Università degli Studi di Firenze

38th Annual WEFTA Meeting

SEAFOOD

from CATCH AND AQUACULTURE

for A SUSTAINABLE SUPPLY

edited by
Bianca Maria Poli
Giuliana Parisi

Proceedings e report

42

Seafood from catch and aquaculture for a sustainable supply

Book of abstracts

edited by
Bianca Maria Poli
Giuliana Parisi

FIRENZE UNIVERSITY PRESS
2008

Seafood from catch and aquaculture for a sustainable supply : book of abstracts / edited by Bianca Maria Poli, Giuliana Parisi – Firenze : Firenze University Press, 2008
(Proceedings e report ; 42)

http://digital.casalini.it/9788884537461

ISBN 978-88-8453-746-1 (online)
ISBN 978-88-8453-745-4 (print)

Progetto grafico della copertina di Alberto Pizarro Fernández

Università degli Studi di Firenze
Firenze University Press
Borgo Albizi, 28, 50122 Firenze, Italy
http://www.fupress.com/

Printed in Italy

WELCOME ADDRESS

It is a great honour and pleasure to welcome all of you to this 38th WEFTA 2008 Meeting in Florence and to be witness to this first time for an Italian representative to host a WEFTA Annual Meeting. The meeting title SEAFOOD FROM CATCH AND AQUACULTURE FOR A SUSTAINABLE SUPPLY was chosen to underline the growing role of aquaculture in seafood supply, now approaching about half of the total sustainable supply.

The WEFTA 2008 will deal with physiological and nutritional aspects related to traditional, novel and underutilised species, both from catch and aquaculture going to examine their safety, quality, freshness, deterioration mechanism and preservation capabilities. Stress from catching, rearing, handling, transport, and killing practices is another pressing item both from an ethical point of view and with reference to the final product quality, in perspective of a future application of animal welfare regulations to the EU fish-farming sector.

The exploitation of milder and more natural seafood preservation, preventing spoilage and oxidation and considering waste and by-products management by turning this resource into value-added products, represent important issues in the perspective of better seafood quality and safety preservation and full utilisation of what is currently wasted for increasing sustainability. Even several kind of restructured foods by dietary components are proposed, testing their physical-chemical and sensory traits before offering the products to the consumer, are topics of high relevance today because of the industry awareness towards innovative products requested by the market.

Seafood quality today does not only mean sensory, chemical and microbiological quality but includes also all their components able to improve human health and well being, so the meeting cannot avoid considering the healthy effects of seafood consumption.

The high number of participants in WEFTA2008, where about 120 full and short papers will be presented, represents a very positive answer of fish technologists to the present difficulties faced by the fishing sector. This answer is also a confirmation that the vision of those who established the WEFTA Association 38 years ago for discussing topics of relevance for the fish processing was right. This informal organisation was able to grow and to establish bridges with colleagues in Europe and across the border of the Atlantic Ocean. WEFTA has been a powerful instrument to promote the relationships among the institutions dealing with fish to participate in multiple research projects in the frame of European research programmes. This association has also allowed the exchange of researchers and knowledge, the strengthening of relationships among research institutions and the formation of teams and networks of excellence, encouraging and mentoring young scientists, educators and policy makers, in fulfilling the goal of sustainable fisheries and aquaculture to ensure a high quality of life.

The WEFTA 2008 organizers and the editors of this Book of Abstracts want to express their thanks to all the authors, participants, WEFTA representatives and colleagues involved in the activities to arrange this event; to all of you, our sincere and personal thanks. We wish you all a very fruitful WEFTA Meeting and a pleasant stay in Florence in this September 2008.

Bianca Maria Poli on behalf of the 38th WEFTA Organizing Committee.

Bianca Maria Poli, Giuliana Parisi (edited by), *Seafood from Catch and Aquaculture for a sustainable Supply. Book of Abstracts*, ISBN 78-88-8453-746-1 (online), ISBN 978-88-8453-745-4 (print), © 2008 Firenze University Press

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Complete Conference Programme

Tuesday, September 16th – room Santa Croce

PM

16:00 Meeting of WEFTA national representatives
18:00 Registration

Wednesday, September 17th – room Ponte Vecchio

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Thursday, September 18th – room Ponte Vecchio

AM

Session 2. Seafood from catch and aquaculture: supply and quality (cont.)

Chairs: Caroline Baron, Hans van de Vis and Gunn Berit Olsson

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20:30		Conference Dinner by Circolo Borghese, Via Ghibellina 110 (close to Bargello)	

Thursday, September 18th – room Pitti – Parallel session

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Session 3. Future challenges in seafood safety and quality assessment
Chairs: Mercedes Careche, Ingrid Undeland and Mark P. Richards

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Social activity

18:15 Visit to the Bargello National Museum (transfer from the conference site)

20:00 Conference Dinner by Circolo Borghese, Via Ghibellina 110 (close to Bargello)

Friday, September 19th – room Ponte Vecchio

AM

Session 3. Future challenges in seafood safety and quality assessment (cont.)

Chairs: Michael Morrissey and Françoise Leroy

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Session 3. Future challenges in seafood safety and quality assessment (cont.)
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Friday, September 19th – room Pitti

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Abstracts

Full & Short communications

Bianca Maria Poli, Giuliana Parisi (edited by), *Seafood from Catch and Aquaculture for a sustainable Supply. Book of Abstracts*,
ISBN 78-88-8453-746-1 (online), ISBN 978-88-8453-745-4 (print), © 2008 Firenze University Press

Keynote lecture

Seafood in the context of the global food outlook

Valdimarsson G.

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Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome, Italy

The current rise in the price of energy is having profound effects on global food production and food markets. In recent decades massive strides have been made in improving food security. Per capita food production, particularly in developing countries, has increased very significantly. The share of people with less than 2200 kcal intake per day has fallen to about 10% from 57% in 1964-1966. Yet, some 776 million people are still undernourished. For the world as a whole, fish proteins constitute some 6% of total protein consumption and 15 % of the animal protein consumption.

Global fisheries continue to set new production records. These have risen to new levels thanks to aquaculture, which is fast approaching 50% in its contribution to fish for human consumption. World fish consumption has reached a new record of 17.4 kg per capita and the global fish trade is approaching US$ 100 billion in value. Fish enjoys a good reputation as a healthy food.

However, despite this good news, the big problems in fisheries remain largely the same. Mechanisms to manage capture fisheries are ineffective in many countries, environmental effects of fishing and aquaculture are negative and Illegal, Unregulated and Unreported (IUU) fishing is common. The picture is, however, not all bleak as considerable work is being done on many fronts to address these problems with positive results.

The paper will give a view of fisheries and aquaculture in the context of the global food outlook with projections on how the sector is likely to develop in the coming decades.

Session 1

Seafood from catch and aquaculture on the same market place: the consumer perspective

Bianca Maria Poli, Giuliana Parisi (edited by), *Seafood from Catch and Aquaculture for a sustainable Supply*. *Book of Abstracts*, ISBN 78-88-8453-746-1 (online), ISBN 978-88-8453-745-4 (print), © 2008 Firenze University Press

F1.01. Consumer evaluation of fresh fillets of cod and salmon: a preliminary study

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From a consumer's point of view the possibilities of a thorough evaluation of fresh seafood at point of sale is limited: Fresh seafood is handled by the shop's personnel, and the products are often exposed behind a glass wall making also visual inspections difficult. Evaluation of pre-packed seafood products, a growing category in many European countries, is also limited. The quality assessment of fresh seafood must therefore be made at home.
Few reported studies have targeted consumer evaluation of fresh seafood, and an experiment was set up to explore this.
Fillets of cod and salmon were stored at three different temperatures (0 °C, 4 °C and 7 °C). The wild cod was caught, bled, gutted and kept on ice 2 days old before filleting. The farmed salmon was slaughtered and kept on ice 1 day before filleting.
2 pieces of salmon or cod fillet (500 g) were put in trays and wrapped in plastic.
30 minutes before the consumer assessment the plastic were removed. The only information given was that the products were fillets of cod and salmon and the consumers were allowed to closely inspect and smell the products. Each product was assessed using a 7-point scale with 1 as top and 7 as bottom rating. Assessment took place after 1, 4, 6, 8, 11 and 15 days of storage and about 40 persons assessed the fillets each time. The results show that some consumers rejected very fresh fillets, while others accepted fillets that were stored for a long time. In general, the fillets of salmon were rejected later than the cod filets at the same storage time and temperature. The results show a great variance in the consumers' acceptance of both fresh cod and salmon fillets.

F1.02. Connecting consumer preferences with technical product specifications in wheat dietary fibre enriched seafood restructured products

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Previous studies have shown that there is an opportunity to develop new seafood products better adapted to consumer preferences. Experts can establish the technical product specifications which determine the ideal quality standards, also called objective quality. But consumers' evaluation does not necessarily have to coincide with this objective quality. Thus there is a need to integrating the two approaches -objective quality and consumer preferences- in order to adapt or improve the physical characteristics of the seafood products based on consumer demands. The overall objective of this research consisted in relating the seafood product's technological characteristics established by the experts with consumer evaluations.

First, the technological properties of a salmon restructured product with (M1) or without (M0) wheat dietary fibre added were established. These included: proximate analyses, colour, water binding capacity, texture profile analysis and stress-relaxation test. The products were used for an experiment with 100 consumers in order to evaluate the sensory acceptability. Evaluation was performed prior to consumption (e.g. appearance, sensory properties), during and after consumption (e.g. taste, naturalness, satisfaction, intention, and willingness to pay for the product).

Technological results showed that the main differences were related to the rheological parameters and texture profile analysis, as well as water retention capacity. Prior to consumption, consumers´ evaluations of sensory properties were higher for the product with dietary fibre. During consumption fatty/lean and texture have presented significant differences between both products. Post consumption evaluation was quite acceptable in both products but significantly higher for the case of salmon without dietary fibre. In conclusion, in this product, consumers can detect some intrinsic properties related with its quality. This knowledge will be used by experts with the subsequent advantage in the improvement steps to be carried out before considering the restructured product enriched in dietary fibre ready for commercial use.

F1.03. Is it possible to 'sell' welfare to EU consumers?

Schelvis R.[1], Martinsdottir E.[2], Sveinsdottir K.[2], Verbeke W.[3], Olson S.O.[4], Hokanen P.[4], Kole A.[5]

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In the ETHICOD project of SEAFOODplus, we address ethical concerns, asking if good fish welfare lead to better quality of farmed fish. The project includes full scale value chain experiments up to the consumer's fork.
Objectives of this project were:
- To measure the effect of consumers' knowledge about welfare information on the consumers' product perception.
- To measure the effect of consumers' use of and trust in welfare information on the consumers' product perception.
Results will be presented from the consumer in-home tests performed in Iceland and the Netherlands in an in-home situation n = 156 subjects from Iceland and n = 202 subjects from The Netherlands tested once a week the 4 -6 samples with various sets of information. The consumers filled out a 'product test' questionnaire and a 'general' questionnaire.
The results show that the consumers hardly use information on welfare when they buy seafood but if they search for information about welfare, they firstly look on the product label.
Consumers think that Government and fish farmers have the equal responsibility to take care of the welfare of the fish. When the source of the information about welfare is mentioned on the label of the tested cod, either governmental or producers organisation, the reliability of the governmental source is higher. The consumers think that though the fish farmers are the experts they also have more interest in hiding the truth about welfare issues. The more reliable the information is perceived, the higher the buying intention of the product labeled as such.
The more detailed the information was provided, the more relevant, important and reliable this information was perceived and resulted in higher selfreported knowledge of the system. At the same time this resulted in higher quality perception of the product.
It can be concluded that it is possible to 'sell' welfare to some specific groups of consumers if attention is paid to the source and content of the product information.

Session 2

Seafood from catch and aquaculture: supply and quality

Bianca Maria Poli, Giuliana Parisi (edited by), *Seafood from Catch and Aquaculture for a sustainable Supply*. *Book of Abstracts*, ISBN 78-88-8453-746-1 (online), ISBN 978-88-8453-745-4 (print), © 2008 Firenze University Press

F2.01. Biochemical changes in muscle, gonad and liver during maturation of farmed cod, *Gadus morhua* L.

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The objective for the present experiment was to investigate the chemical composition, including amino acid (AA) and fatty acid (FA) profile in muscle, gonads and liver of maturing commercial farmed Atlantic cod.
In cod farming, the winter months brings unfavourable growth conditions coinciding with a dramatic increase of gonads in sexually maturing individuals, which involves considerable metabolic reorganisation. Myotomal protein content was reduced by 4.5% and 1.9% females and male respectively. From December to February the female gonad index increased from 4.4% to 15.6%, while male gonad index increased from 12.1 % to 13.5 %. The AA profile in the muscle only showed minor changes with the exception lysine. The lysine content of female gonad increased and paralleled that of muscle tissue. Apart from lysine it was an increase in alanine, valine, isoleucine and trytophan when the aspargine, glutamine, arginine and methionine decreased in female gonad tissue.
From December to April liver index of males was reduced from 17.8% to 12.9% while only being reduced from 17.4% to 15.4% in females. The fat content of the liver in males was stable during the winter (~74%) while fluctuating from 72% to 61% in the females, indicating an uptake of water during maturation of females. The FA composition was relatively stable in the liver consisting of approximately 22% saturated, 41% monounsaturated and 37% polyunsaturated fatty acids with approximately 14% EPA and 11% DHA in the muscle tissue for both males and females. The EPA and DHA content was higher in the muscle and varied from 22 to 27% for EPA and from 31 to 25% for DHA. The lowest values for monounsaturated FA and the highest values for polyunsaturated FA were found in the gonads.

F2.02. Manipulation of growth and quality in underyearling and yearling farmed Atlantic salmon

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The aim of this study was to identify the assembled effect of feed and feeding practices on growth, flesh quality and feed utilisation in underyearling (0+) and yearling (1+) farmed Atlantic salmon (*Salmo salar*). From November 2006 to July (0+) and September (1+) 2007 a large scale feeding trail was carried out under commercial condition at Gildeskål Research Station in the northern part of Norway. Both yearling (1 kg) and underyearling (2.4 kg) fish were divided into a control and a treatment group each consisting of 4 net pens with approx. 25000 fish in each. The control groups were given a commercial high energy feed (38% fat, 32-36% protein) combined with a commercial accepted feeding regime (1-3 daily meals). The treatment groups were given a leaner feed (30% fat, 40% protein) combined with less frequently feeding (1 meal every second day-1 daily meal).
Development of groups mean weight was measured 1-2 times a month using a submerged weight-frame system. Flesh quality traits measured in the Norwegian Quality Cut were red colour by Minolta, fat and protein by near infrared transmission (NIT), texture as shear force by TA-XT2 and muscle fibre distribution by cryosectioning and examination under a light microscope.
Underyearling salmon fed less frequently with a leaner feed resulted in significant stronger red colour, less fat, more protein, softer texture and a higher amount of small muscle fibres (10-40 µm).
Yearling salmon fed less frequently with a leaner feed resulted in slower growth through the winter followed by a catch up growth up to harvest, significant less fat, softer texture, higher muscle fibre density and more intense red colour. All the differences were diminished at harvest when the fish apparently had been able to catch up with the differences established during the winter period.

F2.03. Influence of feeding regimes on fish composition and oxidative stability

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Due to the growing amount of fish produced by aquaculture and the increasing demand for fish oil and fish meal/protein, there is a growing interest in replacing fish based raw materials in fish feed with vegetable based raw materials. Therefore, it is necessary to study the effect of changing diets on the composition of the resulting fish. A difference in composition may lead to differences in quality, possibly sensory attributes or behavior during storage.
For the present study, rainbow trouts were fed with 6 different feeding regimes for 12 weeks – from a diet based exclusively on marine oil and protein to a diet based exclusively on vegetable products.
Proximate analysis, including e.g. protein, amino acid, lipid and fatty acid analyses on feed, faeces and fish was performed.
Additionally, a storage experiment was performed by keeping rainbow trout stored under ice (at 2 ^{o}C) for 3, 5, 7 and 12 days. Lipid and protein oxidation were characterised by the same analyses as mentioned for the proximate analysis plus astaxanthine and tocopherol as antioxidants, primary and secondary lipid oxidation products, as well as carbonyl content, western blot and SH-content for protein oxidation markers.
The proximate analysis showed differences in composition related to feeding, e.g. higher content of n-3 fatty acids in fillets for trout fed with higher content of fish oil in the diet. In the storage experiment this led to higher contents of primary and secondary lipid oxidation products after 12 days of storage. The differences in protein content and amino acid composition as well as protein oxidation markers after 12 days of storage were not very pronounced.

F2.04. Effect of partial substitution of fish meal with plant sources in feed on merchantable and nutritional quality traits of sea bream

Parisi G.[1], Giorgi G.[1], Lupi P.[1], Zampacavallo G.[1], Mecatti M.[1], Messini A.[2], Barsalini L.[1], Poli B.M.[1]

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[2]*Dipartimento di Biotecnologie Agrarie-Sezione Microbiologia, University of Florence, Florence, Italy*

The use of plant protein sources in partial substitution of fish meal, traditionally utilized in diet formulation for carnivorous species of fish, is considered the obliged way to increase the aquaculture production.
The aim of this research was to study the effect of a diet containing plant protein sources in partial substitution of fish meal on quality traits of sea bream of commercial size (600g), in a long duration feeding trial.
Two groups of sea bream (mean initial body weight: 106 g) were reared in floating cages and fed two isoenergetic and isoproteic diets (2 replicates for diet), different for the quality of protein source (100% fish meal/FM or 50% fish meal and 50% of a mixture of plant protein sources, i.e. soybean meal, corn gluten meal and pea protein isolate /PP). After about 460 days of rearing, sea bream (total number: 240) were sampled, ice-water stunned/killed and analysed for zootechnical and marketable characteristics (body weight, dressing and fillet yields, visceral fat incidence, skin and fillet colour), chemical composition of the fillet (proximate analysis, fatty acids composition) and quality parameters behaviour during the shelf life (shape changes of the fillet in the first 24h, rigor index, K freshness index, microbiological analyses including aerobic psychrotrophic bacteria, coliforms and *Escherichia coli* counts). For this last purpose the fish were analysed immediately after death (10 fish per diet) and at different times during the refrigerated storage (1 °C with ice covering) until 15 days post harvesting, at each time analysing n. 6 subjects per diet. The quality of protein sources utilized in the diet formulation did not influence the final body weight (621.51 and 600.07 g, in FM and PP sea bream), fillet yield and visceral fat percentage (about 40% and 1.9% on average in both groups, respectively), while the FM fish had higher dressing (93.87 vs.93.39%) consequently a lower incidence of viscera ($P<0.08$). Some differences emerged for skin and fillet colour and for chemical characteristics, as the FM sea bream showed a lower n-6 PUFA incidence (12.3 vs 21.1%) and a higher n-3 PUFA incidence (about +4%; EPA: +2.5%, DHA:+1.6%), the fat percentage in fillet resulting similar (about 12%) in the two groups of fish. During the refrigerated storage, differences between the fish differently fed emerged only for some parameters and they always resulted quite slight in entity. On the whole, the results of this research demonstrate it is possible to include a high percentage (50%) of plant protein sources in the diet of sea bream with modest effects on the quality parameters investigated, more relevant only in the case of the fatty acid composition profile.

