christelle.kouakou@ifremer.fr

Among possible strategies for upgrading salmon by-products, hydrolysate production seems to be a solution in order to obtain a high protein source. However, its important fish flavour is sometimes associated to rancid odour and/or flavour, and can limit its use in food industry. The aim of this work is to find solutions to reduce or mask off-flavours generated during enzymatic hydrolysis. Impact of process conditions (hydrolysis time and temperature), addition of antioxidant and/or sugar on odour and biochemical properties were evaluated in order to control lipid oxidation and promote the Maillard reaction that generates sweet notes and aromatic compounds able to mask fish note perception.

Biochemical (protein and available amino acids contents) and sensory tests (conventional odor profiling) were performed on 41 hydrolysates established according to a D-optimal experimental design. Sensory profiling were realised by sixteen panellists, trained to score the intensity of ten selected odour descriptors. Two-way ANOVAs and multiple range tests were performed to reveal if hydrolysis conditions could induce significant variations of

odour profiling. Principal component analysis were also performed to highlight the main odours associated with each product.

Grilled, sulfur/gas, global intensity and fat fish odours are the descriptors which have the highest discriminative power between samples. Sensory descriptors have been modeled according to hydrolysis conditions. As expected, the global intensity and grilled odour are strongly associated with sugar addition, while hydrolysis time and temperature have a significant effect on sulfur odour. According to hydrolysis conditions, soluble protein content varied from 7 % to 12 % whereas available amino acid composition varied from 61% to 97%.

Odour potential and nutritional properties of salmon by-products could also be modulated by hydrolysis conditions. This work paves the way and constitutes a promising mean to produce high value-added ingredients issued from enzymatic hydrolysis of marine by-products, which could be used as nutritional and/or flavor ingredients in food or feed.

Effect of heat treatment on enzymes, chemical composition and long time storage in the marine zooplankton *Calanus finmarchicus*

***Maria Bergvik*¹, *I. Overrein*², *M. Bantle*³, *J.O. Evjemo*¹, and *T. Rustad*⁴**

¹Norwegian University of Science and Technology (NTNU), Department of Biology

²Lipotek, Trondheim, Norway

³NTNU, Department of Energy and process Engineering, Trondheim, Norway

⁴NTNU, Department of Biotechnology, Trondheim, Norway

*maria.bergvik@bio.ntnu.no

Calanus finmarchicus is a marine zooplankton of interest for the aquaculture industry, as well as for nutraceuticals and the cosmetic industry. Their storage lipid is wax esters (fatty acid esterified with fatty alcohol), with a high content of n-3 fatty acids that vary with season. The chemical composition of *C. finmarchicus* rapidly changes *post mortem* due to autolytic processes. The phospholipids (PL) have a high content of C22:6 n3 and C20:5 n3 and are rapidly decreasing giving an increased content of free fatty acids (FFA). At the same time the proteolytic activity decompose proteins to peptides and free amino acids (FAA). The aim of the study was to inactivate enzymes in *C. finmarchicus* by heat treatment, and to explore effects of heat and long time storage on the chemical composition.

C. finmarchicus was sampled from the coast of Central Norway (spring 2008) and mixed with boiling water, giving a final temperature of 70 °C. The heat was held for 5, 10, 15, 20, 25 and 30 minutes. After heat treatment, each sample

was separated in a solid and a water phase, and analyzed for enzymes, proteins and lipids. Untreated and heat treated (70 °C, 15 min) biomass were stored at -20 °C, and analyzed for PL, FFA and total astaxanthin after 4, 6, 8, 10 and 12 months.

All enzyme activities measured were inactivated after 5 min heat treatment, and the samples contained the same quantitative amount of PL, total lipid and crude protein as the zero sample, at all holding times. The amount of salt was reduced by mixing with fresh water, which lowered the ash content by 50 %. Storage of untreated biomass led to a loss of all PL and the formation of 13 ± 1 % FFA of total lipid. After heat treatment, the content of PL was kept stable at the initial amount with a low content of FFA. No reductions were found in total astaxanthin in neither untreated nor heat treated material. Heat treatment at 70 °C in 5 min inactivates all tested enzymes and results in a stable *C. finmarchicus* product.

Sodium reduction in minced fish products: Effect on physicochemical properties

Kirsti Greiff^{1,2,*}, I.G. Aursand¹, U. Erikson¹, K.I. Draget², and T. Rustad²

¹SINTEF Fisheries and Aquaculture, Trondheim, Norway

²NTNU, Department of Biotechnology, Trondheim, Norway

*kirsti.greiff@sintef.no

There is an increasing interest for salt reduction in food, due to the known negative health effect of consumption of foods with high amounts of sodium. Food manufacturers are faced with the dilemma of how to reduce the salt content of foods without losing their palatability, texture, processing yield and long shelf-life. This requires a full understanding of the technological problems associated with salt reduction. The aim of this study was to investigate the effect of different cat-ions (Na^+ , K^+ , Mg^{2+}) on gelation and physicochemical properties of minced fillets and cooked haddock (*Melanogrammus aeglefinus*) mince. Fresh or frozen/thawed fillets were minced together with water and different salts (NaCl , KCl , MgCl_2) at different concentrations between 0.4% and 3.2% w/w. The minces were analyzed before and after heating to a core temperature of 80°C. Low field

NMR T_2 relaxation, texture, rheology, water content, pH, water-holding capacity (WHC), and cooking loss were measured. The rheological analyses showed differences in viscoelastic properties between the different minces, and the low field NMR T_2 relaxation data showed differences in water distribution within the mince protein network. Generally, increased salt content seemed to result in a higher water-content in the cooked minces, and to a decline in pH. The water content of the cooked minces was dependent on type of cat-ion added. In addition, the pH of the cooked minces varied due to different salts. The minces containing MgCl_2 had the lowest pH independent of salt content. There were small differences in the water-holding capacity of minces with different salts. A summary of the results will be presented.

Comparative study on protein cross-linking and gel enhancing effect of microbial transglutaminase on different fish muscle proteins

S. Chanarat and Sootawat Benjakul*

Department of Food Technology, Faculty of Agro-Industry Prince of Songkla University, Hat Yai, Songkhla, Thailand

*soottawat.b@psu.ac.th

Microbial transglutaminase has been used to improve gel properties of surimi, however the efficacy varies depending upon fish species used for surimi production. This research aimed to study the effect of MTGase at different levels (0-0.6 units/g surimi) on gel property of surimi from three fish species including threadfin bream, Indian mackerel and sardine in the presence and absence of EDTA (10 mmole /kg). Without EDTA, breaking force of gel from all surimi prepared by 2 step- heating (40°C for 30 min, followed by 90°C for 20 min) increased as MTGase levels increased, except for threadfin bream surimi gel, where breaking force decreased at 0.6 units/g ($P<0.05$). Deformation of threadfin bream surimi decreased continuously as the MTGase increased, while there was no change in deformation for surimi from the other two species. In presence of EDTA, all surimi gels had increased breaking force with increasing MTGase levels and slight increases in deformation were found in gel of surimi from Indian mackerel and sardine. Generally higher breaking force and deformation were observed when EDTA was excluded, suggesting the combined effect of indigenous

transglutaminase with MTGase added in surimi gel strengthening. With the addition of MTGase, the highest increase in breaking force with the coincidentally highest decrease in myosin heavy chain was found in surimi from threadfin bream, followed by those from mackerel and sardine, respectively.

When cross-linking activity of MTGase on natural actomyosin (NAM) from different fish species was determined by monitoring the decrease in ϵ -amino group content and the formation of polymerized proteins, it was found that the highest decreasing rate in ϵ -amino group content was found in NAM from threadfin bream, followed by NAM from mackerel and sardine, respectively. This was correlated with the increase in formation of cross-linked proteins as determined by SDS-PAGE. The reactivity of muscle proteins toward MTGase-induced cross-linking was in agreement with surimi gel enhancement. Thus, the composition and property of muscle proteins of varying fish species more likely determined the ability of MTGase in protein cross-linking, thereby affecting their gel properties differently.

Obtaining a restructured seafood product from non-functional fish muscle by adding konjac glucomannan

B. Herranz, B. Solo-de-Zaldivar and A. Javier Borderías*

Instituto de Investigaciones Department of Products, Institute of Food Science and Technology and Nutrition (ICTAN-CSIC), Madrid, SPAIN

*jborderias@if.csic.es

After certain processing procedures, fish muscle by-products lack gelling properties and are therefore unable to elaborate the structures needed for restructured products. To overcome these problems, the technological properties of Konjac glucomannan (KGM), a neutral hydrocolloid derived from *Amorphophallus konjac* offer the possibility of forming a gel net capable of maintaining the unfunctional mince as a “filler” for making different structures. To get a thermostable gel, glucomannan needs to be deacetylated by raising the pH to 11-11.8 by adding an alkali. The aim of this work was to study the influence of pH in two types of gel elaborated with different proportions of mince (frozen hake muscle- sawdust) and aqueous glucomannan solution (AGD), and their behaviour during chilled storage.

Different ratios (75:25 and 50:50) (w/w) of mince and AGD at 6 and 3% (w/v) were mixed to obtain a final glucomannan concentration of 1.5%. Then 0.6N KOH was added to bring the pH up to a range of 11-11.8. Afterwards, these mixtures were set and finally neutralized. Sensorial analysis, moisture content and water binding capacity (WBC), colour and puncture

tests were performed at 1, 3 and 6 days of chilled storage (5°C).

The moisture content of all samples was around 90%. WBC was slightly higher in 75:25 samples (around 80%) than in 50:50 samples (around 70%), remaining constant during the 6 days of chilled storage. The lightness of all samples was also stable during storage at any pH value. 50:50 samples were less firm than 75:25 samples during 6 days of refrigeration. The rigidity in 50:50 samples increased during chilled storage as the pH increased. However, both types of samples showed similar breaking deformation during storage irrespective of the different pH values.

Finally, the addition of AGD at 6% (w/v) to minced fish muscle in a ratio of 25:75 at pH 11 and then neutralizing it, resulted in a good gel, with flavour and texture similar to that of fish (hake) muscle and appropriate physical characteristics which were maintained during chilled storage. This methodology makes it possible to elaborate fibres, myotomes-like and other structures which will be possible to elaborate restructured seafood products.