Research supported by MURST – FISR (Special integrated research grant).

F2.05. Effect of catching season in the North Adriatic sea on selected fatty acid response to cooking and ensuing true retention values in the edible portion from four species of fatty fish

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The objective of this study was to determine if the behaviour of the most important fatty acids in four species of fatty fish and their true retention values upon cooking were in any way affected by the season of catch.
For each of the following species: anchovy (*Engraulis encrasicolus*, EE), sardine (*Sardina pilchardus*, SP), sprat (*Sprattus sprattus*, SS), and scad (*Trachurus trachurus*, TT), four batches were acquired at local wholesale fish markets either in fall 2006 (anchovy, 105 specimens on the whole; sardine, 80; scad, 27) or in winter 2006 (sprat, 110), and four more batches in spring 2007 (anchovy, 120; sardine, 78; sprat, 75; scad, 34). Within batch, one fillet per fish was retained to constitute the pooled raw reference, while their counterparts were oven baked in a partially covered baking tin and pooled. Raw and cooked samples were analysed in duplicate for lipids, and fatty acid composition (% fatty acid methyl esters) and content (mg/100 g edible portion).
The season of catch significantly affected both the lipid content and its retention value (RV) upon baking for EE, SP, and SS, the first and the third species being fattier, the second leaner in spring. Fall EE had higher saturated (SFA) and n-6 polyunsaturated (n-6 PUFA) fatty acid percentages, while having significantly lower percentages of C20:5 n-3 (EPA), C22:5 n-3 (DPA), C22:6 n-3 (DHA), n-3 PUFA, and total PUFA, without substantial differences between the raw and the cooked state, SFA excepted. TT was the only other species where a season effect on fatty acid composition was evident, especially on the members of the n-3 PUFA family which were significantly more abundant in spring. Except for TT, RV for lipids were inversely related to the lipid content. RV for n-6 and n-3 PUFA were not affected by the season of catch.

S2.01. No significative effect of commercial (animal based) and vegetarian diet on the cytochrome P450 system of farmed gilthead sea bream (*Sparus aurata*) despite a significant increase with size

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To date fish oil and meal are still the main components of commercial feed used in farming of marine species such as sea bream with associated risk of contamination by the accidental presence of environmental contaminants. The increase use of vegetable diet still address such concern both in terms of suitability for fish growth and welfare and also due to the potential presence of contaminants of different origin. In the present study two isoenergetic and isoproteic diets, one based on fish meal and another based on 50% of fish meal and 50% of soybean, maize gluten and pea concentrate (14%, 20% and 16% respectively), have been tested on specimens of gilthead sea bream of two size: 300 g and 600 g approximately. All specimens have been reared on sea cages and their effects on biotransformation system cytochrome P450 have been investigated. Phase I enzymes such as EROD, MROD, PROD and BROD as well as phase II such as GST and UDPGT have been investigated in liver. No differences on phase I enzymes activities (EROD, MROD, PROD and BROD) as well phase II (GST and UDPGT) were observed in gilthead sea bream fed with the two diets. On the opposite, CYP3A-like protein seems to be more activated in fish exposed to vegetable diet. Regarding fish size, a significant increase of both phase I and II P450 activities was observed in fish of 600 g compared to those of 300 g suggesting a potential role of bioaccumulation of compounds able to induce the P450 system. The absence of significative effects on both phases of biotransformation system suggest that both diet might be considered contaminants-free and suitable to be used for in rearing marine fish species such as gilthead sea bream.

S2.02. Dietary protein source affects the non muscular components of fillet in rainbow trout

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The effect of high vegetable protein containing diets on the non muscular components of rainbow trout (*O. mykiss*) fillet was studied. Two isoproteic (44.8%) and isolipidic (19.6%) diets were tested: a fish meal-based diet (FM) and a vegetable protein-based diet (VP) where a mix of pea protein concentrate and wheat gluten meal replaced 80% of protein from fish meal. 336 fish (initial body weight 367±5g) were randomly distributed in 12 tanks and fed for 109 days the test diets at two feeding levels (nearly to satiation vs. -15% satiation) according to a two factors experimental design with triplicate units per treatment.
Fish growth, feed conversion and protein efficiency ratio were not affected by the dietary treatment while fish fed nearly to satiation resulted in higher final body weight (796.0 vs. 710.1g; $P<0.05$).
Dietary treatment did not affected fillet composition but feeding nearly to satiation resulted in higher lipid content (3.5 vs. 4.0 g/100g; $P<0.05$). Fish fed diet VP exhibited higher fillet monounsaturated and n-6 fatty acid contents (1.33 vs. 1.26 mg/100g and 0.42 vs. 0.26 mg/100g; $P<0.05$). Such effect was enhanced in fish fed the higher feeding level ($P<0.05$).
Fillet collagen content resulted higher in fish fed diet VP (1.40 vs. 0.87 g/100 g muscle; $P<0.05$) and also the collagen fractions resulted altered by the dietary treatment. Feeding diet VP resulted in an increase in the acid soluble (0.33 vs. 0.16 mg/100g; $P<0.05$) and the pepsin soluble (0.72 vs. 0.47 mg/100g; $P<0.05$) fractions. Feeding rainbow trout nearly to satiation resulted in an increase of the insoluble fraction (0.30 vs. 0.18 mg/100 g fillet; $P<0.05$). The textural properties measured as shear force at 48 hrs post mortem resulted non significantly affected by the dietary treatment but only by the feeding level ($P<0.05$).

Research funded by MIUR – PRIN05 prot. 2005070451_003.

S2.03. Comparison of qualitative traits of European sea bass (*Dicentrarchus labrax*), of different provenance, commercialized by an Italian supermarket company

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[2]*Centro Piccole Specie, Bologna University, Italy*

In the last years, European sea bass, together sea bream, represents the finfish species from aquaculture that has known the largest development in Italy. About recent statistics (ISMEA, 2007), the largest part of the Italian fish produced with aquaculture practices has sold through the hypermarket and chain stores and tendency is going to increase.
Based on this considerations, European sea bass of different origin, commercialized by an important Italian supermarket, were analysed with particular attention to the proximate composition, fatty acid profile and cholesterol content in order to give detailed information on nutrients of sea bass to the Italian consumers.
At different times, samples were collected from an Italian supermarket chain during a period of 1 year. Three different batches were considered: sea bass reared in Italy, following prescriptions reported in terms of specification that defines sea bass produced with label ("Prodotto a marchio"), were compared with sea bass of foreign provenance and sea bass farmed in Italy in extensive conditions ("valli").
At each sampling time, 5 fish were examined; a portion of skinned dorsal muscle was collected, homogenised and submitted to the following analysis: dry matter (determined at 105 °C for 24 h); total nitrogen (Kjeldahl, conversion factor of N to protein 6.25); total lipids extracted with chloroform/methanol (2:1 v/v) (Folch et al., 1956) and converted to fatty acid methyl esters following Sukhija and Palmquist (1988). Total cholesterol was calculated using as internal standard beta-sytosterol.
From a nutritional point of view, sea bass with label showed an excellent value, similar to that found in fish from extensive farm. Lipid content was significantly higher in the foreign sample than the other two. Total cholesterol resulted significantly higher in the foreign farmed fish than extensive and label fish in all the samples. Significant differences were also found in total saturated monounsaturated and polyunsaturated fatty acids of the three batches.

S2.04. Characterization of main seafood consumed in Portugal

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In a balanced diet seafood products are considered more and more an essential aliment. However, it is very perishable and along the distribution chain it can suffer profound alterations if not duly preserved and conditioned. Being Portugal one of the countries with higher *per capita* seafood products consumption, within EU it is of great importance to know the quality of these products in the retail market. Thus, the main quality parameters regarding canned and frozen fish products are discussed. The levels of some toxic elements (mercury, cadmium and lead) are presented as well.

In general terms, most of samples are of good quality, regarding sensory analysis and chemical index. In addition, the number of samples reaching the recommend EU limits for Hg, Pb and Cd is lower than 1%.

S2.05. The effects of Aegean Sea (Inner Bay) on the microbiological quality of sardines (*Sardina pilchardus)*

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Introduction: The study was performed to investigate to what extent the polluted waters of the Aegean Sea affect microbiological quality of sardines caught in the Inner Bay of the Aegean Sea. Physicochemical (pH, soluble oxygen, Chl-a, temperature and salinity), nutrient (Si, NH_4-N^+, NO_3-N^-, NO_2-N^-, RP) and microbiological (total living creatures, coliform, fecal coliform, *E. coli* and *Salmonella*) analyses of the polluted waters were conducted.
Methods: *Microbiological analyses*. Total viable bacteria count was determined by using pour plate method. Plate Count Agar (Difco, 0479-17,USA) was used as a *medium* according to Harrigan and McCance (1976). Plates were incubated at 30 ℃ for 24-48 h. For coliform bacteria count, Most Probable Number method was used. Lauryl Tryptose Broth (Difco, 0241-17-0, USA) was used as a medium, and confirmation test was made in Brilliant Green Bile 2% (Difco, 0007-17-4, USA). Tubes were incubated at 37 ℃ for 24-48 h. For fecal coliform bacteria count, Most Probable Number method was used. EC broth was used as a medium. Tubes were incubated at 44.5 ℃ for 24h according to Harrigan and McCance (1976). *Salmonella* was determined by the method of Poelma and Silikler (1976).
Physicochemical and nutrient analyses according to Strickland and Parsons (1972).
Conclusion: It has been assessed how pollution parameters affect microbial load of sardines and whether sardines caught in polluted waters pose a risk when they are consumed.

F2.06. Aquaculture and human nutrition: evolution of the nutritional quality and new species on the market

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Fish products are important in the human diet because of their digestibility and high nutritional value, mostly characterised by peculiar features that make them unique among food protein sources. In particular, seafood products are high in polyunsaturated fatty acids (PUFA), mainly those belonging to the n-3 series, like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), present in significant amounts only in seafood. These fatty acids, present in cell membranes and essential in brain and retina development, are fundamental in the prevention of cardiovascular and chronic inflammatory diseases. In the human organism n-3 PUFA undergo metabolic reactions to give biologically active molecules known as eicosanoids (prostaglandins, tromboxanes and leukotrienes) playing a role in platelet aggregation, vasoconstriction and blood pressure.
Today the contribution of aquaculture to the supply of seafood products rich in PUFA has become of extreme importance. However, during the years, the evolution towards a sustainable aquaculture has brought some changes to the nutritional value (in particular to the fatty acid profile) of the reared species, mainly as a consequence of changes in the composition of feeds. In most cases it has been detected an increment of n-6 fatty acids at the expense of n-3 fatty acids.
In addition, on the market are emerged with success fish species like the sutchi catfish *Pangasius hypophthalmus* from Vietnamese aquaculture. This species, commercialized in fillets, compete on the market with the traditional aquaculture species because of the low price. However, the nutritional value of *Pangasius* fillets is rather low because of their high water content and low n-3 PUFA levels.

F2.07. Elemental profile of the edible portion from several species of whitefish caught off the Northern Adriatic coast

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Seventeen whitefish species caught in the North Adriatic sea were sampled from the wholesale fish markets of Cesenatico and Rimini from October 2006 to May 2007 to gain a better knowledge of their potential as a source of macro- and micro-elements, both at the raw and cooked state.
Flathead grey mullet (*Mugil cephalus*, 13 batches, 13 specimens on the whole), annular seabream (*Diplodus annularis*, 3, 19), and conger eel (*Conger conger*, 8, 8) were destined to pan frying (PF). Striped mullet (*Mullus barbatus*, 12, 166), picarel (*Spicara smaris*, 2, 17), bogue (*Boops boops*, 7, 47), yellow gurnard (*Trigla lucerna*, 12, 60), largescaled scorpionfish (*Scorpaena scrofa*, 5, 52), Dover sole (*Solea solea*, 12, 77), stargazer (*Uranoscopus scaber*, 4, 20), and greater weever (*Trachinus draco*, 5, 50) were destined to oven baking in a partially covered baking tin (OB). Red bandfish (*Cepola rubescens*, 2, 35), common Pandora (*Pagellus erythrinus*, 5, 50), rock goby (*Gobius paganellus*, 6, 120), poor cod (*Gadus minutus capelanus*, 9, 54), blue whiting (*Micromesistius poutassou*, 12, 114), and European hake (*Merluccius merluccius*, 14, 85) were intended for cooking in parchment (CP). The content of ten macro- and micro-elements were determined in raw and cooked flesh by ICP-AES. Since each cooked sample had its own proper raw reference, true retention coefficients could be computed for each element.
At the raw state and collapsing the effect of the catching season, the whitefish species examined here proved to be well endowed in potassium (especially bogue, European hake, and blue whiting) and selenium (especially conger eel), while being quite poor sources of iron and zinc. Upon cooking, each element underwent an increase in concentration. In terms of true nutrient retention, CP proved to be the most conservative out of the three implemented cooking methods, while PF was the least one.

F2.08. Compositional characteristics of brown crab (*Cancer pagurus*)

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The Brown crab (*Cancer pagurus*) is a commercially important shellfish species in Norway. In 2004 the amount landed was close to 5000 metric tons. The crab industry in the UK and Southern Europe sell most crabs as whole crab, fresh or cooked. In Norway all crabs are cooked and most of them are used for production of crab meat products, based on the different parts of the crab. These products are exported as semi-manufactured products to foreign processing industry. There is a large potential for development of new products from the different semi manufactured crab meat.
In order to develop new products and find new and improved processing methods there is a need for more knowledge both on the composition and storage stability of the different crab fractions and on how different processing conditions affect the raw material composition and properties.
In this study, the chemical composition of different parts of the crab was analyzed. The different fractions were: brown meat (roe and liver), meat from the large claw, meat from the legs and the meat from the “cage”. The fractions were analyzed for content of ash, water, lipid and total protein. The amino acid composition, lipid class and fatty acid composition and content of taurine and carotenoids were also determined. The different fractions varied in chemical composition with the lipid content being highest in the brown meat and lowest in the claw and leg meat.

The support from the Research Council of Norway is gratefully acknowledged.

S2.06. Water, salt and fat content in commercial 200-g-consumer packages of vacuum packaged cold smoked Atlantic salmon (*Salmo salar*)

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300 consumer packages of vacuum packaged cold-smoked Atlantic salmon of 200 g each have been investigated for water, salt and fat content. Water and fat content varied considerably between consumer packages. Labelled fat content were frequently exceeded. In some samples the salt content in water-phase of 3% which is necessary for safety reasons was not reached. Dry salted samples and injection salted samples differed not in average water, salt and fat content.

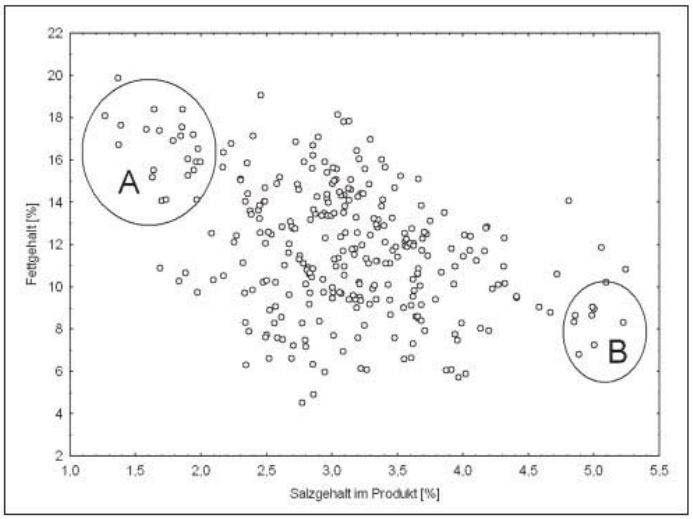

Figure: Correlation between salt content (y-axes) and corresponding fat contents (x-axes) in product (all 300 samples investigated).

S2.07. Frozen raw material for the production of high quality acid marinated herrings

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Today more and more of the herring catch is frozen and stored frozen until it is further processed. This new situation can result in quality changes of salted and marinated herring products traditionally produced from fresh herring. Therefore, the aim of this study was to investigate if frozen herring could be used to prepare high quality acid marinated herring. Acid marinated herring were prepared according to the Scandinavian recipes by placing the herring fillets in an acetic acid solution for a minimum of 35 days. Fresh herring fillets as well as herring fillets that have been frozen for 2 months at -18 °C and at -30 °C were used. A series of analysis were performed in order to evaluate the quality of the frozen fillets as well as the quality of the final product. Biochemical analysis such as water binding capacity, peroxide value, protein content in brine, acid content were performed on the raw material and at regular intervals on the fillets during the marinating period. In addition, the texture of the fillets was also measured using a Texture Profile Analyzer (TPA). Sensory analysis was performed on the final product by a trained panel in order to assess the quality of the acid marinated herring product. The results are presented and the correlation between the quality of the raw material and the quality of the final product highlighted.

S2.08. Development of quantitative rapid methods to asses sensory and physical quality of frozen cod

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The quality of frozen cod depends on biological and technological parameters during catching, processing and distribution. The biological parameters are fish size, catch season and sexual maturation. Regarding technological parameters, the most important ones are catching method, freshness before freezing, rate of freezing and temperature of storage. One of the most undesirable physical changes occurring during prolonged frozen fish storage is the loss of textural attributes, due to denaturation and aggregation of the myofibrillar proteins. As well as textural attributes, other functional properties of muscle proteins could be reduced: Water Holding Capacity (WHC), Water Binding Capacity (WBC) and Cooking Yield (CY). Consequently, theses changes cause a hard, dry and fibrous product.

The aim of the work was to develop a quantitative rapid method to evaluate the quality of frozen cod to take fast decisions in industrial planning productions. Two different frozen cod (*Gadus morhua*) batches provided by two different freezer factory vessel and frozen in different conditions were tested by means of different physicochemical and sensory quality parameters.
As physicochemical parameters, moisture, colour (CieL*a*b*) of frozen and thawed cod fillet, pH, phosphates content, WHC, WBC and CY were analyzed. To measure fish hardness in raw and cooked fillets, a Warner-Bratzler (W-B) shear cell was used. Concerning sensory analyses, the Quality Index Sensory Method (QIM) was adapted by AZTI and it was applied to whole thawed cod, thawed cod fillet and cooked cod fillet.

Regarding to physicochemical parameters, results showed that both batches of frozen cod were significantly different in CY and in hardness (W-B) in cooked muscle. It could be also observed that the lower CY the more hardness it was obtained. In case of sensory analyses the “cooked muscle texture” (CMT) was the only parameter showing significant differences for both batches. CY, Hardness and CMT parameters tested for both batches are well correlated and they could be used as Quantitative Rapid Methods to evaluate the quality of frozen cod.