Thermal denaturation properties of catfish myosin as compared with those of tilapia

Thavaroj Wichulada** and *K Kunihiko

Faculty of Fisheries Sciences, Hokkaido University, Japan

*wichulada.t@rmutsv.ac.th

Hybrid catfish (*Clarias macrocephalus* x *Clarias gariepinus*) is an important species cultured in Thailand that is consumed domestically and exported as chilled or frozen fillets. However there is little information on the properties of its muscular proteins. Thus thermal denaturation properties of myosin in form of myofibrils (Mf) were compared with those of another important fish, tilapia.

Myofibrils were prepared from hybrid catfish and tilapia dorsal muscle and suspended in 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5). Thermal inactivation rate of Ca-ATPase of Mf was measured at 0.1 M NaCl (stability with full protection by actin) and at 2.0 M NaCl (stability without protection by actin, stability of myosin itself). Myosin denaturation at head and tail regions were studied by using change in chymotryptic digestion upon heating of Mf. The digestion cleaved myosin into head (S-1) and tail

(rod) regions. Thermal inactivation rates of ATPase of catfish Mf at 0.1 M and 2.0 M NaCl were quite similar to the ones of tilapia Mf at all heating temperatures. Denaturation pattern of the S-1 and rod portion of catfish Mf was different from tilapia Mf as revealed by chymotryptic digestion. In catfish Mf denaturation of the rod region proceeded faster than that of S-1, while tilapia showed opposite results. The difference was explained by different rigidity of myosin filaments of the two species. Generally myosin forms rigid filaments at acidic pH and fragile ones at alkaline pH. Decrease in pH slowed rod denaturation relative to S-1 denaturation for Mf of both fish species. The S-1/rod denaturation pattern of catfish Mf at pH 7.0 was similar to that of tilapia Mf at pH 8.0. Thus, it was concluded that catfish myosin forms less rigid filaments than tilapia myosin because of its quicker rod denaturation.

Mushy tail or 'skyrhumar' in Icelandic langoustines

Heather Philp* and ***G. Marteinsdottir***

University of Iceland, Reykjavik, Iceland
*hrp@hi.is

The langoustine (*Nephrops norvegicus*) fishery in Iceland is highly lucrative due to the large size of individuals. In the last few years a problem of mushiness in cooked tail meat has been identified. This appears to vary seasonally and in the worst cases, 80% of catch samples may be affected causing considerable loss of value.

Working in partnership with the Icelandic fishing industry, a series of studies were carried out with the first phase being to fully describe the condition – how it was manifested and which lobsters exhibited it (in terms of sex, size, season and storage time). The second explored the possible causative factors including disease, condition and catch quality. Finally, we proposed changes to industry practices to reduce mushy tail and increase the value of the fishery.

We developed an index to describe the severity of the condition and supplied this and a sampling regime to factories processing lobsters to monitor it over the fishing season. Samples were collected at sea and the catch on board the boat followed to determine whether the problem

arose from catching or storing techniques. Both histology and SDS-PAGE were used to understand the ultimate cause of textural breakdown in the cooked meat.

Mushiness in langoustine tails appears to be the result of post-mortem changes in the lobster physiology. Animals can exhibit the condition in as little as six hours after capture and the rate increases significantly during the subsequent twenty-four. No relationship was detected with either sex, size or known diseases. However, the proteolysis observed suggests a diet-related enzyme or group of enzymes which escape from the hepatopancreas due to cellular autolysis.

The industry was advised to separate the heads from the tails of animals destined for scampi production on board the boat, a practice normally carried out in the factory after a four-day fishing trip. Further, new additives to the metabisulfite treatment administered immediately post-capture are currently being explored.

Generating a year-around profitable trade of live handpicked scallops (*Pecten maximus*) with an optimal quality

A.K. Woll^{1,*}, Snorre Bakke¹, K.L. Fjørtoft¹, and H. Myrseth²

¹Møreforsking Marin, Ålesund, Norway

²Seashell AS, Norddyrøy, Norway

*astrid@mfaa.no

In the northwestern part of Norway, approximately 800 tons of scallop (*Pecten maxiums*) are handpicked annually by scuba divers, most of which is exported to the high-end restaurant market in Europe. Because of the gentle harvest, large sizes and clean scallops without sand, the scallops have a reputation for good quality in the market. To maintain the high quality and keep the scallops alive as long as possible, it is essential that the physiological stress is minimized during storage and transport.

The aim of this project was to identify and improve critical points in the value chain from catch to market, with a special emphasis on catch handling, stacking height during water storage, pre-treatment and packing prior to transport. Market analysis was conducted in order to describe customer preferences.

Rough handling and blows significantly increased shell damage and mortality. Experiments with stacking height (10, 30 and 48 cm) were conducted in April (18 days storage) and September (7 days storage). In both seasons the mortality was low after one week of storage.

In April a significant effect of stacking height was found after 18 days of storage, with a mortality of 24, 25 and 58 % for shells stored in heights of 10, 30 and 48 cm respectively. Simulated transport and subsequent revitalization in water showed a significant higher mortality for stored shells (either stacked or in a single layer) compared with shells transported directly after catch. Long storage time (more than 7 days) and stacking height of more than 30 cm in general caused higher mortality both during storage, simulated transport and subsequent revitalization. Packing method and temperature in the transport boxes were of great importance and preliminary experiments showed that using gel-ice and wood wool instead of crushed ice resulted in a higher survival after 3 days transport.

Summarized our investigations show that vitality and survival of scallops can be significantly improved by implementing best practice in terms of catch handling, live storage and transport, and allow delivery of high quality scallops to consumers Europe.

Effect of gelatin hydrolysate on physicochemical properties of surimi subjected to different freeze-thaw cycles

Phanat Kittiphattanabawon¹, S. Benjakul^{1,*}, W. Visessanguan², and F. Shahidi³

¹Department of Food Technology, Faculty of Agro-Industry Prince of Songkla University, Hat Yai, Songkhla, Thailand

²National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Klong Luang, Pathumthani, Thailand

³Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL, Canada

*soottawat.b@psu.ac.th

Cryoprotectants, especially sucrose and sorbitol blend, have been used in surimi to lower the denaturation and/or aggregation of myofibrillar protein during frozen storage. However, sucrose and sorbitol impart a sweet taste to surimi products which may be undesirable to consumers. Thus, an alternative cryoprotectant with reduced sweetness such as protein hydrolysate has gained attention. This study aimed to elucidate the cryoprotective effect of gelatin hydrolysate from shark skin (*Carcharhinus limbatus*) with different DHs (5, 10, 20 and 30%) on surimi from threadfin bream (*Nemipterus spp.*) subjected to 3 and 6 freeze-thaw cycles in comparison with commercial cryoprotectant (sucrose/sorbitol blend, 3:1). Physicochemical properties, including protein solubility in 0.6 M KCl, Ca²⁺-ATPase activity, surface hydrophobicity, sulfhydryl group and disulfide bond contents, were determined in natural actomyosin extracted from surimi. Solubility, Ca²⁺-ATPase and sulfhydryl group

content in all samples decreased with increasing freeze-thaw cycles, while surface hydrophobicity and disulfide bond content increased ($P<0.05$). Among all samples, the highest Ca²⁺-ATPase activity with the coincidental lowest surface hydrophobicity was observed in surimi with added gelatin hydrolysate having 10%DH after freeze-thawing ($P<0.05$). On the other hand, surimi without cyroprotectant showed the lowest Ca²⁺-ATPase and the highest surface hydrophobicity ($P<0.05$). The similar solubility was obtained between samples added with commercial cryoprotectant and gelatin hydrolysate (10%DH) ($P>0.05$). The results suggest that gelatin hydrolysate with 10%DH prevented the denaturation of surimi protein comparably to commercial cryoprotectant, but exhibited the higher protective effect on denaturation of myosin heavy chain. Therefore, gelatin hydrolysate can be a promising cryoprotectant to reduce the sweetness in surimi products.

Quality assessment of fresh fish by biochemical approaches

François Leduc^{1,*}, F. Krzewinski², A. N'Guessan³, P. Malle¹, O. Kol², and G. Duflos¹

¹ANSES, Laboratoire des produits de la pêche, Boulogne-sur-Mer, France

²Unité de Glycobiologie Structurale et Fonctionnelle, Université de Lille 1, Villeneuve d'Ascq Cedex, France

³Laboratoire Paul Painlevé, UMR CNRS 8524 & Ecole Polytechnique Universitaire de Lille, Université de Lille 1, Villeneuve d'Ascq Cedex, France

*francois.leduc@anses.fr

Freshness is essential for assessing fish quality. As fish is a very sensitive matrix to spoilage, the purpose of the freshness assessment is essential for all professionals of the seafood industry as well as for official fish inspection authorities. It involves disposing of high-performance tools to accurately determine spoilage levels. The aim is to propose methods that clearly and objectively determine the quality of the fish from the first stage of spoilage.

Indeed, different chromatographic methods were undertaken: SPME-GC-MS, DH-GC-MS-80, DH-GC-GC-MS-O to identify volatile fractions of several species on day 1, 4, 7, 10 and 15. Species studied in this survey are: whiting (*Merlangus merlangus*), seabass (*Dicentrarchus labrax*), seabream (*Sparus aurata*), cod (*Gadus morhua*), salmon (*Salmo salar*) and tilapia (*Oreochromis mossambicus*). These analytic approaches are complementary and give us important information to objectively characterise olfactory criteria related to fish freshness.

In total more than 150 volatile compounds with an odor active or not have been identified in species belonging mainly to the families of aldehydes, alcohols and ketones. Different statistical studies (principal component analysis, ascending hierarchical classification, ANOVA and Fisher) of the movement of these compounds provide some very interesting data. There is not a reference marker to assess the quality of fish in general but rather a set of volatile compounds that can be considered as potential markers of fish freshness, i.e. compounds in extra fresh fish which tend to diminish or even disappear during storage (such as octanal, nonanal, (E)-2-heptenal and 2-ethylfuran). On the other hand, compounds considered as potential markers of fish spoilage have an increasing concentration during storage (such as 3-methyl-butanol, 3-methyl-butanal, 1-octen-3-one, dimethylsulfide and ethyl-acetate).