S2.09. Determination of quality of marinated anchovy (*Engreaulis encrasicolus*, Linnaeus, 1758) in different sauces during storage at 0-4 °C

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Anchovy is most abundant species which is caught from Black Sea in Turkey. 270.000 tons of anchovy was caught in 2006 in Turkey. Anchovy is generally consumed as fresh, canned or marinade in Turkey.
The term ‘‘marinated fish’’ is used to define semi-preserved fish made by immersion in a solution of organic acids or vinegar and salt. The inhibitory effects of these substances on bacteria and enzymes increase with concentration.
In this study anchovy will be marinated with vinegar (12%) and salt (11%) mixture. After marination will be completed, marinated anchovy will be separated two groups and they filled into plastic containers with sauce. Sauce of first group will be contained red pepper paste and sunflower oil and sauce of second group will be include garlic and sunflower oil too.
For determination of quality chemical quality analysis (thiobarbituric acid, TBA, mg malonadehyde/kg; total volatile base-nitrogen, TVB-N, mg/100g; trimethylamine, TMA-N, mg/100g; pH values, acid and salt concentrations in fish flesh and in brine), color measurements, sensory analysis and microbiological analyses (total aerobic mezophilic bacteria and yeast-mold counts) will be done.
The aim of this study is to compare anchovy marinades which contains different sauce during storage at 0-4 °C.

S2.10. The sensory, chemical and microbiological assessment of smoked and marinated anchovy during the storage of 7 months at 4 °C

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The quality of smoked and marinated anchovy stored at 4 °C was investigated in terms of sensory, chemical (total volatile basic nitrogen, TVB-N; thiobarbituric acid, TBA; peroxide value, PV; free fatty acids, FFA; pH) and microbiological parameters (total aerobic count -TVC, coliform, *E. coli*, *Salmonella*) during 7 months of storage. Anchovy were obtained the local fish company. After that, they were gutted and washed and filleted before smoking for 40-50 minutes. The marinating process for cooked material was carried out in 3% alcohol vinegar and 10% salt for 30 min. at 4 °C. The fish:alcohol vinegar ratio was 1:1.5. Smoked marinated samples were then placed into boxes, followed by sunflower oil addition as a filling to each box and stored 7 months at 4 °C.

The total appearance, odour-taste and texture scores decreased from the initial values of 8.61, 5.5 and 4 to 6.58, 6.33 and 2.75 during storage period, respectively. TVB-N value increased until the 6 months, reaching the maximum value of 41.23 mg/100g and then decreased to 36.59 mg/100g after 7 months of storage period. POV showed fluctuations whereas FFA increased gradually from 1.51 to 9.38 (expressed as % of oleic acid) at the end of storage period. TBA values also increased from the initial value of 1.9 to 4.25 MA/kg.

Salmonella, coliform*, E. coli* and *Staphylococcus aureus* were not detected during storage period of 7 months. TVC increased to 6.2 log CFU/g at the end of storage period. According to the sensory results, it has been found that smoked and marinated anchovy was rejected by panellists after 7 months at 4 °C.

S2.11. Effects of seawater and freshwater washing on microbiological and sensory changes of whole and fillet sardine during refrigerated storage

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Introduction: In this study, the effects of sea water and freshwater washing on microbiological and sensory changes of whole and fillet sardines were determined. Two different treated sardine (seawater and fresh water) has been put into styrofoam tray and wrapped with strech film. Packaged sardine stored for up to 15 days. Microbiological (total mesophilic bacteria count, psychrotrophic bacteria, coliform bacteria, lactic acid bacteria, H_2S producing bacteria, *Pseudomonas* counts and sensory analyses were done.
Methods: *Microbiological Analysis*. Samples were taken from the surface of 10 cm^2 by swapping and mixed with 10 ml of 0.1% peptone water (Difco, 0118-17-0). In all cases, serial dilutions were prepared. Total viable and psychrotrophic bacteria counts were determined by the pour plate method, using Plate Count Agar (Difco, 0479-17) as the medium. Plates were incubated at 30 °C for 24-48 h and 7 °C for 10 days respectively, according to Harrigan and McCance (1976). Coliform bacteria counts were determined as pour plate method in Violet Red Bile Agar (VRBA, Oxoid CM 485) after 24 h incubation at 37 °C. Lactic acid bacteria count was determined by using the pour plate method MRSA (de Man, Rogosa and Sharpe Broth) (LAB M 93) was used as medium. Plates were incubated at 30 °C for 3–5 days. H_2S producing bacteria and *Pseudomonos* counts were determined by the method of Papadopoulos et al. (2003). Sensory analysis. Sensory analysis were performed according to Fernández-Fernández et al. (2001).
Conclusions: In this study, the comparison of different washing method (seawater and fresh water) on the microbiological and sensory quality of whole and fillet sardines have been determined during refrigerated storage. The most effective washing method for sardine was determined.

S2.12. Use of potentiometric measurements in fish freshness determination

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The alternatives to the traditional way for fish freshness evaluation (sensory analysis) are the instrumental methods such as electrical, texture and color measurements, image analysis, VIS spectroscopy and electronic noses.
The objective of this work was to evaluate the correlation of the potentiometric measurements, obtained with gold and silver electrodes, with the instrumental measurements accepted as fish freshness indicators.
The potentiometric measurements were compared with the evolution of ATP breakdown compounds, pH and microbial counts.
The obtained results showed a strong correlation of the potentiometric measurements with the determined changes in fish, obtaining an important correlation with the K_1 index, dependent on the nucleoside degradation, which is used as a good indicator of post-mortem time and so freshness.

F2.09. Chemical changes in farmed Atlantic salmon during freeze storage

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The critical factor limiting the frozen storage life of Atlantic salmon is oxidation of lipids in the muscle tissue. This oxidation results in rancidity of the flesh deteriorating the product. The preservation of nutritionally important omega-3 fatty acids during frozen storage is very important in maintaining the nutritional value of salmon. This study was designed to study the stability and any possible deterioration of the fatty acids in vacuum packed freeze stored Atlantic salmon for up to 18 months at two different temperatures, -24 and -40 °C. A main issue was to follow any changes in the amount of omega-3 polyunsaturated fatty acids in the fish muscle. The effect of freeze storage on total fat content, peroxide values, colour score, texture and sensorial changes was also followed. In addition the flesh quality and fatty acid profile in four different weight classes of salmon was examined.

Colour and texture of salmon fillets remains stable during 18 months freeze storage at both at –24 °C and –40 °C. The peroxide values increased during the first 9 months of freeze storage at -24 °C. Thereafter a rapid decline in peroxide values was seen from 9 to 18 month of freeze storage. Also a rancid flavour were detected by panellists in the fish stored at –24 °C compared with –40 °C and fresh salmon. The fatty acid profile in salmon flesh was unchanged during the first 12 months at both –24 °C and –40°C, but from 12 to 18 months a rise in the percentage of saturated and a decrease in polyunsaturated fatty acids were seen. The amount of DHA and EPA in the muscle was unchanged after 18 months freeze storage.

The total lipid content in the fish increased with fish size. The content of EPA and DHA in the muscle was higher in larger weigh classes being fattier compared with smaller weight classes.

F2.10. Identification of underlying mechanisms controlling cathepsin activity associated to flesh quality deterioration in Atlantic halibut, *Hippoglossus hippoglossus* L.

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The first objective was to investigate cathepsin B, B+L, D, H and collagenase activities, their influence on muscle protein and water holding capacity (WHC). The second aim was to investigate the mechanisms underlying increased catheptic activity in farmed Atlantic halibut.
The average weight of fish increased over the 12 month production cycle by 73% and 50% for females and males, respectively, despite some sexually dimorphic seasonal variation. During the winter, muscle protein content declined by up to 5.7% in females and 17.9% in males. Catheptic activity showed a reciprocal relationship, and was highly correlated with the changes in protein content. WHC, measured as liquid loss, increased from 3-5% in November to 11-13% in May 2005. Cathepsins B+L, D and collagenase explained 73.1% of the total variance in protein content, while cathepsin H was the largest contributor to liquid loss, explaining 49% of the total variance. The results indicate that to obtain the best flesh quality Atlantic halibut should be harvested in the fall or early winter when the liquid loss and cathepsin activities are low and less likely to cause problems during secondary processing and storage.
Changes in cathepsin activity could be influenced by increased activation/ secretion of proenzymes, decreased activity of inhibitors, changes in gene transcription or a combination of these factors. Gene expression of *cathepsins B* (*ctsb*) and *D* (*ctsd*) in fast myotomal muscle was investigated using qPCR in fasted and re-fed juvenile Atlantic halibut. The sampling intervals were as follows: Initial point, 26 and 64 days fasting and then 3, 7, 14, 30 and 60 days after refeeding. The geometric average of *Fau* and *18S rRNA* was used to normalize the data. Both *ctsb* and *ctsd* transcript levels were significantly higher during fasting than refeeding, providing a partial explanation for the mechanism underlying increased cathepsin activity during periods of food deprivation.

F2.11. Nutritional quality of some underutilised fish species from the Italian fishery

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In Italy the underutilised fish species may represent tons of products that, in spite of their undeniable intrinsic quality in terms of nutrient content, are treated as discards and often returned to the sea.
A first step toward a process of valorization of products and divulgation to the market and consumers of the inherent quality of underutilised fish species, is represented by an improved knowledge of their nutritional qualities, generally scarcely studied due to their low commercial interest.
We report the results of a study aimed at evaluating the nutritional properties of bogue (*Boops boops*), horse mackerel (*Trachurus trachurus*) and scaldfish (*Arnoglossus laterna*), some of the low-value species most represented in the Italian trawl fishery. Fish were sampled in different seasonal periods and from different Italian sites. Proximate composition, fatty acid profile by GC and composition of the unsaponifiable lipid fraction by HPLC were evaluated in the three species.
Results showed that all species were characterized by good protein contents and low lipid and cholesterol levels. From a qualitative point of view, the fatty acid profile of the three fish species was characterized by high proportions of polyunsaturated fatty acids (PUFA) and, most remarkably, by high n-3/n-6 PUFA ratio values (from 3 up to 10 depending on the species and seasonal period).
In comparison to other fish species from the wild, and also from aquaculture, present on the Italian market that we have studied over the time, the fatty acid profiles of bogue, horse mackerel are characterised by higher n-3/n-6 PUFA ratio values. This is an element of interesting nutritional implication that should help the promotion of such underutilised species on the market since n-3 PUFA, as precursors of hormon-like substances with antithrombogenic and antiatherogenic properties, play an important role in the prevention of cardiovascular diseases.
The promotion on the market of these underutilised species, especially of *T. trachurus*, would also require a marketing strategy in order to find new forms of commercialization, possibly as value-added products.

F2.12. How to manage fish wastes on board: the compacting solution

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Usually fishermen throw to the seas variables quantities of biological material that is: discards (non targeted species), small-sized species with low commercial value and by-products (heads, viscera, skins, etc) resulting from on board transformations.
Such dumps alter the ecosystem structure, that is why new fishing regulations had been proposed focusing on:

1. Technical regulations improving the fishing gear, in order to minimize the proportion of by-catch or small-sized species in the catches.
2. Zero-discards policies, by introducing a discard ban where all finfish and crustacean must be landed.

The BE-FAIR project (European project under the LIFE environment program) aims to develop efficient waste management and processing practices to recycle fish wastes (on board and in land). In this framework, a pilot plant has been designed and constructed to compact fish wastes directly on board and to treat the resulting effluents.
This pilot plant pursues three main objectives:

1. Minimize the volume of solid by-products stored on board.
2. Treat the effluents so their discharging causes minimal environmental impact.
3. Recover fish oil and part of fish protein for an ulterior up-grading on shore.

Unitary actions of the pilot plant (cutting, compression, centrifugation, filtration and ultrafiltration) have been optimized in order to lead to: - a press cake that can be frozen on board, - oil and protein concentrate that can be stored and “clean water” that can be directly discharge at sea.
This presentation will describe all the process and results will be detailed.

S2.13. Quality assessment of low commercial value fishery species from Western Sicily (Italy) to promote sustainability and local resources distinction, consumption and promotion

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Sea food "commercial success" depends not only on "intrinsic quality" of fish species. In fact it could be also determined by other factors, such as: social, cultural and traditional, that can vary from country to country. High value fish received many attention from the scientific point of view.
However, few data are available for those species considered of low commercial value, due to the low interest from consumers, producers and research.
In the last decades, due to decreasing fish resources and the increasing fish consumption, scientific interest increases, to promote consumption of underutilized fish species, in order to ameliorate fishery sustainability and local resources consumption and valorisation. To reach these results it is necessary the analytical assessment of quality of the selected species, indispensable to achieve consumers confidence.
Our research focused on a three years survey on quality of low commercial value fisheries species from western Sicily (Trapani, Italy), for which few, or no published data, are available. Selected species were: *Boops boops*, *Lepidopus caudatus, Sarpa salpa*, *Trachurus trachurus*, *Coryphaena hippurus* and *Danichthys rondoleti* caught in different season. Quality was evaluated by the assessment of yield, nutritional traits and freshness. All species considered appear characterized by high yield, showing positive quality-price ratio and suggesting minimal processing to increase availability and consumption. Nutritional data show an *optimum* protein content, low/medium lipid level and low cholesterol content. Comparison of fatty acid profile by principal component analysis (PCA) give interesting information to characterize the target species ($P<0.05$). Obtained results could represent the basis to achieve the consumer confidence, contributing to local resources distinction, consumption, promotion and to fisheries sustainability.

S2.14. Characterization of collagenous proteins from different discarded fish species of the West coast of the Iberian Peninsula

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Introduction: Present production of wild fish resources is around 85 millions tons per year, however, not all that is obtained from the sea is adequately used and three clearly differentiated factors can be taken into account to explain this fact: discards, wastes on board and byproducts and wastes ashore.
Discarded fish vary among fishing gear, area of capture and time of the year. Some discarded fish from a trawler were analyzed in terms of yields and collagen content of skin. Also, a comparative analysis of the collagen of discarded species is being carried out.
Methods: Fish samples were obtained from a trawler vessel which operates in the west coast of the Iberian Peninsula. *Collagen acid extraction*. Skin collagen was extracted using an acid solubilisation (acetic acid), previously non collagen proteins were removed with NaOH. Freeze-dried collagenous was referred to wet weight of skin or body part. SDS-PAGE of collagenSDS-PAGE was performed using the Laemmli (1970) method. *Collagen determination*. Collagen was determined according to Woessner (1961).
Results: *Collagen of discarded species*. Collagen content of discarded species was analysed. It becomes apparent that the two species of sharks presented the highest collagen content, especially Galeus, which presents 15%. Other authors have reported similar contents of collagen for skin, like the 8.9% of the elasmobranch Raja kenojei.
SDS-PAGE electrophoresis shows that collagen extracted is of type I, showing $\alpha 1$ and $\alpha 2$ chains and the β component. These results show that among discarded species, elasmobranches are suitable candidates for using their skins as sources of marine gelatin.

S2.15. Characterization of protein hydrolysates and lipids obtained from black scabbardfish (*Aphanopus carbo*) by-products

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The production of black scabbardfish (*Aphanopus carbo*) (BSF) fillets generates a substantial amount of by-products (heads, viscera, skin, cut-offs), which are generally dumped. The preparation of protein hydrolysates represents an alternative for upgrading this raw material. Thus, it was objective of this work the preparation of protein hydrolysates using Protamex™ and the characterization of lipids present in each of the four fractions (oil, emulsion, aqueous solution of the protein hydrolysate and sludge) obtained after centrifugation of the hydrolysed material. The conditions used in the hydrolysis process were: water/fish ratio 1:1, 0.5 and 1% Protamex levels, pH 7.5, 50 ºC for 2 hours. The proximate chemical composition of BSF by-products was: 70.39% moisture, 10.13% fat, 14.92% protein and 3.53% ash. The protein solubilisation achieved with 0.5% and 1% Protamex was 47.3% and 59.0% respectively. The hydrolysates have a low fat content (*ca*. 1%) and a pale cream colour. An important percentage of total lipids remained in the emulsion, which represented 60.3% and 48.8% of the oil present in the raw material when 0.5% and 1% of Protamex respectively was used. The oil released was 15.5% and 37.4%, respectively for the lower and higher Protamex level used. In the sludge the mean percentage of oil was around 12%.
The fatty acid profile in BSF by-products and in the oil of all fractions was similar (monounsaturated fatty acids 60.3%, followed by saturated 24.9% and polyunsaturated 13.4%). The triacylglycerols were dominant in all fractions and the highest percentage of phospholipids was detected in the oil from sludge and hydrolysate, followed by the emulsion but they were not present in the oil released. Cholesterol and cholesterol esters were detected in all fractions at low levels.

This work was done in the frame of the project BE-FAIR (LIFE 05 ENV/E000267-BE FAIR).

S2.16. Utilization of different solid wastes as animal feedstuffs

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Introduction: Five different fractions of the biodegradable solid wastes were evaluated as potential animal feedstuffs. For each source of waste fish waste (FW), meat waste (MW), fruit and vegetables waste (FVW), chicken waste (CW), plant waste (PW) samples were obtained from small shops (butchers, fishmongers, fruit and vegetable shops), The chemical composition (protein %, ash %,moisture %, lipid %), pH, microbiological characterization, sensory analyses were determined for every type of waste fraction. From a microbiological standpoint, a heat treatment at 70 ˚C for 20 min was sufficient to ensure microbiological quality of the samples. This treatment was also advisable to reduce the moisture content: a lower moisture content facilitates the waste handling and processing and, therefore, the inclusion of these waste fractions in commercial animal diets. The most proper waste as animal feedstuffs was determined.
Methods: *Chemical composition analyses*. The chemical composition of different solid wastes was determined as crude protein (N 6.25), crude fat, crude ash and moisture. The pH value was measured as described by Lima dos Santos et al. (1981) using a digital pH meter (Hanna Instruments, Inc., Romania). *Microbiological Analysis*. Total viable count was determined by the pour plate method, using Plate Count Agar (Difco, 0479-17) as the medium. Plates were incubated at 30 ºC for 24-48 h. Coliform bacteria counts were determined as pour plate method in Violet Red Bile Agar (VRBA, Oxoid CM 485) after 24 h incubation at 37 ºC. Coagulase-positive *Staphylococcus aureus* count was determined by using spread plate method. Baird Parker Agar (Difco, 0768-17-3) was used as medium. Plates were incubated at 37 ºC for 24-48 h. *Salmonella* determination were made as according to the method of Poelma and Silliker (1976).
Conclusions: This paper presents a potential alternative for the recovery of organic matter content solid wastes (fish waste, fruit and vegetable waste, chicken waste, meat waste and plant waste). These wastes could be considered as alternatives to animal feedstuffs.

F2.13. Rearing of sea bass *Dicentrarchus labrax* L. and gilthead sea bream *Sparus aurata* L.: welfare assessment and stress

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The Treaty of Amsterdam reflects the concerns of the public about the welfare of sentient animals, including fish. In order to assure high standards of fish welfare, pre-slaughter procedures should be carried out minimizing excitement, pain, fear or stress conditions.

The aims of this study were to examine the effects of routine harvest and *pre mortem* procedures on welfare of off-shore sea cage-reared sea bass and sea bream, and to compare welfare conditions of *Dicentrarchus labrax* L. from two different aquaculture systems, raceway-type tanks and off-shore cage.Before slaughtering sea bass were confined by the harvesting net and then transferred into the tank with the anaesthetic (eugenol 300 ppm) by means of a deep net. Sea bream specimens were directly caught through the deep net and transferred into the tank. 28 sea bream (TW=237.17±75.85g) and 42 sea bass (TW=450.36±60.54) were analyzed. Serum levels of cortisol, osmolality, ions concentration, glucose, total protein, triglycerides (TG) and non esterified fatty acids (NEFA), and whole blood haematocrit (Htc) were assessed. Statistical analyses were performed by means of Mann-Whitney test and Principal Components Analysis (PCA). Results are summarized in Tables 1 and 2 as medians; marked tests are significant at $p<0.05$ (*), $p<0.005$ (**), $p<0.0005$(***).

Table 1	Cortisol (ng/mL)	Osmolality (mOsm/kg)	Htc (%)	Na^+ (mmol/L)	K^+ (mmol/L)	Glucose (mg/dL)	Total proteins (g/dL)	TG (mg/dL)	NEFA (mg/dL)
Sea bream	0.00	395.0	32	184	3.9	81	3.8	208.5	10.79
Sea bass	54.48	421.5	39	205	1.5	114	4.4	204.0	6.30
Significant differences	***	***	*	***	***	**	*	*	***

Most stress indicators resulted to be significantly higher in sea bass if compared with sea bream, so highlighting the differences among both fish species and catching techniques. Although we can infer that cortisol, osmolality and glucose values are not basal in in-land and off-shore specimens, we tested the hypothesises of a bias due to harvesting stressful effects. Furthermore the two groups showed differences in lipid metabolism (NEFA and TG values). The relative importance of each driving force and the relative significance of every stress indicators in the welfare assessment were evaluated.

Table 2	Cortisol (ng/mL)	Osmolality (mOsm/kg)	NEFA (mg/dL)	Glucose (mg/dL)	TG (mg/dL)	Tot. Prot. (g/dL)	Htc (%)
In-land sea bass	66.48	426	3.94	171	283	4.1	40
Off-Shore sea bass	54.48	421.5	6.3	114	254	4.4	39
Signif. differences			**		*		

The authors would like to thank: A.S.A. srl (Civitavecchia, Rome) and Ittica Trappeto SpA, Trapani (Sicily) for the provided hospitality and fish samples, Dr. Emi Cataldi for her precious suggestions and stimulating discussions on the experimental plan. This work was supported by a research grant from MiPAAF (law 41/82).

F2.14. I- Stress assessment of reared meagre *Argyrosomus regius* Asso, 1801

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Stress research in aquaculture is particularly important in the assessment of both the health of farmed fish and seafood quality. Studies of marine fish have highlighted the role of fish species, gear type and killing method in the induction of stress.
This is the first time that haematological parameters of stress were assayed in Meagre.
The aim of this study is to assess the effects of age, sex, size and pre-slaughter procedures (in terms of time of confinement before slaughter) on the serum cortisol and osmolality levels in cultured meagre.
N=89 specimens of in land cultured *Argyrosomus regius* were net-confined in the tank, transferred by a deep net in a mixture of sea water and ice, according to the routine aquaculture practises. Blood samples were collected by hypodermic syringe from the afferent arch artery. Cortisol concentrations were determined by enzyme immunoassay and osmolality was assessed using a cryoscopic method.
In order to compare median values of haematological parameters in control and anaesthetized specimens, the Mann Whitney Test and the Kruskal-Wallis Test were performed.
Results are shown in Table as medians of each group of measures.

	Sex		Age (seeding year)		Size class (kg)					Time of confinement (hours)	
	F (n=49)	M (n=40)	2002 (n=29)	2003 (n=60)	1 (n=21)	2 (n=34)	3 (n=17)	4 (n=14)	>5 (n=4)	1.5 (n=78)	4 (n=11)
Cortisol (ng/mL)	19.84	10.50	24.01	10.58	18.91	10.95	22.12	12.92	22.12	22.68	1.54 ($p<0.005$)
Osmolality (mOsm/kg)	367	364	363	368	362	370	363	364	366	366	379

These preliminary results suggested that all specimens were not stressed by rearing, as resulted from the comparison with “normal values” measured in Teleosts. No significant differences were found among groups in terms of both cortisol and osmolality levels in relation to sex, seeding year and size class. The time of confinement significantly affected cortisol level. In particular, specimens sampled within 1.5 hours seemed to be stressed by the confinement, whereas those sampled after a longer lapse of time (2-4hrs) seemed to become accustomed to being confined.

F2.15. Simulated live shipment of edible crab *Cancer pagurus*: influence of temperature, air exposure and anaesthetic on physiological stress

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The edible crab, *Cancer pagurus* is a high-value species, usually transported live from UK and France to Southern European countries like Spain, Portugal and Italy. During live shipment (tanks with water; 1 kg/L; aeration; no filtration; no water change; 14±1 ºC; 48h duration) animals are subjected to several stressors, such as air exposure, hypoxia, handling and seawater physicochemical variations that promote up to 20% mortalities. Considering the high mortality rates and concerns with animal welfare, the aim of this study was to compare the stress effect and associated mortalities of *C. pagurus* during simulated live transportation. Refrigerated truck transport (8, 12 and 16 ºC) was simulated during 48 h in a refrigeration unit with tanks. Ninety-six crabs were exposed to four treatments: immersion/aeration; immersion/aeration/anaesthetic (AquiSTM; 40 mg/L); emersion/humid conditions; and emersion/humid conditions/anaesthetic (AquiSTM; 40 mg/L). Animal stress was monitored, by sampling hemolymph and vigour assessment at 0, 3, 8, 24 and 48 h. Additionally, seawater quality parameters were analysed: temperature, pH, dissolved oxygen, salinity, ammonia and nitrites. The stress parameters analysed in the haemolymph were: glucose, lactate, protein concentration, pH and hemocyanine concentration. Preliminary results performed at 8 ºC indicate a fast decrease in water quality; mortalities occurred in all treatments but females had no mortalities in treatments without anaesthetic, while immersed males with anaesthetic had no mortalities. Major mortalities occurred in anesthetised crabs exposed to air (females: 12.5% after 24 h, males: 25%, after 9 h) and immersed crabs without anaesthetic (males: 25%, after 24 and 48 h). Mortalities in air exposure treatments only occurred in crabs placed in the bottom, suggesting that pilled crabs cannot be transported in these conditions. Also, the anaesthetic does not seem to be effective to decrease mortalities and males seem to be more intolerant to transportation.

F2.16. Are handling routines during harvesting really important for the consumers perception of Atlantic salmon skin and fillet colour?

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Not available, according to the indications of the Authors

S2.17. II- Stress assessment of reared meagre *Argyrosomus regius* Asso, 1801: effects of anaesthesia

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Meagre history in aquaculture is quite recent and its market is now slowly expanding. The Scientific Panel on Animal Health and Welfare of the EFSA has issued an opinion related to the animal welfare during stunning and killing procedures in farms. Anaesthesia may be used to minimize physical damage and/or as analgesic. Furthermore, certain anaesthetics may reduce or block activation of the HPI axis associated with stressful conditions.
The aim of this study is to elucidate the effects of two different anaesthetizing methods, clove oil and hypothermia, on the cortisol and osmolality levels in land cultured meagre *Argyrosomus regius*.
N=82 specimens (Ittima srl and Cosa srl, Ansedonia, Tuscany) (TW=546±932g) were analyzed. 6 specimens were used as control and not anaesthetized; fish were exposed to different concentrations of clove oil: 50 (n=5), 75 (n=6), 100 (n=5) ppm, and to hypothermia (mixture of seawater and ice) (n=60).
Blood samples were collected by hypodermic syringe from the afferent arch artery within 1 min since the capture. Cortisol concentrations were determined by enzymatic immunoassay and osmolality was assessed using a cryoscopic method.
In order to compare median values of haematological parameters in control and anaesthetized specimens, the Mann Whitney Test, the Kruskal-Wallis Test and the Spearman rank correlation were performed.
Results are shown in Table as medians of each group of measures.

	controls	Cl50	Cl75	Cl100	hypothermia
Cortisol (ng/mL)	8.18	0.00	27.18	0.00	10.58
Osmolality (mOsm/kg)	374	369	361	372	368

All groups of fish did not seem to be stressed, as shown by low cortisol and osmolality levels measured. No correlation was found between cortisol and osmolality measures. The efficacy of clove oil 50 and 100ppm resulted in the lower cortisol values, if compared with controls and hypothermia ones; however no significant differences were found among groups in terms of both cortisol and osmolality levels. Only clove oil 100ppm succeeded in anaesthetising at stage 3 within 1 min and recovery required 9 min maximum. Further studies could be carried on in order to verify our results, enlarging the number of the analysed specimens.

The authors would like to thank Ittima srl and Cosa srl (Tuscany, Central Italy) and the staff for the provided hospitality and meagre samples. The authors would like to thank Dr. Emi Cataldi for her precious suggestions and stimulating discussions on the experimental plan. This work was supported by a research grant from ARSIAT.

S2.18. Quality characteristics of farmed Atlantic cod (*Gadus morhua*) during iced storage, after exposure to handling stress

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Introduction: Atlantic cod is an emerging species in Norwegian fish farming. For Atlantic salmon and several other fish species, it has been shown that handling stress may have large negative impacts on product quality. The purpose of this work was therefore to investigate the effects of handling stress on given quality characteristics of farmed Atlantic cod during iced storage.
Methods: The cod were stressed by a restriction of available water volume for 1.5-2 h (confirmed by slightly increased plasma cortisol levels). Quality characteristics (fillet index, protein solubility, liquid holding capacity, muscle pH and color) were determined after 4 and 7 days of iced storage, and were compared to a control group.
Results: Handling stress reduced the muscle's liquid holding capacity significantly at both sampling days. In both groups, most of the differences were observed due to storage. Among these were increased fillet index (smell, gaping, surface and texture), increased solubility of muscle proteins, increased liquid holding capacity, reduced muscle pH and somewhat increased lightness and whiteness (instrumental) of fillets.
Discussion: Altogether, it is shown that even a relatively mild stress treatment (according to chemical parameters) was enough to significantly reduce the liquid holding capacity throughout the storage period. However, the iced storage resulted in more significant changes than the stress treatment.

The project is financed by the Research Council of Norway.

F2.17. Electrical stunning of farmed Atlantic cod (*Gadus morhua)*: comparison of an industrial and experimental method and the effect on handling stress and fish quality

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The objectives of this study was a) to compare two electrical stunning method in seawater (experimental method) and in air (commercial method) for farmed Atlantic cod, and b) to investigate the impact of exercise and sedation with AQUI-S^{TM} before electrical stunning. The fish were evaluated with respect to stress parameters and flesh quality.

Four procedures for stunning were compared: cod electrical stunned in air with three different pre-treatments; dip-netted, anaesthetized (AQUI-S^{TM}) and exercised, and dip-netted cod electrical stunned in seawater. For instantaneous stun of individual cod in seawater (47 mS/cm) a bipolar square wave current (170 Hz, 33 duty cycle) was applied side-to-side for 5 s. Across plate electrodes at 57 cm distance an overall field strength of 0.93 V/cm resulted in an overall current density of 3.6 A/dm^2. After dewatering the fish was stunned by placing it head-first in a commercial stunner. For stunning on average 41 V, 0.2 A dc was applied on individual cod for 20 s. After electrical stunning, recovery was prevented by gill-cutting followed by chilling in ice water. Blood glucose, haematocrit, high-energy phosphates, muscle pH, *rigor mortis* and muscle excitability were monitored as indicators of stress. Texture, colour, WHC, liquid leakage, gaping, blood residual, K-value and ATP degradation products were monitored after 8 days of ice-storage.

There were no significant differences with regard to the measured parameters in cod stunned with the two methods, except for a higher ultimate pH in cod electrical stunned in air. Exercise before electrical stunning had a significant effect on several of the stress-related parameters; the haematocrit-value was higher, almost no twitch responses, the initial pH, PCr and ATP contents were lower and *rigor mortis* started earlier. In addition, exercise caused higher liquid leakage, lower L*, higher a* and lower hue value. In general, electrical stunning increased the ATP-breakdown leading to a comparatively high K-value already at the time of harvest.

F2.18. Comparing the effects of slaughter methods on rainbow trout and European whitefish

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European whitefish (*Coregonus lavaretus* L.) has emerged beside rainbow trout (*Oncorhynchus mykiss*) to Finnish farmed fish markets. Both species are produced domestically in cages in brackish seawater. An unresolved question about an appropriate stunning method and the species specific difference of the fish motivated the experiments to study the effects of stunning methods on the quality of the given species.

Four methods from highly stressful conditions, that corresponds the industrial practice, to experimental relaxed harvesting, were compared. Fish were divided into four treatments groups according to 2^2 factor design where treatments were sedation and stunning method. Stunning was carried out either manually by percussion (perc) or by carbon dioxide (CO_2). Fish were stunned as such (norm) or after sedation with metomidate (AQ, Aquacalm).

Rigor development, as measured by fillet shrinkage and pH drop, was facilitated as stressfulness of the method increased. However, the response showed species specific difference. Presumably due to less active behavior, European whitefish was more tolerant to handling than rainbow trout which responded dramatically already to quick percussion. In both species sedated fish maintained texture better than unsedated fish. Detailed data on fillet gaping, color and water holding capacity are also presented.

Species specific characteristics may be used to optimize slaughtering practices to enhance the ethical and technological quality of the fish products. Current study suggests that the development of stunning method is likely to be especially beneficial for European whitefish.

F2.19. Effect of different methods for stunning/slaughtering sea bass on stress and quality indicators

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The fish welfare at death time is calling increasing interest at producer level in the perspective of a future application of animal welfare regulations to the EU fish-farming sector. In fact, the custom slaughter method in the aquaculture practice for the Mediterranean fish species is the direct immersion in tanks filled with ice-water, that is considered stressful from the EFSA Panel.
The aim of this research was to compare some alternative killing methods through the study of their effects on stress and quality indicators, always taking the ice-water method as point of reference.
The alternative stunning/killing methods tested in three consecutive trials done in different seasons were: N_2 alone or mixed to CO_2 gas added to ice water, and single and two-stage electrical stunning at different frequency and voltage.
On a complex of 521 sea bass the following parameters were determined: the behavioural responses and the time to die; RI%, fillet contraction, muscular and eye liquor pH, lactic acid, and ATP and relative catabolites at 0, 3, 5, 24 hours after death; RI%, pH, IMP, inosine and hypoxanthine and the K_1 value during 14 days of storage; the sensorial evaluation using the EU scheme to determine freshness classes and shelf life. Data were analyzed by ANOVA (slaughter methods).
The single and two-stage electrical stunning/killing methods showed the slowest times of action (2-4 min), followed by the gas mixtures (13-19 min) and the ice water immersion (17-30 min).
Ice-water immersion generally caused in sea bass highest pH and ATP values at death, delayed full rigor onset (after 6h from death) and one day longer shelf life (14 days), showing a low stress condition in the combination of asphyxiation and hypothermia.
The electrical stunning always gave early full rigor onset (5h after death) and slightly worst quality traits showing to be not so stressless even in the short death time.
The addition of gas mixtures in water and ice slightly differed in stress and quality indicators in comparison to the ice-water method, even with shorter death time.

S2.19. Quality assessment of trawl-caught European hake (*Merluccius merluccius*) during storage in ice

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The European hake (*Merluccius merluccius*) is uniformly present throughout the Mediterranean and most common in the Italian coast where it is traditionally caught by bottom trawling, a harvesting method with a potentially high impact on fish quality. Immediately after capture fish should be refrigerated in order to delay the onset of any deteriorative process resulting in quality loss. Just after death autolytic reactions controlled by endogenous enzymes establish first, followed by enzymatic reactions of bacterial origin.

We report the results of a study on the shelf-life of whole ungutted *Merluccius merluccius* caught by bottom trawling off the Central Thyrrheanian coast (Civitavecchia, Italy) in winter and in summer. This study was designed to individuate the quality changes of whole ungutted hake during storage in ice in refrigerated rooms.

Biochemical freshness parameters of hake of different body size harvested in winter and in summer were monitored over a storage period of up to 13 days in ice. Levels of ATP and its catabolites, K-value, Trimethylamine (TMA) and Total Volatile Basic Nitrogen (TVBN) were evaluated during ice storage.

Compared with fish caught in winter, fish caught in summer, regardless of the body size, were characterised by more rapid and higher extents of inosine monophosphate degradation and hypoxanthine accumulation. The K-values increased with storage time reaching levels as high as 72% and 54% in winter and 95% and 93% in summer, for small- and large-sized fish, respectively. The TMA and TVBN levels in hake caught in winter remained low throughout the experiment regardless of the body size while in summer increased sharply during ice storage. The results obtained indicate a lower quality and shelf-life of hake caught in summer. At the fishing conditions adopted and regardless of the fishing season, fish of large size showed a higher shelf-life than small fish.

S2.20. Effect of killing and handling methods on physical properties of fresh squid upon storage

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East Asian people prefer to consume fish or other marine creatures including mollusk as Sashimi. In this case freshness is the most important factor to determine their quality. It is generally believed that freshness of fish readily lost within a relatively short period of storage even on ice, and decrease in freshness of squid is the most rapid. Japanese consumers and fish distributors generally judge the quality of squid by translucency and firmness of the mantle flesh. In this presentation, we will describe freshness evaluation methods and techniques to preserve squid quality for a long time.

Live squid *Todarodes pacificus* was used in all experiments. Transparency of mantle flesh was measured by reading absorption at 700nm on spectrophotometer and was corrected by the thickness of samples. Firmness was measured as force at 60% strain when the sample was broken across the muscle fibers by a rheometer equipped with a 0.4mm thick plate plunger. ATP–related compounds were analyzed by the HPLC method.

Translucency and firmness of squid when kept at 0 ºC decreased within 24 hours after beheading. These physical properties were much more sensitive indices than well-known freshness index of K-value. ATP content remaining was as sensitive as these changes. As the result of study on the effect of killing and handling method upon the property after storage, it was clear that instant killing, storage at 5 ºC and storage under high oxygen environment were effective to maintain the physical quality of squid during storage.

We made a collaboration work with a fish distributor in Hokkaido to apply these achievements. The system established was like this: Instantly killed squid was packed in plastic bag with a small volume of oxygenated seawater, and transported to Tokyo by ground transportation. The squid was accepted with good reputation at Tokyo Tsukiji market.

S2.21. Lipid damage in farmed rainbow trout (*Oncorhynchus mykiss*) after slaughtering and storage under advanced chilling technologies

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Introduction: Farmed rainbow trout (*Oncorhynchus mykiss*) has acquired a high production as a farmed product in West- and North-Europe, Chile, USA and Japan. With a view to extend its shelf life, a combined refrigeration system consisting of ozone and flow ice was evaluated for the slaughtering and chilled storage of this species (OFI condition). The work focuses on the effect of ozone on lipid damage development.
Methods: An ozone generator (700 mV, 0.17 mg/L) was coupled to a flow ice (60% water/40% ice) prototype working at –1.5 ºC. Icing treatment (slaughtering and storage) was extended for 16 days. Lipid damage analyses were carried out and compared to sensory acceptance and instrumental colour changes. Comparison to individuals processed under flow ice system in the absence of ozone (FI condition) was undertaken. Lipid analyses included lipid oxidation (primary, secondary and tertiary) and hydrolysis events and lipid composition (polyunsaturated fatty acids, phospholipids, tocopherol isomers and astaxanthin) changes.
Results: Rainbow trout slaughtered and chilled under FI and OFI conditions showed a low lipid damage development, according to lipid oxidation and hydrolysis events and lipid composition changes. Additionally, both icing conditions led to large good quality and shelf-life times and to absence of changes for colour properties. The ozone presence has shown some profitable effects as leading to an extended shelf life time by quality retention of several sensory parameters; in contrast, some negligible negative effects could be assessed on the secondary and tertiary lipid oxidation development. However, oxidation values reached by individuals kept under OFI conditions can not be considered especially high.
Conclusion: It is concluded that flow ice as such, or including the ozone presence, can be considered accurate strategies to be employed as slaughtering and storage systems during the commercialisation of the actual farmed species.