Improving sensorial characteristics of *Engraulis ringens* ripened fillets

Raquel Llorente Holgado^{*} and I. Peral Díez

Food Research Division, AZTI-Tecnalia, Parque Tecnológico de Bizkaia, Bizkaia, Spain

*rllorente@azti.es

The salted anchovy fillet is a traditional salted food product in the Mediterranean countries with high nutritional value due to high content of polyunsaturated fatty acids. It shows a tender consistency and a specific pleasant aroma and taste as a result of the enzymatic activity on the fish flesh during the ripening process. In recent times depletion of stocks of the preferred raw material (*Engraulis encrasicolus*) has led to an alternative processing of other species as *Engraulis ringens*. However the external appearance of the ripened fillets is less attractive, as red muscle gets darker.

The aim of this study was to analyze the feasibility of improving the organoleptical properties of the *E. ringens*'s ripened fillets regarding their superficial colour and texture in relation to the reference *E. encrasicolus* fillets.

For this purpose different acid treatments and commercial solutions (approved by the European

Food Legislation and FDA) were applied superficially on ripened anchovy fillets. Process parameters such as temperature, pH or solution contact time were controlled. Visual discolouration and superficial colour was measured by the CIE L*a*b* system. Sensorial analyses were also performed in order to evaluate if these treatments provided unpleasant flavors to anchovy fillets.

Results indicate that it is possible to improve the sensorial properties of the ripened *E. ringens* anchovy whose fillets showed an optimum brownish colour after the acid treatment with citric acid. From a sensorial and textural point of view, the anchovies treated with these acid solutions also showed organoleptical properties similar to the traditional ones without unpleasant flavors.

Session 1:
Towards sustainable sources of seafood
– aquaculture and rest raw materials

Session 2:
Product development and product communication
within the seafood sector

Session 3:
Innovative process and detection technologies for
seafood

Session 4:
Retaining high quality through the seafood processing
chain

Session 5:
Nutritional and safety aspects of seafood consumption

Keynote: **Early fish consumption – key to avoid allergy in childhood**

Agnes Wold

University of Gothenburg, Gothenburg, Sweden
agnes.wold@microbio.gu.se



A number of studies report that early intake of fish is associated with reduced risk of eczema development in the child. The components of fish that exert this beneficial effect are unknown. Long-chain polyunsaturated fatty acids (PUFA) of the n-3 series have shown beneficial effects against atherosclerosis and have been suggested to have a general anti-inflammatory effect. We have investigated the role of feeding n-3 and n-6 fatty acids in mouse models of Th1 and Th2 mediated hypersensitivity. Quite surprisingly, IgE production and IgE-mediated allergy was enhanced in mice fed n-3 or n-6 PUFAs. In contrast, both n-3 and n-6 PUFAs reduced Th1 functions, such as production of interferon-gamma by lymphocytes in draining lymph nodes. These results suggest that a diet rich in PUFAs might, in fact, enhance the risk of developing IgE-mediated allergy. To test this hypothesis, we examined stored cord blood samples from children who later developed

allergy, or remained healthy. We found a linear relation between the proportion of PUFAs in cord blood phospholipids and the risk of allergy development ($p < 0.001$). Both n-3 and n-6 PUFAs levels in cord blood were positively correlated with allergy development, while the proportion of the saturated FA 20:0 was inversely related to allergy development. One reason for the belief that n-3 FAs would be beneficial against allergy development is that individuals with manifest allergy may have lower serum levels of these PUFAs, compared with healthy persons. Most likely, this phenomenon depends on consumption of the PUFAs during the allergic inflammation. Our results suggest that the advice given to pregnant and breast-feeding mothers to increase consumption of PUFAs and decrease the intake of saturated fat may be questioned. Children may instead benefit from a diet with a high proportion of saturated fat.

Effects of a fish or meat diet during perinatal and post-weaning periods on insulin sensitivity, adiposity and lipid profile in adult offspring

Aysha Hussain*, *N. Jansson, R. Jakubowicz, and A. Holmång*

Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

*aysha.hussain@neuro.gu.se

Maternal diet during pregnancy and lactation may affect the long-term health of the offspring. We studied the effects of maternal and post-weaning high-fat diets (~30E %) based on fatty fish (herring, *Clupea harengus*) or red meat (beef) on body composition, plasma cholesterol, plasma triglycerides and insulin sensitivity in the adult offspring. The diets were fed to C57BL/6 dams during breeding and lactation. The pups (n=79) remained on the maternal diet or underwent a cross-over after weaning. Body-fat percentage was measured by DXA at the age of 9 and 21 weeks. Insulin sensitivity was calculated by QUICKY at young age (15 weeks) and later measured by euglycemic clamp. Plasma triglycerides and cholesterol were measured. The statistics were done by two-way ANOVA. Offspring from dams fed herring had less body fat percentage in week 9 compared to those fed beef (-2.2%, $p<0.05$). Mice fed herring

during the post-weaning period had increased insulin sensitivity ($0.03\mu\text{U}\cdot\text{mg}^{-1}$, $p<0.01$) at 15 weeks of age that was no longer present 7-11 weeks later. This diet group also had reduced plasma cholesterol (-1.1mmol/L , $p<0.001$) and triglyceride (-0.3mmol/L , $p<0.01$) and higher brown adipose tissue (BAT) weight (1.2mg/g , $p<0.05$) than those fed beef. The BAT cells from these mice were unilocular and had more fat deposition. Herring-based maternal diet reduced adiposity in the offspring at young age, while this diet during the post-weaning period increased insulin sensitivity and decreased plasma lipids at young age and increased the amount of brown adipose tissue at adult age. These results suggest that the negative effects of a high-fat diet during the perinatal and post-weaning periods might be counteracted by the addition of herring compared with beef.

Bioactive peptides from Atlantic mackerel (*Scomber scombrus*)

Nazlin K Howell* and C. Kasase

University of Surrey, Faculty of Health and Medical Sciences, Division of Nutrition and Food Science, Guildford, Surrey GU2 7XH, UK

*N.Howell@surrey.c.uk

Peptides that make up most food proteins can exert a bioactive effect, for example lowering blood pressure, when released and activated by enzymes, which is over and above that of normal protein function.

The aims of this study were 1) to produce peptides from Atlantic mackerel by hydrolysis; 2) to test their ACE inhibitory activity and antioxidant activity in vitro; 3) to test the safety and absorption in Caco-2 cells and 4) to test their antioxidant properties in the cells.

In this study, Atlantic mackerel (*Scomber scombrus*) flesh proteins were hydrolyzed with digestion simulating enzymes pepsin and pancreatin. The resulting hydrolysate was fractionated using a 2 kDa cut-off ultrafiltration membrane, followed by gel filtration and high pressure liquid chromatography. Angiotensin I converting enzyme (ACE) inhibitory activity of the fractionated hydrolysates and peptides was investigated by the conversion of the substrate hippuryl- histidyl-leucine by ACE enzyme to give hippuric acid which was quantified by an improved HPLC method. Safety, absorption and antioxidant activity of peptides were tested in Caco-2 cells by the MTT cell proliferation assay and in the presence of tert-butyl hydroperoxide.

The ACE inhibitory activity improved with purification resulting in 18.8, 46.3, 68.1 and 83.1% for the 2 kDa fractions, gel filtration fraction, ion exchange chromatography fraction and captopril (control) respectively. The molecular masses and most potent peptides obtained by HPLC were identified by QTOF LC-MS-MS. The fish peptide fractions exhibited competitive inhibition, as determined by Lineweaver-Burk plots. The safety of fish peptides in human colon Caco-2 cells was studied and showed a marked increase in the viability 112% in the presence of 0.5 mg/ml peptides compared to the control (100%). Peptides were absorbed and enhanced the growth of Caco-2 cells and were not cytotoxic. Fish peptides also showed antioxidant activity in Caco-2 cells treated with tert-butyl hydroperoxide (t-BHP) as cell viability was only 52% in the presence of t-BHP and 72% in the presence of t-BHP/peptide fractions.

Peptides isolated from mackerel flesh have ACE inhibitory activity and antioxidant properties that have the potential for reducing hypertension, thus providing health benefits.

Effect of alkaline pH-shift processing on *in vitro* digestibility of herring (*Clupea harengus*) protein

Sofia Marmon** and *I. Undeland

Department of Chemical and Biological Engineering, Food Science, Chalmers University of Technology, Gothenburg, Sweden

*sofia.marmon@chalmers.se

pH-shift processing can be used to isolate proteins with good functionality from complex raw-materials. There is a large interest to use these proteins as a food source. However, during pH-shift processing, oxidation may occur. We have previously shown that the protein salt solubility is decreased by pH-shift processing. One underlying factor for the reduced salt solubility might be protein oxidation. This reaction in turn has earlier been linked to decreased protein digestibility. Digestibility of a protein is a crucial factor for its nutritional quality. Therefore we found it important to investigate the effect of pH-shift processing on protein digestibility.

In this study a static gastrointestinal *in vitro* model was used. Herring (*Clupea harengus*) fillets were used as raw material. Protein isolate was made using the alkaline version of the pH-shift process. The protein salt solubility, lipid oxidation (TBARS, PV) and protein oxidation (carbonyls) of the herring and protein isolate was

determined. In addition to raw herring mince and freshly made protein isolate, heated samples (55°C) thereof were subjected to *in vitro* digestion. The protein's digestibility was measured as degree of hydrolysis before and after *in vitro* digestion, and the protein profiles were analyzed using SDS-PAGE.

It was showed that protein carbonyls did not increase during pH-shift processing although there was an increase in lipid oxidation and a drastic decrease in protein salt solubility. The degree of hydrolysis after *in vitro* digestion was similar for all samples. SDS-PAGE analysis of digested protein isolate revealed a few bands in the region of 20-40kDa that were less prominent in the digested herring mince. No difference was seen between the heated and unheated samples.

We conclude that, despite lowered salt solubility and slight lipid oxidation, the digestibility of isolated herring proteins is retained after pH-shift processing as determined in a static gastrointestinal *in vitro* model.

Comparison of *in-vitro* chemical and cellular based antioxidant assays on bioactive marine peptides

Patricia Y. Hamaguchi¹, H. Sveinsdóttir¹, R. Jónsdóttir¹, and H.G. Kristinsson^{1,2,*}

¹Matís ohf. - Icelandic Food and Biotech R&D, Reykjavík, Iceland

²Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL, USA

*hordur.g.kristinsson@matis.is

Bioactive marine derived peptides are receiving increasing attention as being potential ingredients for functional foods and supplements. Traditionally these peptides have been analyzed with simple *in-vitro* chemical assays which may or may not be relevant to what happens in the human body. Thus, it is important to gain more information by biologically more relevant cellular based assays to properly screen for bioactivity.

The objective was to compare the bioactivity of peptides obtained from different marine species by *in-vitro* chemical assay and a novel cellular antioxidant system and to characterize their properties to demonstrate their benefit as functional ingredients.

Peptide samples were prepared by enzymatically hydrolyzing different marine based raw materials (saithe, blue whiting, clams, scampi, lobster and shrimp) with a novel cod enzyme mixture. Samples were filtrated, purified and subjected to the following *in-vitro* analysis: oxygen radical absorbance capacity (ORAC), DPPH radical scavenging, reducing power and metal ion chelating activity. The effect of marine peptides on cellular ROS level induced by 2-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) in HepG2 cells was analysed. After HepG2 cells were pre-incubated with DCFH-DA (fluorescent probe), cells were

treated with different concentrations of marine peptides for 1 h. DCF fluorescence was monitored at 37°C for 2 hour on 10 min intervals, followed by the addition of 500 µM AAPH. From the chemical based assays results, saithe, lobster, scampi and shrimp peptides showed DPPH scavenging ability higher than 80%. Peptides from scampi and shrimp had also higher than 80% ability to chelate iron. The reducing power of the peptides followed the sequence: shrimp>scampi>lobster>saithe and blue whiting>clam. ORAC values of the peptides were in the following order: lobster and scampi>shrimp>saithe and blue whiting>clam. Blue whiting and scampi peptides displayed no antioxidant activity in the HepG2 cell model. However, peptides from clam, shrimp and saithe showed increasing intracellular antioxidant activity with increasing concentrations. Lobster peptides showed similar antioxidant activity in all tested concentrations. Peptides from saithe and shrimp exhibited the highest intracellular antioxidant activities. Cell viability was not affected when cells were incubated for 24 h with peptides.

This study gives good overview on antioxidant activity of different marine derived peptides by showing the importance of finding other means to support the *in-vitro* chemical based assays.

High levels of long chain polyunsaturated fatty acids in cord serum predict allergy development in childhood

Malin Barman^{1,*}, S. Johansson¹, A.E. Wold², A.-S. Sandberg¹ and A. Sandin³

¹Department of Chemical and Biological Engineering, Food Science, Chalmers University of Technology, Gothenburg, Sweden

²Institute of Biomedicine, Department of Infectious medicine, Clinical Bacteriology section, University of Gothenburg, Gothenburg, Sweden

³Department of Clinical Sciences, Paediatrics, Umeå University, Umeå, Sweden

*malin.barman@chalmers.se

Allergies increased strongly during the 20th century in countries with a Western lifestyle. The cause is unknown, but reduced stimulation by microbes in early childhood, and changed dietary habits may play a role. The consumption of long-chain polyunsaturated fatty acids (LCPUFAs) has increased in the last decades. PUFAs are precursors for lipid mediators with immune and inflammatory effects, but their role in allergic disease development is controversial.

To investigate whether serum levels of LCPUFAs at birth affects the risk of allergy development in childhood.

Children were selected from a birth-cohort comprising children born in northern Sweden in 1996-7 who underwent clinical examination and skin prick test at 13 years of age. Cord blood had been obtained at birth and stored frozen. We selected adolescents with either atopic eczema ($n=44$) or respiratory allergy ($n=58$), and skin prick negative asymptomatic controls ($n=52$). Their cord blood levels of eight PUFAs of the n-3 and n-6 series, and one saturated fatty acid were retrospectively analyzed. A serum sample was also collected at 13 years of age and analyzed for the same fatty acids and a food-

frequency questionnaire was distributed probing for consumption of fat-containing foods.

Cord blood levels of n-3 LCPUFAs, n-6 LCPUFAs, and total LCPUFAs were higher in subjects with respiratory allergy as well as subjects with eczema at 13 years of age, as compared to controls ($p<0.0005$ for each comparison). In contrast, controls had significantly higher levels of the saturated fatty acid 20:0 in cord blood serum than those who developed eczema ($p<0.0005$) or respiratory allergy ($p=0.001$). There was a dose-dependent increase in the risk of developing respiratory allergy in relation to total PUFA levels in cord blood ($p<0.01$). At 13 years of age, allergic subjects did not differ significantly concerning serum fatty acid pattern. Eczematous adolescents consumed more low-fat cheese and low-fat milk compared to non-allergic adolescents.

Exposure to high levels of long-chain PUFAs in early infancy may be a risk factor for allergy development, possibly by modulating the function of the infant's developing immune system. Current recommendations of increased intake of PUFAs in pregnant women and children may be questioned with regard to the risk of allergy development.

Poster 5.1

Extraction and characterization of naturally occurring bioactive peptides from different tissues from Salmon (*Salmo salar*)

Susan Skanderup Falkenberg** and *H. Hauch Nielsen

Technical University of Denmark, National Food Institute, Kgs. Lyngby, Denmark

*sskfa@food.dtu.dk

The aquatic ecosystem represents a large number of organisms adapted to living conditions remarkably different from the land-living ones. For instance fish generally possess an eminent bioactive defense to protect them from the high bacterial load in water, and must be expected to harbor a large number of bio-components such as bioactive peptides for this purpose.

Tissue and proteins from e.g. fish gills, skin and viscera could be a new source of peptides that could have a nutritional and pharmaceutical value, and be used in health and functional foods and thereby increasing the value adding of secondary marine products.

Only few naturally occurring bioactive peptides have been characterized such as the antimicrobial polypeptide piscidines from gills. It is therefore hypothesized, that fish tissue also contains numerous other peptides with other bioactive properties.

The approach in this project is therefore to extract and identify naturally occurring bioactive peptides from different tissues from salmon.

A number of aqueous extracts were made from gills, skin and belly flap. In order to preserve the bioactivity of the peptides mild extraction procedures as acidic, basic and aqueous solutions were used. Combination of different extraction conditions such as with/without boiling, with/without inhibitor and variation of pH resulted in a total of 36 extracts. The activity of the extracts was analyzed *in vitro* for ACE (angiotensin-converting enzyme) inhibiting activity, and anti-oxidative activity (Free Radical Scavenging assay).

A number of extracts showed high ACE inhibiting and anti-oxidative activity. The extracts were then size fractionated by ultrafiltration using a 10 kDa filter, and relevant fractions below 10 kDa from gills, skin and belly flap were further fractionated by gel-filtration on a Superdex peptide HR 10/30 column. Peptide fractions were collected and freeze-dried and will be further characterized with LCMS and MS/MS.

Poster 5.2

Effect of sunlight on the survival of pathogenic *E. coli* in freshwater and sea water

***Alagarsamy Surendraraj*^{1,*}, *K.H. S. Farvin*², and *N. Thampuran*³**

¹Institute of Food and Dairy Technology, TANUVAS, Alamathi, Chennai, India

²Division of Industrial Food Research, DTU Food, Technical University of Denmark, Kgs., Lyngby, Denmark

³Retired Principal Scientist and Head, Microbiology and Fermentation Division, Central Institute of Fisheries Technology, Matsyapuri (PO), Cochin, India

*asurendraraj@rediffmail.com

An enteropathogenic group of *E. coli* are the emerging category of pathogen of public health significance. Several recent pathogenic *E. coli* outbreaks are associated with drinking water. Aquaculture, the fast emerging food production sector also poses a pathogenic EHEC outbreak risk, as it regularly uses cow dung, a reservoir of this organism. Hence, an experiment was set up to study the duration of survival of pathogenic *E. coli* under sunlight and darkness. Eight pathogenic *E. coli* isolates from clinical (EPEC, ETEC, EHEC, EAEC), veterinary (CTE3, CTE4) and environmental sources (ASHE3, Rao II) were studied for their survival under sunlight and darkness in fresh water and seawater. Effect of direct sunlight on the viable but non-culturable (VBNC) state of cultures was also studied. The results of the study indicated a distinct pattern between freshwater system and

seawater system. Pathogenic *E. coli* from different sources showed significantly higher level of destruction under direct sunlight than in complete darkness. A reduction of 1.1 to 5.7 log CFU was seen in fresh water after 90 to 105 min under direct sunlight and only 0.2 to 2 log reduction was observed in complete darkness in 5 to 96 h period. The effect of sunlight was severe in seawater and in selective media, where a higher cell reduction to an extent of 3 log CFU was observed. Survival period was least for environmental pathogens followed by veterinary and finally clinical pathogens with an exception of Shiga-toxigenic *E. coli* (STEC) from veterinary sources. Findings of this study, may aid the public health specialist to evolve better strategies to control these organism in the above systems.

Poster 5.3

The effects of sex and seasonality on the metal levels of different muscle tissues of mature blue swimmer crabs (*Portunus pelagicus* Linnaeus 1758) in Mersin Bay, north-eastern Mediterranean, Turkey

***Deniz Ayas*^{1,*} and *Y. Ozogul*²**

¹Department of Seafood Processing Technology, Faculty of Fisheries, Mersin University, Mersin, Turkey

²Department of Seafood Processing Technology, Faculty of Fisheries, Çukurova University, Adana, Turkey

*ayasdeniz@mersin.edu.tr

The objective of this study was to investigate the seasonal and sexual effects on metal levels of lump crabmeat (LCM) and chela crabmeat (CCM) of mature blue swimmer crabs, *Portunus pelagicus*, caught in the Mersin Bay, the north-eastern Mediterranean. The annual range of carapace length (CL), carapace width (CW) and weight of blue swimmer crabs caught for the present study were 54.0-83.0 mm, 125.0-175.0 mm and 107.37-390.83 g, respectively. The findings indicated that the annual ranges of metal levels in the LCM of blue swimmer crabs were: 0.50-1.01 µg Cd g⁻¹, 0.37-0.45 µg Cr g⁻¹, 0.10-0.40 µg Pb g⁻¹, 9.64-33.85 µg Cu g⁻¹, 37.80-78.16 µg Zn g⁻¹, 9.35-25.51 µg Fe g⁻¹. The annual range of metal levels in the CCM of blue swimmer crabs were: 0.63-1.47 µg Cd g⁻¹, 0.31-

0.53 µg Cr g⁻¹, 0.12-0.52 µg Pb g⁻¹, 25.13-72.50 µg Cu g⁻¹, 89.85-178.61 µg Zn g⁻¹, 7.31-20.08 µg Fe g⁻¹. Cd, Cu, Zn levels in CCM of blue swimmer crabs were higher than LCM (p < 0.05). Cu and Zn metals specifically accumulated in CCM. In addition, it was observed that the accumulation of metals in muscle tissues (LCM and CCM) of blue swimmer crabs did not depend on sex and specimens size. Metals such as Cu, Zn and Fe showed seasonal variations. It was found that Cu, Zn and Fe levels of muscle tissues of the blue swimmer crab in spring and summer seasons were higher than in autumn and winter seasons. The results of this study showed that blue swimmer crabs caught from Mersin Bay are contaminated with Cd, Cu and Zn metals.