S2.22. Attempt to identify a multi-factorial index for the evaluation of stress and quality in sea bass

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A significant pressure to identify the best fish stunning/slaughtering methods currently emerged in the perspective of the future application of the animal welfare regulations to the a EU fish-farming sector. In these last years several research groups worked on this topic and proposed stunning/slaughtering methods adapted to different species. However none of them seems to completely satisfy both the ethic and the qualitative requirements.
There are a number of stress and quality indicators that can be used to verify the stress condition at death and its reflexes on the product quality evolution. One of the most indicative parameters of the stress suffered and of the potential evolution of freshness/ quality is the onset speed of *rigor mortis.* Other good indicators proved to be ATP and its catabolites degradation rate, pH measured in muscle or in eye liquor, dielectric properties, muscular lactic acid. However, none of them can be exhaustive if used alone. Following a sequence of trials with several stunning/slaughtering methods differently stressful, carried out on sea bass, by using all these parameters, it seemed interesting to exploit the complex of data to identify multi-factorial indexes as useful tools for the evaluation of stress and quality.
The complex of data came from 765 sea bass used in carrying out seven trials by which the following killing methods were compared: asphyxia, knocking, spiking, CO_2 saturated water, ice-water (1:2), ice-water saturated with CO_2 100%, or N 100%, or 40, 60 e 70%, N and CO_2 mixture, one stage and two stage electrical stunning at different frequency and voltage. At 0, 3, and 5 hours after death the following parameters were determined: muscular and eye liquor pH, RI%, dielectric properties, muscular lactic acid, ATP, ADP, AMP, IMP, inosine, hypoxanthine and ATP/IMP and AEC rates were calculated. The most useful and indicative stress and quality parameter was confirmed to be the speed of rigor onset, more frequently and negatively affected by stress also with the use of the electric stunning. Being the rigor onset speed not easy to determine in rigorous way - even if macroscopically evident in full rigor - it was chosen as the dependent variable of the multi-factorial indexes set up attempts.

The research was supported by a research grant from MiPAAF (Low 41/82).

S2.23. Strategies to minimize pathogen damage in commercial aquaculture species using the model organism zebrafish

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Infectious diseases are the cause of important economic loss in aquaculture. One of the best approaches to reduce the impact of disease is the development of breeding programs aimed at increasing the natural resistance against infection. This approach will also minimize the use of antibiotics in aquaculture species.

Zebrafish (*Danio rerio*) is a small teleost fish that offers several advantages as a vertebrate model species: i) small size, ii) rapid life cycle, iii) ease of breeding, iv) short generation time v) low maintenance cost vi) sequenced genome vii) high genetic homology with vertebrates. All these characteristics make it an innovative biological model that can be advantageously applied to aquaculture species.

One of the objectives of this project was to identify a pool of resistance genes involved in the immune response against infection. To this aim we infected zebrafish with one of the most notorious pathogen for aquaculture species (*Vibrio anguillarum* etc…) and we studied the animal response by detecting changes in genes expression profiles. From the profiling results we selected a number of genes to carry out a genetic association study based on the identification of Single Nucleotide Polymorphisms (SNPs) as markers.

This information will then be applied to aquaculture species in order to set up breeding programmes that will increase the natural immune response.

S2.24. Partial characterization of antimicrobial peptide from the marine Gastropoda: *Littorina littorina*

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Finding new strategies to fight against pathogenic bacteria had become an emergency in front of the serious consequences of epizootic diseases in fish farming. Antimicrobial peptides (AMPs) have been described as excellent candidates to renew the therapeutic arsenal. To date, only a few AMPs were described in the marine kingdom. We have searched for such compounds in *Littorina littorina* because this marine gastropod is living in the tidal zone being exposed to both marine and terrestrial bacteria.
An acidic extract was prepared and pre-purified on C-18 cartridges. A three step elution was performed with 10, 40 and 80% acetonitril. Antibacterial activity was assayed against aquaculture pathogens (*eg Lactococcus garviae*, *Vagococcus salmoninarum, Listonella anguillarum* and *Yersinia ruckeri*) and classical bacteria for antibacterial testing (eg *Bacillus megaterium*, *Micrococcus luteus*, *E. coli* and *Pseudomnas aeruginosa*). Antibacterial activity was detected in both 40 and 80% ACN fractions against *L. anguillarum*, *B. megaterium* and *M. luteus*.
Active fractions incubated in appropriated buffer at 37 °C with endoproteinase (enzyme to protein substrate ratio of 1 to 50 (w/w)) have lost antibacterial activity after 1H incubation. A SDS-PAGE gel overlaid with broth agar inoculated with the sensitive bacteria shows an inhibition zone in the 3.5 kDa migration zone. Size exclusion chromatography has confirmed this result. Moreover, its cationic character has been demonstrated using cation exchange chromatography. These results taken together suggest that the active compound is a small sized cationic peptide.
Antibacterial kinetics study was performed with the most sensitive strain, *Micrococcus luteus* at 10^4 CFU.mL^{-1} incubated with 40% fraction (protein concentration: 940 µg/ml). A bactericidal effect was clearly showed since no CFU was detectable after 1H incubation.
We describe here evidences for the presence of AMP(s) in *Littorina littorina.* Purification is in progress. Then synthetic peptide will be used to quantify antibiotic potency against aquaculture pathogens.

S2.25. Characterization of chitosan extracted from crayfish waste by different deacetylation processes

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Freshwater crayfish (*Procambarus clarkii*) production in Andalusia exceeds 2.000 t per year, generating 470 tonnes of waste. Thus crayfish industry has shown interest in turning waste into value-added products, such as the biopolymer chitosan. Chitosan is produced by deacetylation of chitin, a polysaccharide found in the shells. The aim of this work was to find optimal conditions for the chitosan extraction for obtaining a high purity product with the lowest reagent and energy cost.

Chitin was first extracted from crayfish waste by the traditional chemical method that involves three steps: deproteinization, demineralization and decolouration. The conversion of chitin into chitosan was made by a deacetylation process, which was performed in three different ways: by autoclaving process (DA1); by autoclaving process after stepping the chitin in a strong sodium hydroxide bath (DA2) and by two autoclaving processes (DA3).

The three chitosans obtained had comparable low nitrogen, ash and lipid content, showing their high purity. Deacetylation was effectively achieved by treatment of chitin in a bath of 50% NaOH (w/v) at a solid/solvent ratio of 1:15 (w/v) under autoclaving conditions for 15 min. Both the prior stepping time to the autoclaving process and the repetition of the autoclaving process improved the solubility and the degree of deacetylation of the obtained chitosan. The DA2 process made the extraction more profitable as the chitosan yield was higher and the process saved energy cost when compared to DA3. To confirm the quality of the DA2 product, its antibacterial activity against *L. innocua* was confirmed and compared to a commercial one (Primex) by *in vitro* tests.

In general, chitosan extracted by DA2 deacetylation process shows antimicrobial activity, high purity, good solubility and high degree of deacetylation. The results reveal that especially DA2 is valid to be used in food applications as edible films and coatings.

S2.26. Protein isolation from cod (*Gadus morhua*) heads and frames using the pH shift technology

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Filleting of cod results in protein rich by-products as frames and large heads. These materials are often used for feed, directly or via fish meal production. Since cod (*Gadus morhua*) is an over-utilized fish specie, a more sustainable strategy would be to use the proteins for human consumption. Methods based on pH-shifts have recently been developed to isolate functional muscle proteins that can be used for human consumption. The methods have been used on complex matrixes such as whole fish, mussels and fish by-products. The aim of this study was to apply the pH-shift methods on cod by-products.

In this study, cod heads and frames ware minced and subjected to pH-shift protein isolation with either pH 11.5 or 2.5 used for solubilization and pH 5.5 for precipitation. The centrifugation force used was varied between 800, 4000 and 8000 g. The highest protein yield, ~55%, was obtained with alkaline solubilization and 800 g. Generally, a trend was that higher protein yields were given with the alkaline process. However, there were no significant ($p < 0.05$) difference in protein yield between the acid and the alkaline method or the different centrifugation forces. In addition to protein, the ash, fat and water content as well as colour was analysed in the starting material and protein isolates. The protein isolates were all significantly whiter than the starting material, and the alkali-produced isolates also were significantly more red than acid isolates ($p < 0.05$). The latter indicating stabilization of heme-proteins. The alkali isolation also gives a trend towards lower ash content than the acid isolation, something which shows better removal of bones and other impurities.

These results suggest that pH-shift processing, in particular the alkaline version, might be a very useful method to increase the use of proteins from cod by-products for human consumption.

S2.27. Influence of the partial substitution of NaCl on the quality of dry-cured tuna loin

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Dry-cured tuna loin is a dry-salted typical product of Spain. Traditionally, Atlantic red tuna loins are cleaned, stacked and smothered in sea salts, washed in bins with fresh running water, pressed and hung to dry in chambers with controlled temperature and relative humidity.

The aim of this work was to study the influence of the partial substitution of NaCl by other salts on the physicochemical properties of the dry-cured tuna loins. A total of 18 thawed loins were used in the study with an average weight of 1504±274 g. 3 loins were used to characterize the raw material. Three batches of 5 loins each one were salted using different salt mixtures: 100% NaCl (batch 1), NaCl (50%) and KCl (50%) (batch 2) and NaCl (55%), KCl (25%), $CaCl_2$ (15%) and $MgCl_2$ (5%) (batch 3). Salting procedure was carried out by scrubbing the salt mixture at 3±1 ºC. The salt added was calculated to reach the same salt concentration than the commercial dry tuna loins. After salting, loins were dried to achieve moisture of 0.48 (about 15 days), at 13 ºC and an air relative humidity close to 75%.

The tuna loins physicochemical values at the end of the processing was determined (salt, moisture, water activity), and in addition to that the sensory characteristics were evaluated.

The obtained results show a lineal relation ship between the added salt and the total uptake by the samples. Nevertheless, the increase in the added amount of salt implied a higher deviation from the diagonal. As regards the physicochemical analyses, the main influence of the salt formulation was observed on the colour (higher redness values in batch 3) and on the flavour (more off-flavours in batch 3).

S2.28. Prevalence of *Listeria* spp. in fresh water fish and the environment of fish markets in Northern Greece

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Information about the incidence of *Listeria* spp. in fresh water fish in Greece is rather limited. The purpose of the present study was to investigate the presence of *Listeria* spp. in fresh water fish, the personnel and the environment of fish markets in Northern Greece. 204 samples from fish, personnel and environment were collected from supermarkets, fish markets and open-air markets of two cities in Northern Greece and were tested for the presence of *Listeria* spp. using a two step enrichment procedure, followed by plating on two selective agars and biochemical identification of the isolates by Microgen Listeria ID system. From the 100 (flesh and skin) fresh water fish samples, 50 were from rainbow trout (*Oncorhynchus mykiss*) and 50 from gibel carp (*Carassius auratus*). From the 104 personnel and environmental samples, 20 samples were from workers' hands, 19 from workers' knives, 19 from working board surfaces, 21 from wooden boxes, 7 from plastic boxes and 18 from floor surfaces. *L. seeligeri* was isolated from 1% of the samples from the flesh of fish. *Listeria* spp. was isolated from 9% from the skin surface samples (4% *L. seeligeri* and 5% *L. innocua*). *Listeria* spp was isolated from 14.42% of personnel and environmental samples examined (1.92% *L. monocytogenes*, 5.77% *L. seeligeri*, and 6.73% *L. innocua*). *Listeria seeligeri* and *Listeria innocua* were the dominant species being detected in fish as well as in personnel and environmental samples. The lower incidence of *Listeria* ssp. in fresh water fish (9%) compared to the higher in personnel and environmental samples (14.42%) emphasises the need for enforcement of sanitary conditions of the establishments and personal hygiene conditions in order to reduce the risk of contamination of raw fish and fishery products by *Listeria* spp. and *L. monocytogenes* at the retail level.

S2.29. Determination of the hygienic conditions of fishing vessel by using 3M Petrifilm™

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Introduction: In this study, the microbiological contamination of fishing vessel during operation was determined. For this purpose samples were taken as follows: from the demersal trawl when the trawl was lifted up aboard, after the separation by the crews, from the crews' hands and clothes, from the deck, from the fish handling areas, from the storage areas and from the all equipments were used, from the fish surface and boxes. The samples of water used for washing fish and ice samples were also investigated.
Methods: *Microbial Enumeration*. Samples were taken from the surface of 10 cm^2 by swapping. Aerobic plate counts (APC) and psychrotrophic plate counts (PPC) were obtained using aerobic count plates (3M Petrifilm™, St. Paul, MN). *Enterobactericeae* (modified VRBG, 3M Petrifilm™, St. Paul, MN), *E.coli* (EC plates 3M Petrifilm™, St. Paul, MN) of appropriate dilutions. Samples, prepared as stated previously, were serially diluted in sterile 0.1% peptonewater. One-milliliter aliquots of appropriate dilutions were plated in duplicate and then incubated at 32 ˚C for 24 h (APC) or at 21 ˚C for 48 h (PPC). For *Enterobactericeae* count petrifilm plates were incubated at 35 ˚C for 24h. For *E. coli* petrifilm plates were incubated at 35 ˚C for 48h. Following incubation, colony-forming units (CFU) were counted.
Conclusions: Personal hygiene working in fish vessels are very important. They must keep themselves clean and wear clean protective clothing to prevent contaminating the fish. Decks, storage areas, cool rooms and any other areas where fish are handled, should be clean and in good condition to minimise contamination of the fish. After they are landed on the vessel, and transferred the fish immediately into chilled storage to maintain its quality and prevent deterioration. Clean water should be used for washing the fish and decks. Sanitation is very important for cleaning fish vessel. Sanitation should be used for eliminating the bacterial pathogens and microbial contamination.

S2.30. *Calanus finmarchicus* – A new raw material for the bio-marine industry

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The herbivorous zooplankton *Calanus finmarchicus* is present in large amounts in the North Atlantic where it plays an important role in the food web. The annual production has been estimated to be several times higher than the total biomass of fish and mammals in the same oceans. A unique trawling system has been developed for harvesting this copepod in a sustainable and environmentally friendly manner. *Calanus finmarchicus* has an interesting biochemical composition reflecting its marine habitat. This presentation describes the properties of the *Calanus finmarchicus* harvested and the oil and protein fractions that can be obtained in an industrial process. The harvested zooplankton contains approximately 80-82% water, 10-11% proteins, 1% chitin, 5-8% lipids and 2% minerals on wet weight basis The oil contains high amounts of omega-3 fatty acids including stearidonic acid (18:4n-3), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3). The neutral lipids are dominated by wax esters. The high content of astaxanthin (> 1500 ppm) results in an excellent oxidative stability of the oil. The astaxanthin is present mainly as mono- and diesters. The position of *C. finmarchicus* in the food web results in a very low content of persistent organic pollutants in the produced oil. The protein fraction produced is of high quality with a well balanced amino acid composition, and with unique organoleptic properties. Other bioactive components are also present. The products derived from *C. finmarchicus* may have potential use as dietary food or feed supplements, flavouring and colouring agents, and as a source for bioactive compounds of more specific nature.

S2.31. Sea lettuce (*Ulva lactuca*) as a human food

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Ulva is a genus of algae that includes species that look like bright green sheets and live primarily in marine environments. They can also be found in brackish water, particularly estuaries. They live attached to rocks in the middle to low intertidal zone, and as deep as 10 meters in calm, protected harbors. *Ulva* are usually seen in dense groups. Commonly known as the sea lettuce or the green laver, *Ulva* species can be eaten in soups and salads. Ten species of *Ulva* exist worldwide.
In this study, the chemical composition of sea lettuce (total water, moisture, ash, water-soluble carbohydrate, total protein) cultured in tanks in Urla-İzmir, were determined and compared for six months (March, April, May, June, July and August 2006) and its usage as a human food was discussed.

F2.20. Detection of antibacterial and antiviral activity in marine molluscs: new alternative strategy to substitute antibiotics in aquaculture

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Infectious diseases are the major limiting factor in aquaculture. Controlling diseases difficulties mainly coming from the differences susceptibility of animal, according to their developmental stage and from the pathogens diversity. Evolution of antibiotic-resistant pathogenic bacteria leads the laboratories to search for new antimicrobial agents from alternative sources.
Marine invertebrates and molluscs drew the attention due to their importance in aquaculture, fisheries and in marine environmental ecosystem equilibrium. As there are constantly exposed to relatively high concentration of bacteria and viruses, there have developed natural self-defence mechanisms. There might be a rich resource for the discovery and the development of new antimicrobial compounds.
The aim of this work consisted in screening for antimicrobial compounds in commercially important bivalves and gastropods molluscs species in aquaculture (*Cerastoderma edule, Ruditapes philippinarum, Ostrea edulis, Crepidula fornicate, Buccinum undatum* and *Littorina littorea*). After acidic extraction and reverse-phase separation, fractions were assayed *in vitro* against aquaculture pathogens bacteria and an enveloped DNA virus, *Herpes Simplex* Virus Type 1 (HSV-1), using Vero Cells.
All acid extracts presented *in vitro* antibacterial or antiviral activity. Significant both antibacterial and antiviral activities have been mainly detected in *C. edule* Acetonitril fractions with MICs of 79 µg/mL for the 40% ACN fraction against *Y.ruckeri* bacteria and of 32 µg/mL for the 80% ACN fraction against *B. megaterium* and *M. luteus* bacteria. Antiviral activity was revealed too with EC_{50} values of 919.5 µg/mL and 67.4 µg/mL respectively for the 40 and 80% ACN fractions without cytotoxicity. Further work could be now elaborated to purify and identify the compounds responsible for the activity. Indeed, these cultured aquatic species study, should help the development of new approaches for the control of microbial infections and be used as a way for potential novel antimicrobial compound in order to find new specific therapeutic agents in controlling infections.

F2.21. "Quality of fish from catch to table", a value chain project for longline caught fish

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There is a growing concern in the retail marked about climatic and environmental impact from the fishing industries. For instance Sainsbury's has changed their purchase terms for wild caught white fish to only include long line catch.

Long line fisheries are a more gentle method than other commercial methods, e.g. trawl. Long line provides a more selective catch, giving less by-catch, the impact on the sea floor is much gentler and studies has shown that the fuel consumption per kg catch is less in long line compared to trawling.

In 2007, a project was founded, with amongst others focuses on the environmental impact of long lining and the quality of the catch. The aim of the environmental part is to document the carbon footprint, and evaluate the by-catch impact on endangered species.
The quality of the fish will be investigated and documented. In addition, the handling of the fish from the boat to the store will be investigated and improved such as the quality is preserved.

Another focus of the project is development of the fishing technology and a system traceability system for the whole value chain.

Results from the first trials will be presented.