Poster 5.4

Benefits and risks associated to the consumption of salmon commercialized in Portugal

*C. Afonso**, *S. Costa*, *C. Cardoso*, *H.M. Lourenço*, *N. Bandarra*, *M.F. Martins*, *S. Gonçalves*, and *Maria Leonor Nunes*

Instituto Nacional dos Recursos Biológicos (INRB, I.P./L-IPIMAR), Unidade de Valorização dos Produtos da Pesca e Aquicultura, Lisboa, Portugal

*cafonso@ipimar.pt

The consumption of fish, including wild and farmed, has grown in recent decades due to the claimed health benefits and contribution for the well-being. Several researches have also stressed their unique role in providing omega 3 fatty acids (n-3 PUFA), namely eicosapentaenoic (EPA) and docosahexaenoic (DHA), that are beneficial to cardiovascular health and also to foetal development. In general, dietary recommendations advise weekly consumption of one to two portions of fatty fish. However, contaminants commonly present in seafood may pose a risk, especially to susceptible groups of people. In fact, it is normally accepted that seafood represents one of the main sources of non-occupational mercury (Hg), especially methylmercury (MeHg), since more than 90% of the mercury in fish is found as MeHg. Methylmercury is implicated in hepatotoxicity, neurotoxicity, mutagenicity, and teratogenicity in organisms including humans. Most of the information published about n-3 PUFA and Hg is related to raw products, being the data associated to cooked products quite scarce and very often contradictory. Thus, there is still lack of knowledge relatively to n-3 PUFA profile and Hg/MeHg contents in relevant fish species, consumed by Portuguese consumers, and the

effect of culinary processes on their abundance and stability. Since farmed salmon is a species highly appreciated in Portugal, the current work aims to provide new insights about the benefits and risks associated to the consumption of raw and cooked salmon (boiled, steamed, grilled, and roasted) by Portuguese population. The risks were quantified taking into consideration the probability of exceeding the provisional tolerable weekly intake (PTWI) for contaminants, and the reference daily intake (RDI) of n-3 PUFA regarding the benefits. Usual culinary treatments were used to boil, steam, grill, and roast and suitable validated methods were used for all determinations. Salmon showed a good level of n-3 fatty acids in all cooked samples, especially EPA and DHA. Despite the higher levels of MeHg in grilled and roasted salmon in comparison to other culinary treatments, it can be concluded that this species presents no serious health concern with respect to MeHg.

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Poster 5.5

The influences of natural zeolite (cliptinolite) on ammonia and biogenic amine formation by food-borne pathogen

S. Gokdogan¹, Y. Özogul¹, Y. Uçar¹, E. Kuley¹, F. Özogul¹, C. Kacar¹, and Deniz Ayas^{2,*}

¹Cukurova University, Faculty of Fisheries, Department of Seafood Processing Technology, Adana, Turkey

²Mersin University, Department of Seafood Processing Technology, Faculty of Fisheries, Mersin, Turkey

*ayasdeniz@mersin.edu.tr

The influence of natural zeolite on biogenic amines (BAs) and ammonia (AMN) production by eight common food-borne pathogens (FBP) were investigated in histidine decarboxylase broth (HDB). Presence of 1% zeolite in the HDB resulted in significantly higher AMN production, but zeolite at dose of 5% suppressed AMN accumulation by *Staphylococcus aureus*. Histamine (HIS) production by Gram-positive bacteria was as low as 0.5 mg/l, whereas *E. coli* produced 18.96 mg/l of HIS. The use of zeolite also significantly suppressed HIS accumulation by *E. coli*, *P. aeruginosa*, *S. paratyphi* A (P<0.05), although zeolite addition stimulated

HIS production by *K. pneumonia* and *A. hydrophila*. The range of tyramine (TYR) production by Gram-positive bacteria was 1.19 mg/l and 4.06 mg/l for *Enterococcus faecalis* and *Listeria monocytogenes* respectively. The zeolite had an inhibiting effect on TYR production by *Salmonella* spp., though stimulating effect on TYR production by Gram-positive bacteria, especially for *Staph. aureus*, with a 12-fold increase of TYR was found in the presence of zeolite. The results of this study show that the effect of zeolite on BAs and AMN production is dependent of bacterial strains, as well as zeolite concentrations used.

Poster 5.6

Regional occurrence of heavy metals in the common whelk *Buccinum undatum* – Implications for an evolving industry in Norway

***Snorre Bakke*^{1,*}, *S. Frantzen*², and *C. Børnes*³**

¹Møreforsking Marin, Ålesund, Norway

²National Institute of Nutrition and Seafood Research, Bergen, Norway

³Norwegian Food Safety Authority, Section for fish and seafood, Brumunddal, Norway

*snorre@mfaa.no

Preliminary resource investigations indicate that there is potential for commercial harvesting of common whelk in several areas along the coast of Norway. Growing demand for whelk, especially from Asia, has sparked an interest from both fishermen and producers that see the potential in harvesting and exporting this species. However, due to lack of information about the content of heavy metals in common whelk from Norwegian waters it has been difficult for the industry to document that contaminant levels do not exceed legal limits. The current investigation aims to fill this gap in

knowledge and produce documentation that can be used to help Norwegian exporters gain access to existing markets. Data will be presented showing occurrence of heavy metals in whelk from five different geographic locations. Sampling and analysis is to be conducted in May/June 2011 where content of heavy metals will be analyzed in soft tissues both *in toto* and white muscle. Findings will be compared with published results from other countries and discussed in relation to national resource management, fisheries, export and consumer health.

Poster 5.7

Beta-hydroxy-gamma-trimethyl amino butyric acid (L-carnitine) contents of commercially important raw and cooked seafood by HPLC method

Deniz Ayas^{1,*}, Y. Özogul², F. Özogul², E. Kuley², and A. Şimşek²

¹Mersin University, Fisheries Faculty, Adana, Turkey

²Çukurova University, Faculty of Fisheries, Mersin, Turkey

*ayasdeniz@mersin.edu.tr

Beta-hydroxy-gamma-trimethyl amino butyric acid (L-carnitine) content of raw and cooked seafood was determined using HPLC method. Thirty-two different fish species and nine different crustaceans were used to compare L-carnitine content of raw and cooked seafood. Significant differences in L-carnitine content were found among species, regardless of if the seafood was raw or cooked ($P<0.05$). There was also significant differences between raw and

cooked species ($P<0.05$). The levels of L-carnitine in raw fish samples ranged from 17.98 mg/kg for sand smelt to 73.07 mg/kg for European conger. Squid and green tiger prawn was found as the best source of L-carnitine among the tested seafood. Microwave cooking also significantly reduced L-carnitine content of some seafoods ($P<0.05$). The study showed that L-carnitine content of raw and cooked seafood is above daily requirement for human.

Functional ingredients in brown seaweed, *Fucus vesiculosus*

**Hólfríður Sveinsdóttir¹, J.Ó. Jónsson¹, P.Y. Hamaguchi¹, H. Benediktsson¹,
H.G. Kristinsson^{1,2}, and R. Jónsdóttir^{1*}**

¹Matís ohf. - Icelandic Food and Biotech R&D, Reykjavík, Iceland

²Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL, USA

*rosa.jonsdottir@matis.is

Seaweeds are a rich source of proteins, minerals, iodine, vitamins and non-digestible polysaccharides in addition to bioactive compounds like polyphenols and fucoxanthin. The brown seaweed *Fucus vesiculosus* grows in abundance in the North Atlantic but is highly underutilized.

The aim of the project was to develop functional ingredients from *Fucus vesiculosus* for use in nutraceutical and functional foods by optimizing extraction of bioactive compounds and to characterize their properties to demonstrate possible health benefits.

Seaweed was collected each month of a calendar year to characterize seasonal variations in composition and activity. Seaweed samples were analyzed for proximate composition, minerals and ascorbic acid. Total polyphenols were determined using the Folin-Ciocalteu method. Fucoxanthin content was measured with HPLC. Functional and bioactive seaweed extracts were prepared and their bioactive properties studied using Oxygen Radical Absorbance Capacity (ORAC), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity together with cell-based immunomodulating assays. Intracellular antioxidative activity of seaweed samples was determined by a novel cellular antioxidant assay using HepG2 cells.

The seaweed was found to vary significantly in composition and activities depending on the season. Protein content was highest in April (13.3%) and lowest in July (6.6%), while fat content was highest in July (3.3%) but lowest in May (0.7%). Ascorbic acid content was lowest in December (40mg/100g) and gradually increased until July (180mg/100g). Fucoxanthin content varied from ~30 µg/g d.w. in the winter to 40-50 µg/g during the summer. The seaweed had high levels of polyphenols (71.3-96.6 g phloroglucinol equivalents/100 g), which did not vary greatly with season. The samples were found to have very high radical scavenging activity measured as ORAC values from 8200 to 9300 µmol TE/g and DPPH capacity from 66% to 81%. All tested seaweed samples showed intracellular antioxidative activity in the HepG2 cell system. Cell viability was not affected when cells were incubated for 24 h with seaweed samples.

Seaweed harvested in different seasons can show significant variations in composition and activity. This study presents antioxidant properties of brown seaweed polyphenols assessed by chemical as well as cellular based methods. This information is of great significance for producers of functional and bioactive seaweed ingredients.

Uncovering global impact of fish and enriched-polyunsaturated fatty acids diet in mice by systems biology

***Intawat Nookaew**, B.G. Gabrielsson, A.-S. Sandberg, and J. Nielsen**

Department of Chemical and Biological Engineering, Systems Biology and Food Science, Chalmers University of Technology, Gothenburg, Sweden

*intawat@chalmers.se

Dietary fish is an excellent source of several bioactive compounds such as vitamins, minerals and long-chain polyunsaturated n-3 fatty acids. Epidemiological studies show an inverse association between high dietary intake of fish and disease, in particular cardiovascular disease. However, the cellular mechanisms that mediate the health beneficial effects of a high dietary intake of fish are not fully elucidated. In this work, we used mice to study the effects of dietary intake of fish vs beef or of different polyunsaturated fatty acids. Mice were fed diets supplemented with beef or herring (matched in fat and cholesterol content) or, in another experiment, high-fat diets enriched in polyunsaturated n-3 (menhaden oil) or n-6 fatty acids. Gene expression of tissues important for whole-body metabolism (liver, adipose tissue,

skeletal muscle) were performed with microarray analysis. Gene expression data was mined using advanced integration analysis with metabolic networks. This approach enabled the identification of specific key pathways that were affected in each tissue by the diet. Furthermore, by simultaneous analysis of transcriptome data from all tissues it was possible to identify basic cellular mechanisms, e.g. calcium handling, that were affected by the diet in all tissues studied. The approach of applying advanced integrated data analysis to nutritional studies can improve the understanding how diet affects processes that underlie disease development. This means that in the future, knowledge obtained from animal studies can be transferred and applied to clinical studies.

Investigations into fish lipid oxidation during gastrointestinal conditions

***Karin Larsson**, L.R. Cavonius, M. Alminger, and I. Undeland**

Department of Chemical and Biological Engineering, Food Science, Chalmers University of Technology, Gothenburg, Sweden

*karin.larsson@chalmers.se

Long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), like EPA and DHA, have for a long time been associated with preventive effects e.g. on cardiovascular disease (CVD) and positive effects on cognitive function. Unfortunately LC n-3 PUFA are also susceptible to oxidation, which alter their chemical properties and yield highly reactive oxidation products. It has lately been recognized that the conditions of the stomach appear to be very pro-oxidative, indicating that lipid oxidation may proceed not only during storage of the PUFA-containing food item, but also during the gastrointestinal (GI) passage. Using a static *in vitro* GI-model, we recently demonstrated that cod liver oil in different forms oxidized both in the stomach step and in the intestinal step. We hereby hypothesize that GI-oxidation may be a reason for the many contradictory results from studies of fish/fish oil and health effects.

The aim of this study was to investigate how certain fish-derived and digestive compounds (e.g. lipase, bile acids and hemoglobin) affect n-3 PUFA oxidation in a static *in vitro* GI tract model. We also compared the susceptibility of isolated oil and intact fish muscle from cod and

herring towards GI-oxidation. All samples (oil/emulsion/fish) were mixed with electrolyte solution, digestive enzymes and bile and incubated at 37°C for 3 h with stepwise adjustments of pH and oxygen pressure to simulate physiological conditions. The peroxide value (PV) and thiobarbituric reactive substances (TBARS) were measured after the gastric and the intestinal steps.

The results indicate that the lipolytic action in the static GI-model did not follow the same kinetics as lipid oxidation why free fatty acid formation does not appear to be the main reason for GI-oxidation of cod liver oil. Trace elements in oil/emulsion and bile acids could however contribute to the noted oxidation in the stomach and intestinal step respectively. Further, cod hemoglobin (Hb) was highly activated as a pro-oxidant by the stomach conditions, which resulted in major TBARS increases in a Hb-enriched emulsion in both the stomach and small intestine. This effect was, however, not observed in Hb-enriched cod muscle. Significant TBARS formation occurred in herring mince when subjected to the static GI-model, but only slight formation was seen in herring oil.

Septic mortality is decreased in mice fed a diet rich in n-3 fatty acid or treated with n-3 resolvin metabolites

***Sara Svahn*^{1,*}, *L. Grahnemo*¹, *S. Nilsson*², *J.O. Jansson*¹**

¹Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

²Department of Mathematical Statistics, Chalmers University of Technology, Gothenburg, Sweden

*sara.svahn@neuro.gu.se

We have previously found that following inoculation with *Staphylococcus aureus* (*S. aureus*) mice fed a high-fat diet with polyunsaturated fatty acids (HFD/P) have decreased mortality and bacterial load compared with mice fed a high-fat diet with saturated fatty acids (HFD/S). It has been postulated that the polyunsaturated n-3 fatty acid metabolites resolvins can mediate biological effects of n-3 fatty acids.

The purpose of this study was to determine which component in HFD/P that had the beneficial effect on septic mortality, and to find a possible mechanism for this effect. Mice were inoculated with *S. aureus* after 8 weeks on: (1) HFD/S, (2) a HFD rich in polyunsaturated n-3 fatty acids (HFD/n-3), or (3) a HFD rich in polyunsaturated n-6 fatty acids (HFD/n-6). We found that the mortality of HFD/n-3-fed mice

with *S. aureus* induced sepsis was lower than in mice fed HFD/S. Mice were then inoculated with *S. aureus* after 8 weeks on HFD/S. The mice were treated with vehicle, resolvin D1 (RvD1), resolvin D2 (RvD2) or 17(S)-hydroxydocosahexaenoic acid (17(S)-HDHA; an inactive precursor of resolvins) i.v. on day 1-5 after *S. aureus* inoculation. We found that the bacterial load in the kidneys on day 6 after bacterial inoculation was lower in mice injected with RvD1 or RvD2 than in mice injected with vehicle. There were no differences between mice given vehicle and 17(S)-HDHA.

In summary, *S. aureus*-induced septic mortality/bacterial load is decreased in mice fed n-3 fatty acids or treated with the biologically active n-3 metabolites RvD1 and RvD2.

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Fatty acid profiling of European seabass (*Dicentrarchus labrax*) flesh as a means of authenticating production origin?

M. Pirini¹, D. Brandolini², D. Remondini³, M. Trentini¹, Magda Rotolo¹, A. Badiani^{1,*}, and S. Testi²

¹Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Ozzano Emilia (BO), Italy

²Corso di Laurea in Acquacoltura e Igiene delle Produzioni Ittiche, Università di Bologna, Cesenatico (FC), Italy

³Dipartimento di Fisica, Università di Bologna, Bologna (BO), Italy

*anna.badiani@unibo.it

Seafood traceability and labelling as imposed by EU Regulation No 2065/2001 (REG in the following) requires that, in addition to commercial designation, both fishery and aquaculture products bear specification of production method and either catch or farming area. This information is all the more crucial when it comes to widely appreciated species, such as European seabass, which may be available in a wild state or derive from aquaculture, retaining quite a different intrinsic value in the consumers' opinions and therefore commanding widely different prices. A large study was therefore funded by the Italian Ministry of Agricultural, Food and Forestry Policies to test the usefulness of several analytical methods, taken either alone or in combination, to authenticate European seabass origin, the part reported here exploring the possibility to confirm product label specifications based solely on the fatty acid profile of flesh total lipids.

A total of 160 European seabass specimens were collected from November 2009 to December 2010. Aquacultured specimens (n=115) were obtained from 13 farms (Italy 8, Greece 2, Turkey 2, Croatia 1), one of the Italian farms

adopting quite a typical extensive production system in brackish lagoons named "vallicoltura", the others predominantly the floating cage intensive system. Wild specimens (n=45) were obtained from 5 main areas for seabass sourcing in Italy, 4 of which in the Mediterranean Sea. Fish were received on ice and promptly filleted, skinned and deboned. Total lipids were extracted (chloroform/methanol 1:1, v/v) from the homogenised flesh of each specimen and fatty acid methyl esters were separated on 30-m DB-23 capillary column. Supervised methods (quadratic discriminant analysis with leave-one-out cross-validation) were performed on the identified fatty acid data set (22/specimen).

As to the "production method" (REG, Article 4), multivariate statistics allowed the correct classification of specimens according to their farmed or wild status, with the only exception of those deriving from Italian "vallicoltura" (n=10). As to the "catch area" (REG, Article 5), about 70% of the wild specimens was allocated to their proper FAO fishing area, whereas whereas around 85% of the farmed specimens coming from Italian waters were properly labelled within the intensive farmed group.

Multi-elemental analysis by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES) of European seabass (*Dicentrarchus labrax*) from several sources

Marina Silvi¹, A. Guerrieri², D. Remondini³, M. Trentini², M. Rotolo², S. Testi¹, M. Pirini², and A. Badiani^{2,*}

¹Corso di Laurea in Acquacoltura e Igiene delle Produzioni Ittiche, Università di Bologna, Cesenatico (FC), Italy

²Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Ozzano Emilia (BO), Italy

³Dipartimento di Fisica, Università di Bologna, Bologna (BO), Italy

*anna.badiani@unibo.it

A large study centred on the authentication of origin (*sensu lato*) of European seabass and funded by the Italian Ministry of Agricultural, Food and Forestry Policies was set up with the aim of identifying the most cost-effective assemblage of analytical methods towards product authentication. The discriminatory power of each of those methods, when taken alone, was also assessed at an intermediate stage of the project. The part reported here explores the possibility of correctly identifying European seabass sources based exclusively on flesh multi-elemental analysis by ICP-AES.

A total of 160 European seabass specimens were collected from 18 sources in the period November 2009 - December 2010. Aquacultured specimens (n = 115) were obtained from 13 farms (Italy 8, Greece 2, Turkey 2, Croatia 1), one of the Italian farms adopting quite a typical extensive production system in brackish lagoons named "vallicoltura", the others predominantly the floating cage intensive system. Wild specimens (n = 45) were obtained from 5 main areas for seabass sourcing in Italy, 4 of which in the Mediterranean Sea. Fish were received on ice

and promptly filleted, skinned and deboned. The homogenised flesh samples, once microwave-digested, were individually analysed for six macro (Ca, K, Mg, Na, P, S) and twelve trace elements (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Zn) by ICP-AES. Supervised methods (quadratic discriminant analysis with leave-one-out cross-validation) were performed on the whole data set, partial results from an exploratory round of statistical analyses being reported here.