Participants in the project are Mustad Longline, Domstein Fish AS, Trace Tracker, Østfoldforskning and Norconserv.

F2.22. Effects of modified atmosphere packaging and vacuum packaging "skin" on chemical and sensory changes of fresh gilthead sea bream (*Sparus aurata*) fillets

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Introduction: Currently, the consumer demand for fresh, refrigerated foods with extended shelf life. Modified atmosphere packaging (MAP) in combination with refrigeration has proven to be an effective preservation method for the extension of shelf-life of fresh fish and fish products.
Methods: Cultured gilthead sea breams (average weight 470g) were gutted and filleted by hand. Fillets were packaged in Air, Vacuum "Skin" (Skin) and three (MAPs): 60%CO_2/40%N_2, 50%CO_2/50%N_2 and 40%CO_2/60%N_2. The conditions of storage were 19 days, 4±1 ºC and standard supermarket lighting (16h). The analyses were: exudate loss; pH by homogenization (Crison Basic 20, Barcelona, Spain); lipid oxidation (TBARS) method according to Pfalzgraf et al. (1995); microbiological count for aerobic psychotrophic flora in PCA (Merck; Darmstadt, Germany) and sensory analysis with six-member trained panel which carried out the sensory evaluation of raw and cooked samples (72 ºC) method according to Gimenez et al. (2005).
Results: No differences were found in pH values for any condition ($p > 0.05$). Exudate loss of Skin was higher than the rest of samples. For TBA values either for fresh or cooked, a similar pattern was followed by Skin and different MAPs, whereas the Air packaging had upper values. Bacteria grew most quickly in the samples stored in Air followed by Skin, having a longer shelf life for the different MAPs. In the sensory analysis for fresh and cooked, the majority of attributes were marked with similar values for the different MAPs and Skin. The Air was rejected of analysis at 7 day. Skin samples shown off-flavours which had a negative influence on the global appreciation at the end of storage.
Discussion: Results obtained in the chemical and microbiological analysis were in accordance with the sensory analysis. This study draws than MAPs with high CO_2 levels could extend significantly the shelf-life of gilthead sea bream by preserving quality.

F2.23. Practical challenges, solutions and incentives for traceability in wet salted fish and dried salted fish production. A case study from Norway

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Many food sectors are being subjected to tighter regulation regarding the origin and processing histories of their products. Some of this regulation is intended to improve sustainability and combat environmental threats such as over fishing. Illegal, unregulated and unreported (IUU) fishing is an example of such a threat to sustainability. In order to combat this, regulatory authorities require detailed information about fish products. Wet salted fish and dried salted fish (clip fish) production is challenging in this respect because it is complex and the raw material undergoes many stages of processing. Traceability is a tool which it is thought can be used to document the origins of the fish.

This study is focused on the practical challenges during production of wet salted fish and dried salted fish which are important when implementing traceability. The study also assesses which challenges the producers may encounter when attempting to implement the suggested changes. In order to analyze critical points for traceability process mapping was carried out at two different production facilities (one facility is a wet salted fish producer and the other is a dried salted producer). The scope of this mapping was from the landing of the fish, through production to the consumer packaging.

A detailed analysis of information flow and material flow through a wet salted fish and a dried salted fish producer is presented. This study outlines what practical measures are necessary to achieve both internal and chain traceability. The main challenges were identified with regards to implementing effective electronic traceability in this supply chain. These include: investments related to the implantation of traceability, alteration of production routines, the use of salt and the human factors involved in implementing traceability.

SEAFOODplus – An American Perspective

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The SEAFOODplus program is a mega-project funded by the European Union from 2004 through 2008 involving 16 countries, 70 partners and over 200 scientists. This project was unique in its scope and its multidisciplinary efforts involving disciplines from food science to medical intervention. An inside window to the workings of SEAFOODplus was provided to the External Advisory Board who were assigned different pillars within the program. Direct interaction with different program leaders was encouraged through research activities, meetings and conferences. This presentation will give an "American. perspective" of the SEAFOODplus program. While the metrics have been strong in terms of publications, book chapters, presentations, etc., the positive benefits of networking, scientific collaboration, and development of working groups have been an outstanding component of the SEAFOODplus program. Although U.S. universities and research centers have shown moderate success in inter-institutional cooperation, it has not been to the degree of teamwork shown in SEAFOODplus. Clearly, having a strong base through organizations such as WEFTA has facilitated this collaboration. The SEAFOODplus program also benefited from capable and fair administration and an organizational structure that promoted good science and technology transfer. A delicate balance of maintaining project goals while encouraging creativity within the programs was evident. While funding challenges will remain for continuation of various aspects of the SEAFOODplus program, inter-institutional bridges between organizations have been established which should prove useful in grantsmanship and collaborative research projects over the next decade.

Shaping the future of seafood research collaboration – Establishing the SEAFOODplus Research Platform

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The EU supported Integrated Project SEAFOODplus, running in the period 2004-08, has been a great success. SEAFOODplus has delivered several research results being breakthroughs in the areas of (1) seafood and health, (2) consumers and seafood, (3) seafood safety, (4) seafood quality and product development and (5) aquaculture. The management structure developed for the 20 projects has been blueprint for many other integrated projects. This structure has contributed to the integration of many different scientific disciplines of relevance to the seafood area. SEAFOODplus has become a brand label for highly qualified seafood research managed in a professional way.
These achievements have been highly acknowledged, and many requests have been received for a continued operation of SEAFOODplus. It has thus been decided that the **SEAFOODplus Research Platform** will be developed as a new collaborative concept after the closing date of the present SEAFOODplus project period, which is by the end of 2008. The new research platform will thus start its operation 1 January 2009.
The new SEAFOODplus Research Platform will be operated on a membership basis, welcoming research institutions, universities, industry and other stakeholders wishing to support the further development of the seafood area and promotion of seafood to the benefit of human health and well-being.
The vision of the SEAFOODplus research platform is (1) To be the preferred research platform for major stakeholders in all aspects of the seafood science, industry, policy makers and financing bodies in Europe, particularly addressing research supported by the European Union. (2) To work for continued and effective integration of the best research environments in academia and industry with the aim of having European seafood research to be recognised as the world leaders in seafood science.
This vision will be realised by the mission adopted where **international integrated multidisciplinary** research will be stimulated and encouraged. Every opportunity will be explored to influence the European research agenda with focus on seafood research. Project ideas from the members of the research platform will be brought forward and promoted for funding wherever possible. Research priorities will be communicated via channels at the scientific level of the European Commission and to existing Technology Platforms in relevant areas.
The research platform will be operated by a management structure based on principles where project leaders in charge of new projects, called satellite projects, fostered by the research platform will have a seat in the management committee. A small management team will take care of the day to day management, and all stakeholders will have a seat in the general assembly, being the governing body of the SEAFOODplus Research Platform.

Session 3

Future challenges in seafood safety and quality assessment

Bianca Maria Poli, Giuliana Parisi (edited by), *Seafood from Catch and Aquaculture for a sustainable Supply*. *Book of Abstracts*, ISBN 78-88-8453-746-1 (online), ISBN 978-88-8453-745-4 (print), © 2008 Firenze University Press

F3.01. Aromatic upgrading of marine by-products: sardine (*Sardina pilchardus*) hydrolysates example

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With changes in waste legislation in general and marine discards in particular, waste management will become more and more difficult and expensive. Among the different upgrading strategies of marine wastes (by-products) studied worldwide, enzymatic hydrolysis is one of the most promising as it allows the recovery of compound with medium or high added value (peptides of nutritional interest, with functional and/or biological activities,…).
In France, nearly 21000 tons per year of marine byproducts are processed for an aromatic purpose and the resulting compounds are added into the formulation of many food products like cooked dishes, soups, sauces… If the typology of marine aroma is huge as the biological diversity of the raw material is great (marine species, type of by-products…) and the extraction procedures are numerous (enzyme, time, temperature, fractionation process…), the control of the aromatic balance of these extracts remains a major issue.
The purpose of this study is to determine the effects of different processing parameters (enzyme, time, temperature, presence or not of antioxidant…) on the aromatic properties of sardine (*Sardina pilchardus*) hydrolysates. Thus, volatile compounds are extracted, identified and quantified while the sensory properties of the final products are described by sensory analysis.
This study is related to the objectives of the SEA^{pro} network (Sustanaible Exploitation of Aquatic PROducts, www.seapro.fr) which aims to promote a full use of marine biomass by proposing biotechnological upgrading tools.

F3.02. Effects of fish protein isolate on physical and sensorial properties of haddock mince balls

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Introduction: Fish protein isolate (FPI) manufactured by pH-shift technology from co-products can be used as an ingredient for production ready to eat fish products based on mince or surimi. Developing new products from mince and isolate requires a comprehensive analysis of isolate characteristics with respect to the physical and sensory properties of final product such as texture-forming properties, flavour and taste characteristics. The present study, describes an attempt to develop an innovative seafood product based on mince and isolate for domestic consumption.
Methods: In this work FPI made from haddock (*Melanogrammus aeglefinus*) cut-offs was added to haddock mince in different proportions (50:50, 25:75) in manufacturing two types of fried fish balls. A mince fish ball product also prepared as a control. The products were assessed for physical properties and sensory changes within a period of 12 weeks of freezing storage at -18 ˚C. The rheological properties of fish flesh (mince with and without isolate) were investigated by using Brabender Viscometer in a range of 40-45 ˚C in order to establish the optimum temperature for setting fish balls. Cooking loss of samples was measured. Sensory analysis of fried products was carried out.
Results: These results suggest that optimum temperatures for setting of mince and mince: isolate (75:25 and 50:50) are 45 ˚C, 45 ˚C, and 40 ˚C respectively. The results show that viscosity of mince significantly decreased ($P<0.05$) as FPI added to mince increased. Samples containing isolate had the same cooking loss after two thermal settings. Data analysis of sensorial results indicates that FPI can affect organoleptic properties significantly ($P<0.05$). Among 20 attributes which were evaluated by sensory panellist, grainy texture and soapy taste were the most important detected defects in fish balls. They depend on proportion of isolate to mince and also freshness level of mince.
Discussion: Recovering protein from fish filleting by-products and using it as an ingredient for developing fish products is a new innovation to increase fish muscle yield as well as fully utilize aquatic food recourses. Although added isolate to the mince decreased the viscosity, the products was still had good viscosity for shaping and setting. Since there is few information and published research about FPI products, this work can introduce how to utilise FPI as an ingredient in mince or surimi industries for production value added fish products.

F3.03. Marinating herring in berry extracts improves storage stability and nutritional value

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The aim of this study was to investigate novel berry extracts with the purpose to preserve and prolong shelf-life of herring, add nutritional value as well as enhance utilisation possibilities of a low price fish species. During storage, the quality of fatty fish decreases among others due to lipid and protein oxidation. Berry extracts have shown to have a good antioxidant capacity due to their content of phenols, anthocyanins, ascorbic acid and other possible bioactive compounds. In addition, berries are known to be protective against cancer, oxidative stress in the body, enhance the immune system, reduce inflammation and reduce blood pressure.
Herring (*Clupea harengus)* was frozen, thawed and marinated in either elderberry (E), cranberry (C) or blackcurrant (B) berry extracts for 24 hours. Fillets were vacuumpacked and stored on ice for 7 days before freezing at – 20 ˚C for 6 months. Total lipids, TG, FFA, PL, lipid oxidation (TBARS), protein oxidation and α-tocopherol were determined after storage.
All investigated oxidation parameters showed lower values in the treated groups in comparison to the control samples, although not all differences were significant. The total content of typical oxidation products such as alkanals and 1-penten alcohols were higher in control fish compared to treatment groups. In agreement with these findings, the antioxidant α-tocopherol was higher in the treated samples compared to control. pH-values were slightly lower in the treated fish samples and no differences in lipid class composition were detected. Our results suggest that marinating herring with the chosen berry extracts will increase storage stability and nutritional value of this fish specie.

F3.04. Functional properties of a protein-rich paste produced from blue mussels (*Mytilus edulis*) using an acid and/or alkaline solubilization technique; gelling, foaming, emulsifying capacity and salt solubility

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One third of the world's production of blue mussels is being thrown away or is used as mussel meals due to small size or damaged shells. The need for sustainable growth of the mussel industry dictates the necessity for new technologies that will allow full utilization of what is currently wasted. Recently, we have described an acid and alkaline solubilization technique that allows the production of a low-lipid, protein-rich paste from mussel meat. The aims of this work were to (i) apply this technology to whole mussels including the shells, (ii) improve the solubilization process in terms of protein yields and (iii) investigate the functional properties of protein isolates (PI) from blue mussels.

Protein isolates were produced from a meat-water homogenate by the acid and alkaline technique, with a few modifications to improve the yield. Protein and lipid contents were determined by standard methods. Proteolysis was followed by SDS-PAGE electrophoresis before and after processing. Surimi was made from the PI by adding cryoprotectants and freezing for 24 hours. Gels were made from surimi by adding 2% NaCl and cooking at 90 °C for 30 min.

The improved alkaline technique exhibited a maximum protein yield of 58%, which was significantly higher than the acid process (43%). When applied to whole blue mussels, including the shells, alkaline process had 48% protein yield, while the acid process 31%. The yields could probably be further improved by preventing the proteolysis occurring during the process as was revealed by electrophoresis. The amino acid profile of both acid- and alkaline-produced isolates was improved compared to mussel meat. Alkaline-produced gels had significantly higher strength values compared to acid-produced gels (240 grams x 7.9 mm vs. 174 grams x 7.5 mm respectively). Salt solubility, emulsification activity index (EAI) and foaming ability were also significantly higher in the alkaline-produced PIs.

S3.01. Instrumental texture and sensory evaluation of frankfurters formulated with different proportions of washed minced from Nile tilapia by-products

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The filleting yield of the Nile tilapia is about 30%, producing large amount of residues, that cause economic losses and environmental impact. The use of by-products from fish processing can reduce industry costs and increase the added value to its products. An alternative for a good use of the filleting residues is the production of minced fish (MF) that may be included in a series of products, between them the frankfurter. However, the heme pigments presence leads to a dark coloration in the MF that may be unpleasant to the consumer. The MF washing can promote better acceptance The objective of this work was to evaluate the effect of the percentage of inclusion (60, 80 and 100%) and the number of washings (0, 1 and 2) of MF on instrumental texture (hardness) and sensory evaluation (texture and flavor) of Nile tilapia frankfurters. The experimental design used was a complete factorial 2^2 with 3 central points. The washing number affected ($p<0.05$) the instrumental texture, causing reduction in the hardness of products. This effect was most markedly when twice washing and 100% of MF were used in the frankfurters production (Table 1), probably due to the loss of soluble proteins during the washing process that caused a decrease on the water retention capacity. The washing process also caused reduction ($p<0.1$) in the texture sensory acceptance, changing from 6.3 (liked slightly) to 5.3 (neither like nor dislike) in sausages formulated with 60% of MF and 0 and 2 washing and from 6.1 (liked slightly) to 4.7 (disliked slightly) in sausages formulated with 100% of MF with 0 and 2 washing. The washings number and MF inclusion percentage (independent variables) had not significantly affected the flavor, which presented an average value of 6.0 (Table 1). It was concluded that MF washing decreased the hardness and the texture sensory acceptance of the frankfurters evaluated, not influencing its flavor.

runs	*Number of washing*	*MF (%)*	*Instrumental Texture*	*Sensory acceptance*	
			Hardness (g/cm)	*Flavor*	*Texture*
1	0 (-1)	60 (-1)	3426.00	6.1	6.3
2	2 (+1)	60 (-1)	1787.90	5.8	5.3
3	0 (-1)	100 (+1)	3890.60	6.3	6.1
4	2 (+1)	100 (+1)	1384.80	5.5	4.7
5	1 (0)	80 (0)	2607.00	6.4	5.2
6	1 (0)	80 (0)	3145.70	5.9	5.8
7	1 (0)	80 (0)	2192.20	5.7	5.4

Table 1. Instrumental texture and sensory evaluation obtained from frankfurters varying the number of washings and percentage of inclusion of Nile tilapia minced fish (MF) using experimental design 2^2 with 3 central points.

Support: CNPq nº 55.1652/2005-5.

S3.02. Tensio-active and organoleptic characterization of a glycated marine by-product hydrolysate

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New food ingredients are needed to improve the stability of the microheterogeneous compositions of foams and emulsions. Furthermore, consumers require that such new ingredients be produced from natural sources. The use of sugar-protein conjugates deals with this attempt. They come from natural sources (proteins and sugars) and they also show different surface-active properties in comparison to native proteins. In this context, this study focuses on the tensio-active and organoleptic properties of a marine by-product hydrolysate (**PH**), and its sugar-conjugate (**GPH**). The latter was obtained by heating PH in the presence of a reducing sugar. In comparison to PH, it was shown that GPH had: i) the same surface-tension values (44.8 mN/m ± 0.3), ii) a slight different behaviour as a function of pH, iii) better foaming capacity, and iv) lower emulsion stability. It was hypothesized that PH had a sufficient presence of free hydrophobic amino-acid residues to be easily and/or rapidly adsorbed to the oil/water interface in emulsions. From this, the lower emulsion stability shown by GHP was explained by a reduction in the proportion of free hydrophobic amino-acid residues due to chemical reactions between sugar and PH. This would also explain the better foaming capacity of GPH. The decrease in the free hydrophobic amino-acid content would increase the hydrosolubility of GPH, which thus probably improved the hydrodynamic properties of the foam's film. Finally, in GPH, the native fish flavour of PH disappeared but a chicory flavour was detected. Perspectives of this work are to compare the molecular compositions of PH and GPH, and to check the absence of cytotoxicity for GPH.

F3.05. Improvements in cold and thermic induced gelification of giant squid (*Dosidicus gigas)* surimi

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Introduction: Squid surimi that has been processed by isoelectric precipitation frequently has low gel strength which is not suitable for elaborating certain products that require specific textural properties. On the other hand, this kind of surimi does have other properties that are suitable for elaborating products resembling not only cooked products but also raw products (marinated, smoked…). The objective of this work was to improve the physicochemical characteristics of cold and thermic induced gels by adding microbial transglutaminase (MTGase) and using isostatic high pressure.
Methods: Giant squid surimi was homogenised with 3% salt (adjusted moisture: 75%) and the resulting samples (C: 0.0% MTGase, A: 0.5% MTGase, B:1.0% MTGase) were stuffed into cases and received different treatments (1:30ºC/1h-5 ºC overnight; 2: 30 ºC/1h+90 ºC/30min-5 ºC overnight; HP: application of 300MPa).
The mechanical properties analyzed were Texture Profile Analysis (TPA): at a deformation of 40% hardness and cohesiveness were analyzed and, at 80%, strength and deformation at rupture; Puncture Test: Breaking strength and breaking deformation; Water binding capacity (WBC); Colour analysis: Lightness; Electrophoresis analysis.
Results and Discussion: In samples induced at 30 ºC, whether subjected to high pressure or not, all mechanical properties and WBC increased with higher MTGase content. In gels induced at 90 ºC, the addition of MTGase was not so important. The treatment with high pressure at 300 Mpa improved all mechanical properties (except cohesiveness) and WBC, for gels induced at 30ºC and 90 ºC.Lightness was slightly higher in samples treated at 90 ºC than in those treated at 30 ºC. Neither the addition of MTGase, nor high pressure treatment induced changes in lightness. Electrophoresis analysis revealed a slight decreasing trend in the MHC when MTGase concentrations increased due to specific bonds being established.

Batch	Hardness (N)†	Cohesiveness†	Rupture force (N)‡	Rupture Distance (mm)‡	Breaking force (N)	Breaking deformation (mm)	WBC (%)	L*
C1	12.69 ± 1.53	0.61 ± 0.10	14.89 ± 0.65	11.21 ± 0.19	0.47 ± 0.02	4.42 ± 0.87	44.87 ± 3.21	73.49 ± 0.19
C2	71.43 ± 3.80	0.69 ± 0.01	83.86 ± 16.16	13.82 ± 1.31	2.81 ± 0.20	5.64 ± 0.23	48.79 ± 4.11	83.11 ± 1.21
A1	22.70 ± 0.46	0.81 ± 0.00	61.63 ± 4.39	18.94 ± 0.16	1.27 ± 0.01	7.40 ± 0.14	69.46 ± 5.87	74.80 ± 0.31
A2	66.95 ± 6.87	0.72 ± 0.00	76.95 ± 8.71	12.97 ± 0.97	2.52 ± 0.14	6.41 ± 0.23	48.66 ± 3.89	82.39 ± 0.67
B1	26.31 ± 0.99	0.86 ± 0.03	112.94 ± 12.17	19.49 ± 0.38	1.73 ± 0.03	8.20 ± 0.12	70.53 ± 1.84	76.60 ± 1.08
B2	74.65 ± 0.73	0.74 ± 0.01	87.60 ± 6.48	13.95 ± 0.72	2.87 ± 0.10	6.81 ± 0.29	45.00 ± 0.32	80.56 ± 0.94
C1 HP	15.85 ± 2.00	0.71 ± 0.01	20.55 ± 1.02	14.85 ± 0.35	0.84 ± 0.03	5.82 ± 0.07	61.49 ± 4.27	70.42 ± 1.23
C2 HP	110.37 ± 2.72	0.72 ± 0.00	132.76 ± 13.80	14.49 ± 1.56	4.01 ± 0.09	5.70 ± 0.09	55.92 ± 3.32	80.59 ± 1.29
A1 HP	22.07 ± 2.24	0.80 ± 0.00	70.06 ± 5.28	18.80 ± 0.59	1.92 ± 0.15	10.04 ± 0.43	75.47 ± 3.15	72.10 ± 1.14
A2 HP	97.42 ± 3.34	0.75 ± 0.00	161.97 ± 19.01	15.37 ± 0.32	3.95 ± 0.35	6.74 ± 0.56	53.31 ± 4.60	81.25 ± 1.05
B1 HP	21.85 ± 2.10	0.85 ± 0.03	332.54 ± 33.48	22.36 ± 0.72	2.35 ± 0.09	11.28 ± 0.09	73.12 ± 2.56	70.81 ± 1.20
B2 HP	92.74 ± 5.60	0.73 ± 0.01	169.15 ± 7.69	16.27 ± 0.67	4.16 ± 0.17	7.40 ± 0.37	50.44 ± 3.52	82.89 ± 0.50

† TPA - 40 % compression; ‡ TPA - 80 % compression

F3.06. Understanding the fish matrix-dietary fiber interactions in restructured seafood

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The interest of wheat dietary fibre (WDF) as ingredient for the formulation of seafood restructured products has been recently demonstrated. At high (~6%) WDF concentrations there are several technological parameters related to gel elasticity and strength, and water retention, which change upon addition of this fibre. In order to study the causes of the above effects, there is a need to understand the interactions between the matrix constituents and WDF. The objective of this work was the rheological and spectroscopic study of the formulations consisting of gels with 0, 3, and 6% of WDF Vitacel® WF200.

The main results reveal that the rheological changes caused by the WDF on the gel are a balance of its filler effect and the heterogeneity of the gel network caused by its addition. The heterogeneity can stem from the high water holding capacity of the fibre, which could cause dehydration of the proteins. This was confirmed by FT-Raman spectroscopy. It was shown that β-sheets formation resulted upon addition of 6% WDF. On the basis of the spectral features of the WDF νCH band as a function of humidity and its relative intensity within the sample matrix, it was concluded that water transfer from protein to WDF can occur in *surimi* gels. The said WDF hydration can be interpreted in the sense that this fibre either takes water that is delivered from the gel protein upon heat-mediated formation of β-sheets and/or or acts as active dehydrating agent. The H/D exchange kinetics of the gels with WDF are slower that those of samples without fibre. This can be interpreted in terms of either stronger fibre-water hydrogen bonding or smaller interstitial water domains. The spectral results of the νOH band suggest that the former mechanism corresponds to the true situation, LT-SEM supporting the above conclusion. These results constitute molecular data to be considered when designing fish restructured food with these fibre ingredients.

F3.07. Myosin denaturation of bluefin tuna *Thunnus orientalis* under acidic conditions

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Introduction: Whitish Bluefin tuna meat is sometimes found at Japanese fish markets, which is a serious problem in market because of its low commercial value. A cause for it is believed to be protein denaturation by exposure to acid and high temperature. Lactic acid production from glycogen through anaerobic metabolism reduces the pH and struggling of fish when hooked raises body temperature. In this presentation, a quantitative analysis of the effect of lowering pH and increasing temperature on myosin denaturation using model system of myofibrils (Mf) and myosin.
Methods: Mf or myosin suspended in 0.1 M NaCl at various pHs was treated with low pH or heated. Myosin denaturation was accessed by measuring Ca-ATPase inactivation rates (*k*).
Result and Discussion: *k* for Mf increase with decreasing pH. A linear relationship between Log *k* and pH gave a breaking at pH 5.8 indicating a two-step effect of pH. *k* at pH 5 was 27 times greater than at 7. Raising temperature also increased *k* obeying Arrhenius' equation; Log *k* against 1/termperature gave straight lines. *k* at 40 ℃ was 180 times greater than at 25 ℃. Combined effect of lowering pH from 7.0 to 5.0 and raising temperature from 25 to 40 ℃ was 4300 times. The effect of pH and temperature on the *k* of myosin alone without the protection by F-actin was also studied. Plotting Log *k* against pH also gave a straight line with a breaking point, while the breaking point (pH 6.5) was higher than that for Mf. Acceleration of the myosin denaturation by lowering pH from 7 to 5 (800 times) was greater than that for Mf. Thus, it was demonstrated that myosin is strongly stabilized by F-actin at acidic pH.

F3.08. Impact of variables through the value chain on the end product quality of sea food

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Salmon (*Salmo salar*) is an important species in Norwegian aquaculture and the Brown crab (*Cancer pagurus*) is a commercially important shellfish species. The industry export the main part of the salmon gutted or as fillets, and sell the crab as whole crab, fresh or cooked. Roe from various species in the cod family is commonly used in spreads, dinners and tarama.

These raw materials are rich in omega 3 fatty acids, taurine and phospholipids, and more product development should be done to increase the human consumption. A good sensory and nutritional quality is of importance for the consumer acceptance of the end product. In order to prevent oxidation and loss of nutrients, there is a need for more knowledge about critical variables influencing sensory and nutritionally quality in the value chain.

An investigation of a value chain starting with production of salmon feed and ending with consumer products was carried out using a complex experimental design (combined fractional split plot and mixture design with time series). Salmon was fed with different levels of long chain omega 3 fatty acids and vitamin E. Meat fractions from each group were processed and stored under various conditions and then further processed into end products including various mixtures of salmon, crab and/or cod roe (*Gadus macrocephalus*).

The raw materials were analysed for amino- and fatty acid composition and contents of ash, water, lipid, total protein and vitamin E. The taurine content and parameters of protein and fat oxidation were followed through the value chain. Odour, headspace oxygen and carbon dioxide/carbonyl, pH and microbial growth were also measured during the storage of end products.

The stability of the products were influenced by several variables through the entire value chain.

The support from the Research Council of Norway is gratefully acknowledged.

S3.03. Effects of fibers on the quality of pre-cooked fish fingers stored at 0-4 °C

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Fiber is an essential compound in the diet, which has health benefit effects in certain disorders. At the same time, dietary fibers can be an effective tool in seafood processing for improving functional properties. The aim of the study is to evaluate the effect of wheat fiber in different size and apple fiber on pre-cooked fish fingers stored at 0-4 °C according to texture, chemical, sensory and microbiological quality parameters. With this respect rainbow trout will be used as raw material and after being minced, ingredients (spices and salt) will be added and minced fish will be divided into 7 groups. Groups will be formulated as follows: control without fiber, 3% wheat fiber 200 (which will be dispersed in water according to formulation of the company Vitacel), 3% wheat fiber 200 (without being dispersed in water), 3% wheat fiber 400 (which will be dispersed in water according to formulation of the company Vitacel), 3% wheat fiber 400 (without being dispersed in water), 3% apple fiber (which will be dispersed in water according to formulation of the company Vitacel), 3% apple fiber (without being dispersed in water) and after being shaped (Adhesive Batter + predust, 18405506) and (Yellow Crumb,18614115) patented products will be used as pasting and coating materials, respectively. They will be fried in oil (pre-cook 30 seconds at 180 °C) and then after being cooled they will be stored at 0-4 °C during the storage period.

S3.04. Application of wheat fiber in to rainbow trout fish burgers and determining their quality

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Fisheries products have high nutritional values and important functional properties. But they do not have fibers. Fibers are the components which have advantageous and positive effects in some specific illness with these effects they are indispensable in diets. And also they can be used for increasing the water binding capacity and gelatin properties of sea food products. In recent years dietary fibers became more important and many scientific studies have been done in seafood sector.

The aim of this study was application of insoluble wheat fiber in to fish burgers and determining their effects on their quality while kept in refrigerator (0-4 ℃). As fish material fresh skinless butterfly type rainbow trout fillets were used. And two different size of wheat fiber were chosen. The size of the fibers were 250µm (Vitacel®-WF200) and 500µm (Vitacel®-WF400). Fish burgers were prepared in both sizes with using 1.5% and 3.0% fibers in their formula. And also control groups were produced to compare. Both groups were stored in refrigerator conditions. For determining their quality, textural parameters, color measurements, chemical (TVB-N, TBA, pH], microbiological (Total aerobic mezophilic bacteria count, Psychotropic bacteria count) quality control analysis and sensorial parameters were monitored, during the storage.

S3.05 Influence of vegetable proteins on the functional properties of hake protein powder

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Fish protein powder has a potential market as protein ingredient and healthy product. The high biological value of fish proteins is recognised and the health benefits of vegetable proteins consumption have been also described. Thus, it has been studied the interaction between muscle and vegetable proteins particularly on the gelation properties. The dried fish proteins used in this work were recovered from Cape hake by-products by alkaline solubilisation followed by isoelectric precipitation and freeze-drying. The objective of work developed was to study the effect of soy and pea proteins on the functional properties of Cape hake protein powder (HPP).
The HPP isolate had higher protein content (90%) and also lower fat (0.53%) and ash (1.44%) content than that of soy protein isolate (SP) and pea protein concentrate (PP).
Breaking force and deformation of gels prepared with the mixtures of HPP and SP or PP decreased with the increase of the percentage of both vegetable proteins. However, the gels prepared with 10% SP + 90% HPP and 5%SP + 95% HPP were more elastic than those prepared without the addition of these vegetable proteins. The gels prepared with HPP and preheated SP at 90 ºC for 3 min showed higher breaking force and deformation while the reverse effect was observed with preheated PP.
Increasing levels of vegetable proteins in the mixtures led to an increase of the emulsifying capacity, which was generally higher in the HPP/SP than in HPP/PP mixtures. In contrast, fat absorption decreased with increasing levels of soy and pea proteins and this decrease was more accentuated in the case of SP.
Gels dynamic behaviour of protein mixtures was studied by a sequence of oscillatory measurements of temperature, time and frequency sweeps. Linear viscoelasticity of emulsions prepared with the mixtures was also evaluated.

S3.06. Physicochemical properties of restructured sea bream products

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Gilthead bream (*Sparus aurata*) is an abundant fish species in the Mediterranean sea. His filleting produces byproducts. The use of the remnants of flesh in the form of sausage is a solution attractive and accepted for the consumer. The konjac gum is derived from tuber of *Amorphophallus konjac* C. Koch. It is a kind of neutral polysaccharides. It is an interesting ingredient because it has a high capacity for water holding capacity and an ability to lower blood cholesterol and sugar level. The objective of this work was to determine the feasibility of obtaining restructured product from this fish using two concentrations of Konjac gum (5 or 10 g/kg) with microbial transglutaminase (MTGase -3g/kg) and low-salt concentration (10g/kg).
Changes in mechanical properties (texture profile analysis), pH, expressible water or water holding capacity, humidity, water activity and colour attributes were measured. Minced fish from Gilthead bream yielded good gels at both konjac gum concentration with salt and MTGase.
The results showed that they were appropriate for improving the properties of restructured product obtained from *Sparus aurata*.

S3.07. Fatty acid profile of *Dicentrarchus labrax* and *Sparus auratus* fillets packaged with different technology

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Introduction: Aim of this study was to evaluate the fatty acid profile, with special reference to n-3 polyunsaturated fatty acids (PUFA), in fillets of cultured sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus auratus*) packaged with different technology.
Methods: Samples of sea bass and sea bream (average weight of 500 g) coming from a off-shore breeding were killed in water and ice and transported, under ice, in refrigerated box to a fish processing plant where they were mechanically filleted and packaged with different systems, including vacuum packaging and modified atmosphere (MAP), as described: 1) DARFRESH 2) STEAM COOKING (a particular typology of MAP packaging) 3) MAP A (CO_2 70%, N 25%, O_2 25%) 4) MAP B (CO_2 63%, N 22% and O_2 15%). The fatty acid composition was determined at regular time intervals (1^{st}, 3^{rd}, 5^{th}, 7^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th}, 23^{rd} and 25^{th} day) by GC according to Reg. UE 796/2002. Data was subjected to analysis of variance (ANOVA).
Results: The total lipid content for was 5% and 11% for sea bass and sea bream fillets respectively. The fatty acids most represented were: palmitic acid (16:0), myristic acid (C14:0), stearic acid (C18:0) among satured (SFA); palmitoleic acid (16:1), oleic acid (C18:1) among monusatured (MUFA) and linoleic acid (18:2ω6), eicosapentaenoic acid (EPA 20:5 ω-3), docosahexaenoic acid (C22:6ω-3) and C22:5 ω-3 among polyunsaturated fatty acids (PUFA). Palmitic acid (C16:0) contribute to the 18% and to the 16% of the total SFAs content for sea bass and sea bream respectively. Oleic acid was the most represented MUFA accounting for 16.80% of total MUFAs content. The most important PUFAs detected were EPA (C20:5ω-3) with percentage varying between 6.66%-7.14% and 5.88%-6.32% and DHA (C22:6ω-3) with levels ranging between 14.87%-16.08 % and 13%-13.78% for sea bass and sea bream respectively.
Discussion: The levels of oleic acid were lower than those reported in literature and this should be due to seasonal influence . Data showed no statistical differences ($P<0.05$) among the different packaging typologies and during the whole experimental period. In conclusion these packaging systems were able to maintain organoleptic characteristics and fatty acids content for a longer time than the usual storage systems.

S3.08. Microwave defrosting technology: effects on canned tuna quality

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Canned tuna is one of the most important fish products in many countries, including Italy and Spain. This type of product supports a significant market demand and it plays an important role as component of the Mediterranean diet. Sometimes, canning industry utilizes frozen precooked tuna blocks as raw materials in order to increase the daily industrial production and to reduce the water waste generation caused by cooking raw tuna loins. In this case, it is necessary to develop a profitable defrosting process for tuna blocks before canning. The main method for thawing is the immersion in hot salted water which requires high electricity and water consumption. In contrast, there are novel technologies as microwave thawing which are faster, more economical and they improve temperature distribution. The objective of this research was to investigate the effect of both thawing methods on the lipid oxidation and protein degradation of precooked tuna blocks (*Thunnus obesus*) after the canning process and sterilization.

Samples of canned tuna were taken at 0, 45, 90 and 180 days and they were tested by means of Total Volatile Base Nitrogen (TVBN), Peroxide Value (PV), Thiobarbituric Acid Index (TBA-i) and Conjugated Diene and Triene formation.

The results shows that TVBN in tuna after defrosting and canning is lower when a microwave method is used, producing a less protein degradation comparing with the traditional thawing. Same results could be observed when lipid oxidation was analyzed. Although significant differences were not found in PV and TBAi between both defrosting treatments, it was found that the formation of conjugated diene and triene compounds was delayed and significantly lower in microwave thawing.

It can be concluded that the microwave technology applied to defrost precooked tuna blocks could be an alternative to the traditional process for the industrial production of canned tuna.

S3.09. Changes in lipid oxidation and omega-3 fatty acids in MPC-added nugget products from salmon and mackerel mince during frozen storage

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The effect of milk protein concentrate (MPC) at 4% on lipid oxidation in 4-month frozen stored mackerel and salmon mince was investigated in developing mince-based batter-breaded seafood products. The mince was prepared from skin-on fillets using a Baader 694 meat-bone separator, immediately mixed with MPC, and stored frozen. The formulated fish nuggets were also prepared with necessary ingredients with and without par-frying. The extent of lipid oxidation and changes in omega-3 fatty acids were assessed by measuring fatty acid composition, thiobarbituric acid reactive substances (TBARS) and peroxide values (PV). The addition of 4% MPC to mackerel and salmon mince resulted in significantly lower TBARS values (0.92±0.05, 1.62±0.02 for salmon; 1.65±0.02, 3.44±0.01 µmole/100g for mackerel) and PV values (5.6±0.5, 6.8±1.2 for salmon; 5.59±0.2, 7.4±0.7 meq/kg for mackerel). MPC also helped retain significantly higher amounts of DHA (2.55±0.2, 1.05±0.09% for salmon; 3.52±0.3, 1.23±0.7% for mackerel) and EPA (0.70±0.2, 0.59±0.3% for salmon; 0.93±0.08, 0.51±0.1% for mackerel ($p<0.05$). There were no significant differences in PV and TBARS values between batter-breaded nugget products with and without par-frying after the 4 month frozen storage. The levels of EPA and DHA significantly decreased with frozen storage for all treatments in less extent for par-fried products. The reductions of EPA and DHA in salmon were 67 and 53% for batter-breaded and 57 and 39% for par-fried, while in mackerel they were 63 and 73% for batter-breaded and 57 and 56% for par-fried, respectively. Results suggest that MPC may effectively retard lipid oxidation and improve retention of omega-3 fatty acids in formulated mince-based seafood products during frozen storage.

S3.10. Investigation of color change with lipid oxidation, chemical decomposition and microbial growth of cold stored sea bass (*Dicentrarchus labrax* L.) fillets by machine vision system

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Introduction: Temperature and time are most effective parameters for lipid oxidation. They change some physical and chemical specifications of fish. One of them is color which affects consumers at first sight and also may show the level of lipid oxidation especially in fatty fishes. The purpose of this study was to investigate the relations of color change with lipid oxidation, chemical decomposition and microbiological growth of refrigerated sea bass fillets with Machine Vision System.
Material: Fish material was obtained aquaculture plant in Çeşme-İzmir
Methods:
Color Analysis
The color change was measured for L*, a*, b* values using color machine vision system.
Chemical Analysis
TBA, pH and TVB-N analysis
Microbiological analysis
Total viable mesophilic and psychrophilic bacterial counts.
Discussion: Correlation between color values, chemical and microbiological analysis were discussed.
Discriminant function analysis were done for color values with oxidation, decomposition and microbiological loads.