In this first attempt, elemental fingerprinting seemed not to be able to fully discriminate among the 18 European seabass sources. Slightly less than 40% of all the specimens were allocated to their own source (n = 63, 84% of which had been collected during the autumn and winter months). Slightly more than 62% of the wild specimens (28 out of 45) were allocated to the proper FAO fishing area. Significant differences between wild (W) and farmed (F) specimens were found in the content of K (W/F = 0.9625), Na (1.1272), P (0.9476), Cu (0.8680), Mn (0.8692), Se (1.2212), Zn (0.9378).

Selenium response to diverse household cooking methods as applied to 15 species of finfish and shellfish caught in the Mediterranean Sea

R. Orletti¹, E. Rocchegiani¹, A. Guerrieri², M. Silvi³, L. Tarsi³, S. Testi³, and Anna Badiani^{2,*}

¹Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Sede di Ancona, Italy

²Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Ozzano Emilia (BO), Italy

³Corso di Laurea in Acquacoltura e Igiene delle Produzioni Ittiche, Università di Bologna, Cesenatico (FC), Italy

*anna.badiani@unibo.it

Total selenium contents were determined in the edible portion of several species of blueback fish (European anchovy, European pilchard, sprat, horse mackerel), whitefish (European hake, common sole, red mullet, flathead grey mullet, tub gurnard), molluscs (cuttlefish, Mediterranean mussel, Manila clam, striped Venus clam) and crustaceans (Caramote prawn, mantis shrimp). Within each species, specimens were analysed both in the raw state and cooked by the most appropriate technique [selected among the following: oven roasting in a partially covered tin (ORO), pan frying (PAF), cooking in parchment (PAR), cooking in a covered non-stick pan (CNS), pressure cooking (PRE), steam cooking (STM)], to gain knowledge about selenium true retention values. Several considerations prompted and justified this study at the same time: the essentiality of selenium, the medium-to-high level of this element known to be present in raw seafood, the attention recently focused on the influence exerted by processing (cooking in particular) on such issues as selenium bioavailability and speciation, the variety of culinary approaches to finfish and shellfish which have been fine-tuned in centuries in the Mediterranean countries and possibly bears on selenium retention.

From October to May, 2-to-5 batches per season were collected for each species, the number of specimens per batch depending on their average size and merceological nature. A species-specific procedure was devised in order to have a representative raw reference for each cooked sample. Within species and batch, both raw and cooked flesh was microwave digested and analysed in duplicate by ICP-MS for total selenium content. True retention values (TRVs) of selenium were determined for each species within its own cooking procedure and cooking yield.

Season of catch effect was significant for two-thirds of finfish species and for mantis shrimp, the lower levels being in autumn. Upon cooking, selenium content increased significantly with the exception of PRE cuttlefish, CNS mussel and CNS Manila clam. The richest sources of selenium were ORO pilchard within blueback fish, ORO tub gurnard and ORO red mullet within whitefish, CNS mussel within molluscs and PAF mantis shrimp within crustaceans. When comparing cooking methods as to the selenium TRVs (%) they generated, significant differences did emerge, the average being 106a, 103a, 100a, 95.1ab, 83.9b, and 46.6c, for ORO, PAF, PAR, STM, CNS, and PRE, respectively.

Fresh start: HACCP 2011 for seafood commerce in the USA

Steve Otwell

National Coordinator for Seafood HACCP Alliance, Food Science & Human Nutrition Department
University of Florida, Gainesville, USA
otwell@ufl.edu

On April 26, 2011 the U. S. Food and Drug Administration, the leading authority for seafood safety, released their new, fourth edition of the Fish and Fishery Products Hazards and Controls Guidance (FDA.gov). This document contains the recommendations governing the commerce of all seafood sold in the United States. The original intent remains the same as initially mandated in 1996, but the revised version contains additions, changes and specific clarifications based on over 15 years of commercial and regulatory experience under the HACCP concepts. Required compliance is immediate relative to statues calling for current updates and HACCP plan reviews, but actual enforcement will become more increasingly evident through the following months. Adjustments will involve both domestic and international processing operations and importers based in the USA. Some of the more

significant changes will focus more attention for time-temperature controls during transit, increasing validations for thermal processes, care in monitoring for proper use of antibiotics with farm-raised products, and specific product identity for potential allergen hazards. The format is more user-friendly and progressively outlines more controls intended to prevent scombrototoxic poisoning, *Clostridium botulinum* growth in reduced oxygen packaging, and potential *Vibrio* pathogens in molluscan shellfish destined for raw consumption. New action levels are introduced for natural ciguatoxins from both Pacific and Caribbean waters, and post-harvest processing (PHP) methods are more formally recognized for use to retain raw product characteristics. The details and implications for international commerce will be discussed along with predictions for impacts on product availability and costs.

Food safety of salt-cured products. Effects of salt-curing, rehydration on survival, growth and invasiveness of *Listeria* spp.

***Grete Lorentzen*^{1,*}, *S. Mennen*¹, *R.L. Olsen*², *I. Bjørkevoll*³, *H. Mikkelsen*¹, and *T. Skjerdal*⁴**

¹Nofima, Tromsø, Norway

²University of Tromsø, Norwegian College of Fishery Science, Tromsø, Norway

³Møre Research, Ålesund, Norway

⁴National Veterinary Institute, Oslo, Norway

*grete.lorentzen@nofima.no

In the North Atlantic countries, salting and drying of cod have continued for centuries up to the present time. In Norway, about 50% of the cod harvested is salt-cured. In 2009, approximately 120 000 tonnes of salt-cured cod was exported from Norway and this implies a consumption of about 150 000 tonnes of rehydrated cod. Salt-cured products are traditional and highly regarded products, especially by the Mediterranean, Caribbean and Brazilian populations. In addition, it is a growing market in the Scandinavian countries.

In our work, survival of *Listeria innocua* and *Listeria monocytogenes* in muscle of cod during salt-curing and growth during chilled storage of the rehydrated product was studied. Fresh cod was inoculated with each strain at different levels before salt-curing. After salt-curing and rehydration, *Listeria* spp. was detected at levels within 1 log₁₀ CFU/g lower than prior to salt-curing. During storage of the rehydrated product, growth of *Listeria* spp. was observed. These experiments demonstrated that long term

exposure to very high salt concentrations did not eliminate *Listeria* spp., and that *Listeria* spp. being present in the fish prior to salt-curing could recover and grow in the rehydrated salt-cured cod products.

Afterwards, the ability of salt-stressed *L. monocytogenes* to cause listeriosis, measured as its ability to invade human Caco-2 cells, was studied. *L. monocytogenes* was cultivated in BHI at 4 °C to stationary phase and exposed to either no salt or salt stress conditions comparable to that applied in the production of salt-cured and rehydrated salt-cured cod. The results show that extreme salt-stress exposure attenuated the invasiveness of *L. monocytogenes* whereas the ability to invade Caco-2 cells was significantly higher for the non-salt stressed strains. As the ability to invade the Caco-2 cells correlates with bacterial virulence, the results suggests that *L. monocytogenes* exposed to salt-curing with extreme NaCl concentrations represent a lower food safety risk compared to no salt stress exposure.

Microbiological properties of sushi during storage

H. Herland*, M.S. Wesmajervi Breiland, Heidi A. Nilsen, G. Lorentzen, and M. Cooper

Nofima, Tromsø, Norway

*hilde.herland@nofima.no

The Japanese delicacies, sushi and sashimi, are experiencing widespread growth in popularity in Europe and Scandinavia. Sushi and sashimi are seen as nutritious, natural foods with beneficial health effects.

Sushi rice is cooked using a combination of boiling and steaming followed by marinating in a mixture of rice vinegar, sugar and salt. The pH of the rice mixtures used in these studies were 4.2 - 4.3. Sushi rice ingredients, whole, fresh salmon and sliced, frozen halibut were purchased locally.

In the first experiment, samples of fish were taken for pH measurements and microbial culture (total viable counts, TVC and sulphur-producing bacteria, SPB) at the point of preparation and at timed intervals. Fish pieces stored without rice served as controls and experimental samples were stored either on a bed of sushi rice (sushi nigiri) or packed in sushi rice (sushi maki). Sets of six fish pieces with and without rice were stored at 4° C in single use plastic containers covered with aluminium foil. One container was prepared for each sample point.

Fish pH remained stable at around 6.3 for up to six days in the controls and fell by 0.5 - 1.0 pH

units in fish pieces in contact with sushi rice. The pH change appeared depended on fish species and length of exposure to the rice. For both salmon and halibut TVC were significantly lower than controls in fish pieces exposed to rice for 48 hours or more, $p \leq 0.01$.

The aim of the second experiment was to study the viability of *Listeria monocytogenes* in a model of nigiri sushi comprised of either halibut or salmon. Sushi is not free from health risks and food borne illness linked to this product has been attributed to *L. monocytogenes*. Samples of halibut (frozen and thawed) and salmon (fresh) were inoculated with the pathogen and subsequently placed on top of vinegar marinated sushi rice followed by storage for 7 days at 4 and 8 °C. Inoculated seafood without rice served as controls.

The experiments demonstrated that the level of *L. monocytogenes* in simulated nigiri sushi was significantly lower during storage over 7 days at 4 and 8 °C compared to controls. The pH drop in the fish muscle, caused by the sushi rice, is believed to be the reason for the decrease in viability of *L. monocytogenes* in nigiri sushi.

Effect of CO₂-enriched atmospheres on the survival of *Listeria monocytogenes*, *Salmonella enteritidis*, *Vibrio parahaemolyticus* and *Aeromonas hydrophila* in sea bream (*Sparus aurata*) fillets

L. Provincial, E. Guillén, M. Gil, V. Alonso, P. Roncalés, and Jose A. Beltrán*

Department of Animal Production and Food Science, Laboratory of Science and Technology of Meat and Fish, Faculty of Veterinary Sciences, University of Zaragoza, Zaragoza, Spain

*jbeltrán@unizar.es

The conditions used in MAP that avoid the suppression of indigenous flora and reduces some degradation reactions combined with refrigeration temperatures, extend the shelf life of fish and fish products but could enhance the growth of some pathogens. *Listeria*, *Salmonella*, *Vibrio* and *Aeromonas* spp. are widely distributed in marine environments and foods. Important gastrointestinal diseases may be caused by them, through the consumption of contaminated raw or undercooked fish.

The objectives of this research were to determine the effect of different CO₂/N₂ atmospheres on the survival of two strains of: *Listeria monocytogenes*, *Salmonella enteritidis*, *Vibrio parahaemolyticus* and *Aeromonas hydrophila* during the storage of fresh sea bream fillets at 0°C and 4°C during 16 days. Fillets were packaged with three different modified atmospheres: 60%CO₂/40%N₂, 70%CO₂/30%N₂ and 80%CO₂/20%N₂. Headspace gas composition, total mesophilic and psychrotrophic viable counts, *Enterobacteriaceae*, *Salmonella*, *Listeria*, *Vibrio* and *Aeromonas* investigation were carried out in control samples and the same counts were performed in inoculated ones.

The highest absorption of CO₂ appeared in 80/20 samples and the lower the temperature used, the

higher CO₂ absorption. At 0°C *Enterobacteriaceae* showed no growth and mesophilic and psychrotrophic flora showed some difficulties to grow. At 4°C all the microorganisms studied were able to grow exceeding the legal limits. None of the control samples were contaminated with *Listeria*, *Vibrio* or *Aeromonas* and only one sample of 72 was contaminated with *Salmonella*. All the pathogens studied were inhibited and counts decreased 2-4 logarithmic cycles depending on the strain and the atmosphere used in inoculated samples at 0°C. During the storage at 4°C *Salmonella* and *Vibrio* showed a great decrease although *Salmonella* was able to grow after day 12. On the other hand, *Listeria* and *Aeromonas* were able to grow from the beginning, especially in samples with lower CO₂ concentrations.

The antimicrobial effectiveness of 60-80% CO₂ concentrations was very similar against the four pathogens; showing isolated differences where richer CO₂ batches presented lower microbial counts. Storage at 0°C was the most effective factor to control the growth of microorganisms although the strains tested had different sensitivity to CO₂ and temperature.

Parvalbumins in herring (*Clupea harengus*): characteristics and stability during processing

Hartmut Rehbein^{*} and M. Schwarz

Max Rubner-Institute, Hamburg, Germany

^{*}hartmut.rehbein@mri.bund.de

Parvalbumins are water-soluble, calcium-binding, heat-stable proteins, which have been detected in the fillet of many fish species. Parvalbumins have a low molecular mass (10-12 kDalton), can resist heat denaturation, and possess an isoelectric point (pI value) between pH 3.5 to 5.5. Parvalbumins are considered to be the major allergens of fish.

The aim of our study was to characterize the parvalbumins of the light muscle of herring, and to determine their stability against heat and acetic acid. By isoelectric focusing of sarcoplasmic proteins, in raw fillet of herring three isoforms of parvalbumin were detected, having pI values of 4.1, 4.2 and 5.2. Their

molecular mass was between 6.5 to 11.5 kDalton, as determined by SDS-PAGE.

The parvalbumins of herring turned out to be resistant against heating up to 90 °C, and were nearly stable during storage in acetic acid. However, during incubation of acidified muscle extract, parvalbumins were degraded by proteases, presumably of lysosomal origin.

Parvalbumin concentration was very low in marinated herring like “Roll mops” or “Bismarck herring”, offering the possibility to develop hypo allergic herring products.

Retention and viability of *Anisakis simplex* larvae in the mechanical separation of muscle during surimi process simulation

***Fabiola Olivares*^{1,*}, *C. de las Heras*¹, *A. Mendizábal*², and *M. Tejada*¹**

¹Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN), Consejo Superior de Investigaciones Científicas (CSIC). Madrid, Spain

²Technical Unit of Mercamadrid, Public Health Institute, Madrid City Council, Madrid, Spain

*fabiola.olivares@ictan.csic.es

Surimi, a mechanical deboned, minced and washed fish flesh, is thoroughly used as an intermediate product in the preparation of a variety of imitation seafood products. Basically, surimi processing can be divided into two main stages: the first phase prepares the fish mince for the second one, washing and refining of mince. The fish mince preparation, depending on the raw material, includes heading, gutting, filleting and removal of the backbone to eliminate viscera and impurities. One common meat separator is a belt-drum type, by which meat is squeezed through a drum with large number of holes (3-5 mm in diameter) enclosed in a squeezing disc or roll. In surimi and surimi products, adequate freezing or heating would kill *Anisakis* larvae; however dead larvae can still cause allergic symptoms in sensitized consumers.

The aim of this study is to assess the retention and viability of *Anisakis simplex* larvae in simulated mechanical deboning and separation of muscle during the first stage of surimi process. Fresh hake (*Merluccius merluccius*) fillets were artificially parasitized with live *A. simplex* larvae (20 larvae in 100 g muscle). The retention of larvae in the sieves and the presence of larvae in the fresh minced muscle was measured after passing the muscle through single or successive sieves (simulation of

mincing and refining) of different hole diameter (3,5; 3,0 and 2,0 mm). The same treatments were applied to frozen-thawed hake belly flaps with a natural heavy infestation. (116 larvae in 100 g muscle).

The yield of mince muscle obtained after sieving ranged between 60 and 80% depending on the sieve used and the raw material. The percentage of entire larvae (alive or dead) in the minced muscle decreased significantly at lower hole diameter, especially when it passes through the three sieves at decreasing hole size. Nevertheless, the high content of broken larvae found in the minced muscle after passing through sieves with smaller hole diameter might indicate a higher spread of larval somatic antigens.

Immunological studies are in progress in order to elucidate how this first phase of the process influences the rate of antigens in the surimi obtained.

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Serotypes of *L. monocytogenes* isolated from RTE seafood in northern Greece

Nikolaos Soutos^{*}, *E. Iossifidou, Th. Lazou, D. Sergelidis, G. Drakopoulos, and I. Konstantelis*

Laboratory of Hygiene of Food of Animal Origin, Department of Hygiene and Technology of Food of Animal Origin, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

*soutos@vet.auth.gr

Serotyping has been widely used to characterize *L. monocytogenes* isolates and is important from the epidemiological point of view. For the food industry, where the presence of *L. monocytogenes*, is a big concern, tracing contaminating strains within the food chain and the plant environment is of primary importance. According to previous reports, although 13 serovars are described for the species *L. monocytogenes*, at least 95 % of the strains isolated from foods and patients belong to serovars 1/2a, 1/2b, 1/2c and 4b. The aim of this study was to identify the serotypes of *L. monocytogenes* isolated from ready-to-eat (RTE) seafood samples collected from different markets in Thessaloniki, northern Greece. A total of 15 *L. monocytogenes* isolates recovered from 132 RTE seafood samples were used in the present study. For the identification of the

serotypes, a rapid and practical multiplex PCR assay was used which clusters *L. monocytogenes* strains into four major groups (group 1: serovars 1/2a, 3a; group 2: 1/2b, 3b, 7; group 3: 1/2c, 3c; and group 4: 4b, 4d, 4e). Out of 15 *L. monocytogenes* isolates tested, 7 (46.7 %), 6 (40 %) and 2 (13.3 %) were serotyped as 1/2c (or 3c), 1/2a (or 3a) and 4b (or 4d, 4e), respectively. Multiple serotypes were not observed in any of the samples tested. The presence of this human pathogen in RTE seafood should be considered as having significant public health implications, particularly among persons at greater risk. Therefore, RTE seafood should be produced under appropriate hygienic and technological conditions since the product does not undergo any treatment to ensure its safety before consumption.

Determination of selected toxic elements by ICP-MS in Atlantic mackerel *Scomber scombrus* imported to Turkey

T. Yurdusever^{1,*}, C. Altinelataman², and Ufuk Celik²

¹Ministry of Food, Agriculture and Livestock, Izmir, Turkey

²Ege University, Faculty of Fisheries, Izmir, Turkey

*tyurdusever@hotmail.com

Contamination in fish is one of the major problems for consumers' health. Heavy metals include toxic elements that cause harmful effects to the human body which were well investigated in last two decades by many researchers. The main objective of this research is to determine lead, cadmium, mercury and arsenic contents of imported mackerels in Turkey.

In this study, selected toxic (lead, cadmium, arsenic, mercury) element contents of mackerel which was imported as frozen in 2010, were determined by ICP-MS with method EPA 3052 and EPA 6020A. Presented are the results of annual monitoring by the Ministry of Food, Agriculture and Livestock Izmir central control laboratory between January and November 2010. All analyses were done for edible parts of

fish. All elements were determined in "total" forms.

Cadmium, lead and mercury levels were under the legal limits for all samples. Arsenic levels of 24 out of 34 samples exceeded the limit (1 mg kg⁻¹). The highest As content was found in samples of September (3.059 mgkg⁻¹). Samples of October all exceed the limit. Lowest As value belonged to samples of February (0.627 mgkg⁻¹).

Results showed that all values were in the range of legal limits for Pb, Cd and Hg. 70% of all samples were above the legal limit for total arsenic. Two arsenic species As (III) and As (V) were toxic. Therefore some further, separate analyses should be done for these As species for frozen pelagic fishes.

When meat meets fish – muscle as food, some personal reflections

Bo Ekstrand

Department Food Science, Aarhus University, Denmark

bo.ekstrand@agrsci.dk

Fish and meat are two examples of muscle based food, with both great similarities and differences.

This paper will be based on some research activities in the areas of post-mortem proteolytic changes in muscle structure, of lipid composition and stability, and of effect of feed on fish and meat quality and composition.

For the post-mortem proteolysis in fish and meat there has been a development in the last 30 years of the models for the proteolytic mechanisms that are effective after death, in which order they come and which is the most important, etc. For meat quality this is of immense importance, and there have been some recent findings that changed our view of the combined effect of proteolytic enzymes, pH and Ca release.

For fish the lipid composition and content of polyunsaturated n-3 fatty acids (PUFAs) has always been a central aspect for nutritional value and shelf life stability, the important content of the fatty acids threatened by oxidation and

rancidity. For meat production the possibility to increase the content of n-3 fatty acids by special feed has been of great interest, and how this would influence the sensory quality and storage stability. There is a tendency in modern meat production to change lipid sources in the feed, both for economic and practical reasons, and this will influence the lipid content and maybe the functional properties of meat, but how?

And this leads to the final theme, how fish and meat quality is influenced by the composition of the feed, in many levels, and how the same is valid for food and human health. From the basic view that we are what we eat, we have come to understand that the metabolism and omic regulation in animals and in ourselves is affected at four or five omic levels by components in feed and food. If we can learn to master these complicated synergy mechanisms, it might give us new tools to achieve good quality fish and meat products.



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