S3.11. The relation between temperature and ice formation during freezing of several fish species as measured by differential scanning calorimetry

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A consequence of the heterogeneity of fish muscle tissue is that several compartments exist and the muscle water (60-80% of the total weight) is distributed among these pools. The freezing and thawing behavior of muscle tissue thus is far more complicated than that of a watery solution of proteins and salts. Differential scanning calorimetry (DSC) is a valuable technique for studying ice formation and thawing in small pieces of "intact" muscle. By this technique, it is possible to calculate how much water has been frozen as a function of e.g. the frozen storage temperature and the muscle system's integrity. We found that the freezing process is 'all or none': below a certain temperature, all freezable water becomes ice and above that temperature no ice formation occurs at all. This is in contrast to common beliefs that the amount of ice varies smoothly with temperature. The "actual freezing temperature" was about -15 °C in muscle from several fish species, and the amount of freezable water was only 75-90% of the total muscle water, dependent on the later quantity. The amount of frozen water, but not the freezing temperature, was slightly, but significantly, higher in pre-frozen than in fresh cod.

S3.12. Auto-fluorescence of fish muscle juice. Resolution into components by robust PARAFAC with automatic scatter correction

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Fish muscle juice from cod contains substances that exhibit auto-fluorescence. They are well represented in a fluorescence landscape with an excitation wavelength range from 250 nm to 370 nm and an emission wavelength range from 270 nm to 550 nm. From this landscape, three components can be identified by parallel factor analysis (PARAFAC). One component has an excitation spectrum with maximum around 340 nm and an emission spectrum with maximum around 430 nm suggesting that it may be attributable to the metabolically important coenzyme NADH. The two other components are heavily overlapping with excitation wavelength maximum around 280 nm and emission wavelength maxima around 320 nm and 350 nm characteristic for the aromatic amino acids (tryptophane, tyrosine, and phenylalanine).
Due to the considerable overlap of the excitation and emission wavelength ranges, the fluorescence landscape is dominated by first-order Rayleigh scatter and second-order scatter also contributes. This has to be taken care of in order for the PARAFAC solution to be meaningful. Several approaches have been tried to handle these scatter effects. However, a common problem is that all the methods depend on a subjective identification of the scatter band. Recently an automatic scatter identification procedure was published based on a robust principal component analysis of an unfolded and transposed version of the 3-dimensional data array (the landscape). In that way, the scatter considered as variable-wise outliers is transformed to sample-wise outliers suitable for the robust algorithm used. After having identified the outliers, they are substituted with missing values, the data matrix reformed to three dimensions and PARAFAC performed.
The procedure may be combined with a modified PARAFAC method that is robust to sample-wise outliers. The combined procedure is presented and applied to a set of measurements on cod muscle juice with different amounts of NADH, tyrosine and/or tryptophane added.

F3.09. Iron and haemoglobin induced oxidation of polyunsaturated fatty acids

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Marine lipids are rich in long chain n-3 fatty acids that have beneficial effects on human health. However, due to the high content of polyunsaturated fatty acids, marine lipids are also highly susceptible to oxidation. Different seafood products usually contain different prooxidants. Knowledge of how different factors affect oxidation induced by prooxidants could help to find better ways to protect seafood products from lipid oxidation.

The oxidation rate of fatty acids was evaluated using a model system consisting of marine phospholipid liposomes (cod roe phospholipid dispersion). The consumption of dissolved oxygen by liposomes in a closed vessel was used to measure the rate of lipid oxidation, the lipid oxidation was initiated by iron ions (Fe^{2+}, Fe^{3+}), or haemoglobin (Hb).

Different oxidation mechanisms are involved in iron and haemoglobin induced oxidations. With regard to pH, the highest oxidation rate was observed at pH 4-5 for iron induced oxidation. However, for Hb-induced oxidation, the rate increased with increasing pH (up to pH 6). The oxidation rate increased with increasing temperature and the activation energy for iron and Hb induced oxidation were observed to be 60-86 and 48 kJ/mol, respectively. Iron chelating compounds, such as EDTA and phosphate, reduced iron induced oxidation, but had no effect on Hb induced oxidation. The effect of different phenolic compound on oxidation was studied. Both iron and Hb induced oxidation was reduced by propyl gallate and ferulic acid. Coumaric acid had no effect on oxidation rate induced by iron or Hb. Caffeic acid was an antioxidant for Hb induced oxidation. However, it acted as a strong prooxidant on iron induced oxidation.

These results show that selection of antioxidative compounds has to be performed after careful analysis of which prooxidants that are the most important in the relevant seafood product in order to successfully reduce lipid oxidation.

F3.10. Revealing mechanisms of lipid oxidation mediated by fish hemoglobins using protein crystallography

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Lipid oxidation is a major cause of quality deterioration in fish muscle. Fish hemoglobins (Hb) are particularly effective at promoting lipid oxidation compared to avian and mammalian Hbs. Understanding the mechanisms by which the fish Hbs are highly pro-oxidative can be used to develop effective strategies to inhibit color deterioration and lipid oxidation during cold storage.

Our work indicates that release of the heme moiety from the globin is a key determinant in the onset of Hb-mediated lipid oxidation. The autooxidation of oxy/deoxyHb to metHb is also relevant since heme affinity is around 60-fold lower upon oxidation from the red to brown pigment.

We compared autooxidation and hemin release rates from trout IV and perch Hb compared to bovine Hb. In addition each Hb was crystallized at post mortem pH (5.7-6.3) and high pH values (8.0-8.5). X-ray data were collected using the 19-BM beamline and various programs were used for structure analysis.

The fish Hbs autooxidize and release hemin 50 to 100-fold more rapidly than bovine Hb. The stereochemical mechanisms for these dramatic differences have been revealed upon examination of the crystal structures. Five specific amino acid replacements in the CD corner and along the E helix appear to cause the increased susceptibility of fish Hbs to oxidative degradation compared to mammalian Hbs. These sites are CD3, CD4, E10, E11 and E14 in alpha and beta chains. The mechanisms by which autooxidation and hemin loss occur more rapidly in the fish Hbs involve i) steric displacement of bound ligands, ii) weak anchoring of the heme propionates to the globin, iii) larger channels for solvent entry into the heme pocket, and iv) weakened interactions with the distal histidine.

Novel antioxidant strategies should focus on maintaining the heme moiety within the globin and preventing the interaction of heme with lipids.

F3.11. Factors affecting the interaction of trout (*Onchorhynchus mykiss*) hemoglobin (Hb) with washed cod mince

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Introduction: Presence of hemoglobin (Hb) leads to oxidative changes of lipids and proteins in fish and meat products. Such oxidative changes are accelerated in the presence of preformed hydro-peroxides. Minimising Hb-mediated oxidation necessitates either removal of Hb or prevention of Hb from interacting with lipids and proteins. Earlier studies have revealed that it can be hard to extract Hb from oxidized matrices. This indicates that oxidized Hb binds firmly to the muscle matrix. However the exact factors that influence interaction and non extractability of Hb are not understood. In this study we intend to evaluate factors that are governing the binding of Hb to a muscle matrix such as washed cod mince. Among these factors are pH, salt and presence of antioxidants. Further we intend to study the kinetics of Hb interaction with washed cod mince.
Materials and Methods: The muscle source used was washed cod mince and the source of Hb was blood from trout. The Hb was tested both as a hemolysate and as individual forms of Hb (component IV). Following adjustments of pH, addition of salt, antioxidants etc., changes in redness, development of rancid odour, and extractability of Hb were followed. Also, the percentage of different forms of Hb during oxidation was closely followed. In some studies attempts were made to keep Hb in reduced state.
Results: Preliminary trials have shown that the extractability of trout Hb from the washed cod mince matrix decreases rapidly along with formation of met-Hb. Kinetic studies of binding indicate that the binding of Hb occurs rapidly within 24 hours after which extractability of Hb is almost negligible. Further results on Hb extractability as a function of the above said conditions will be presented and discussed in detail.

F3.12. Evaluating intrinsic factors involved on lipid oxidation promotion by fish haemoglobin

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Introduction: Hemoglobin (Hb) is a main active catalyst of lipid oxidation in fish muscle-based foods during storage and processing. However, the mechanism involved on the oxidative role of Hb is still unclear spite of its negative repercussion on sensory and nutritional quality.
The present investigation was aimed to discover the intrinsic factors controlling Hb-mediated lipid oxidation. To this purpose, the activity of three fish Hb species to promote lipid oxidation was evaluated in liposome and washed minced fish muscle, and that was correlated with intrinsic properties of Hb, such as, the susceptibility to release heme group, and to form methemoglobin (MetHb) and hypervalent ferryl hemoglobin (ferryl Hb). Those inherent properties were also studied in the presence of lipid oxidation by-products as hydroperoxides and aldehydes. The activity of Hb was also compared with the corresponding MetHb.
Methods: Hb was isolated from pollack (*Pollachius pollachius*), horse mackerel (*Trachurus trachurus*) and sea bass (*Dicentarchus labrax*). Lipid oxidation was followed by peroxide value, conjugated dienes and volatiles (TBARS).
Results and Discussion: Hb from pollack was more active promoting lipid oxidation in both liposome and washed minced fish muscle systems, followed in decreasing order by horse mackerel Hb and seabass Hb. Moreover, metHb was a better catalyst of lipid oxidation that the corresponding Hb. This result is according with the tendency found to generate metHb either in absence (autooxidation) or presence of hydroperoxides, pollack Hb> horse mackerel Hb > sea bass Hb. The heme group was also faster released by pollack Hb > horse mackerel Hb > sea bass Hb. Since the reduced Hb form posses a more compact structure, and therefore, lower accessibility to the heme group, metHb should released more easily the heme group. Additionally, metHb could mediate lipid oxidation via formation of ferryl Hb, and such formation was also investigated and discussed.
Conclusion: The extent of Hb-promoted lipid oxidation showed a high correlation with Hb oxidation up to metHb form and the liberation of heme group. Therefore, antioxidant procedures conducted to reduce either metHb formation or heme group release should be potentially active to inhibit Hb-mediated lipid oxidation in seafood products.

This work has been performed into the Integrated Research Project SEAFOODplus, contract No FOOD-CT-2004-506359 and the research project AGL2006- 602-C03-01.

S3.13. Use of lactic acid bacteria for the biopreservation of sea bream fillets (*Sparus aurata*)

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The shelf-life of fresh seafood is short and this represents a substantial practical problem for the distribution of these products. Spoilage reactions can be inhibited by traditional processing and preservation with milder and more natural preservatives. The biopreservation include the use of microflora natural or controlled or its antibacterial metabolites to extend the shelf life and enhance the safety of foods. As stated above, LAB can protect food from microbial spoilage by competitive growth, by the production of antagonistic metabolic products, or by the formation of other antimicrobial compounds.

The aims for this work was find the *optimum medium* for antimicrobial compounds production of *Lactobacillus lactis* and *Entorococcus faecium* for their application at fresh gilthead sea bream fillets (*Sparus aurata*). The antimicrobial efficacy at this microorganisms and metobolites products on two target bacteria (*L. innocua* and *L. sakei*) and on the native microflora was tested.

The LAB were grown in four medium: MRS; YGPLB, optimal 1 and 2. The optimal medium was formulate with (saccarose, peptone, yeast extract, KH_2PO_4, NaCl, $MgSO_4 \cdot 7H_2O$). The sensibility of target microorganisms was determined by the agar diffusion method front nisin patterns.

The results show a higher antimicrobial efficacity when the LAB are growth in optimum 1 and optimum 2 medium. *L. lactis* only shows antimicrobial activity against *L. sakei* but no against *L. innocua*. By the other hand *E. faecium* shows activity against both target microorganisms. For the LAB studied similar behaviour was observed in the study on the native microflora of fresh gilthead sea bream fillets.

S3.14. Properties of hake muscle subjected to high hydrostatic pressure to kill *Anisakis simplex* L3 larvae

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Introduction: The application of high hydrostatic pressure to fish infested with *Anisakis* sp. larvae has been proposed as an alternative to freezing in order to kill the larvae and avoid anisakiasis in consumers. This work aims to evaluate the effect exerted by high pressure on the properties of hake muscle after applying pressure-time conditions that kill *Anisakis simplex* larvae in infested fish.
Methods: Sandwiches of hake steaks (1cmx8cmx6cm) were prepared by placing 12 live *A. simplex* L3 larvae inside each sandwich. Samples were stored (5±1 ºC, 24 hours) before applying 200 MPa for 1min, 2min, 2min+2min (repeated cycles of 2min, 6min between the cycles) or 5min and 300 MPa for 1min. After treatment, the larvae were transferred to distilled water where mobility (spontaneous and stimulated) and emission of fluorescence (366 nm) were measured at room temperature immediately and at intervals over a space of 2 h. Muscle properties were evaluated by measuring shear strength, color (*L*a*b**), apparent viscosity, protein content and electrophoretic pattern of proteins isolated from muscle homogenates.
Results and Discussion: *Anisakis simplex* larvae did not present spontaneous neither stimulated movements immediately and up to 2 hours in all pressure-time conditions applied. All the treated larvae emitted fluorescence. These results showed that *Anisakis* larvae in artificially infested muscle were killed by high pressure at 200 MPa in less time than previously reported in other fish species. A decrease in viscosity of muscle homogenates and an increase in shear strength were apparent after 1 minute at 200 MPa. High pressure exerted at 200 MPa did not modify the appearance of the fresh muscle; however at 300 MPa the muscle visibly appeared as partially cooked and higher *L** values were measured. The protein bands obtained in the electrophoretic pattern from muscle homogenates subjected to pressure treatments did not change at all pressure-time conditions applied. Nevertheless, the hake muscle tissue was less altered by pressure than previously reported in other species, probably due to the short time in which the treatment was applied. Work is in progess to evaluate the effect of high hydrostatic pressure in naturally infested fish.

Work financed by Spanish projects AGL2005-05699-C02-01/02 ALI, PIE 2004 7 0E 160 and PIE 2004 7 0E 340 CSIC.

S.3.15. The potential use of high pressure treatment for shelf life extension in the Norway lobster (*Nephrops norvegicus*)

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The Norway lobster (*Nephrops norvegicus*) is the target of the most valuable fishery in the UK. The product market has evolved from a frozen tailed product or scampi to fresh whole animals or langoustines. This increase in demand for a high quality product has stimulated the need to find processing methods in order to extend shelf life. Therefore, the aim of the present study was to assess the possibility to use high-pressure processing to extend the shelf life of whole langoustines.
To this end, whole langoustines were pressurised at 150, 300 and 500 megapascals (MPa) for 3 min at ambient temperature and were subsequently stored at 0-2 °C for up to 21 days. Visual assessment, melanosis score, total bacteria counts, K-values (used as freshness indicator) and concentrations of trimethylamine (TMA) and hypoxanthine were measured. Furthermore, an independent professional sensory panel performed an organoleptic evaluation of the samples.
The results show that the bacterial load was reduced in a pressure-dependent manner for up to 21 days. However, microbial growth was resumed in all the lots after a delay which was also pressure-dependent. Sensory and biochemical data will highlight the potential of using this technology on langoustines, but will also identify its limitations.

This work was supported by a grant from the EU Financial Instrument for Fisheries Guidance (FIFG) scheme through the Scottish Executive, and by Young's Seafood Ltd.

F3.13. Contaminants in two populations of edible crab *Cancer pagurus*: environmental and human health implications

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The edible crab *Cancer pagurus* is much appreciated in Southern European countries and the edible tissues are consumed separately or as a mixture. This species is mostly harvested along the Scottish Coast (SC) and English Channel (EC) and has different market prices depending on the catching area and animal sex. The aim of this study was to quantify and characterize the contents of S, As, Br, Sr, Cd, Hg and Pb in the muscle, hepatopancreas, gonads and gills of female and male crabs from both catching areas. Additionally, the accumulation patterns were evaluated according to the risks for human consumption and from an environmental point of view. The elements S, As, Br and Sr were quantified by energy-dispersive X-ray fluorescence and Cd, Hg and Pb by flame atomic-absorption spectrometry. Statistical differences were found between catching areas, sex and tissues. Crabs caught off the SC had higher S, As and Hg concentration in gonads but lower Cd than EC crabs. Concerning sexes, females had higher Cd (muscle and gills) and As (hepatopancreas) concentration but lower levels of S (muscle and gills), Sr (hepatopancreas), Br (gills) and Pb (gills). Regarding tissues, generally higher concentrations were found in hepatopancreas (S, Sr, Cd and Hg), gills (Br and Pb), gonads (S) and muscle (Hg). Cadmium in the hepatopancreas of both crabs' populations was the only contaminant above the level set by international regulating organizations, therefore consumers should avoid its consumption. The accumulation pattern is dependent on the intake route, seawater and food, and the proportion of each element is related to the crabs' physiological needs and the elemental bioavailability in seawater and diet. This study highlights the importance to determine contaminants' concentrations in all tissues.

F3.14. pH-shift protein isolation decreases toxicity caused by dioxin-like PCBs and PCDD/PCDFs of Baltic herring (*Clupea harengus*)

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Baltic herring (*Clupea harengus*) has a problem of often having high levels of polychlorinated dibenzo-*p*-dioxins (PCDD), dibenzofurans (PCDF) and biphenyls (PCB). According to EU-regulations, fish must have a PCDD/PCDF-TEQ-value (TCDD-equivalent) below 4pg/g and below 8pg/g (fresh weight) in a combinatory value for both PCDD/PCDF and dioxin-like PCB. Fish that exceeds these values are not allowed for consumption in EU. Based on detailed intake recommendations Sweden and Finland has an exception from this rule and are allowed to sell fish with higher PCDD/PCDF-TEQ values on the domestic market. The exception is limited to the end of 2011. Methods to reduce the dioxins in fish meal and fish oil exists but so far has no study has showed a method that gives food-grade proteins with reduced toxicity. The pH-shift protein isolation technique has previously been shown to give good recoveries of muscle proteins. The proteins have a good functionality and a reduced fat content compared to the starting material.
The hypothesis of this study was that pH-shift protein isolation should yield protein with significantly lowered PCDD/F and PCB toxicity load due to removal of fat. To test this both acid and alkaline pH-shift protein isolation was investigated on gutted Baltic herring.
In this study the starting material had PCDD/F-TEQ value as well as the combined PCDD/F-PCB-TEQ value above EU-limitations. The pH-shift method (solubilization pH 11.2 /2.7 and precipitation pH 5.5/6.1) reduced the PCDD/F-TEQ per gram protein with around 80% and the PCB-TEQ with around 85%. On a wet weight basis the reduction was even greater, mostly due to higher water content in the protein isolate. Different modifications to further increase the reduction were tried, resulting in different PCDD congener profiles in the isolates. Also, a mass-balance was made to investigate in which fractions of the process the different congeners ended up.

F3.15. Effectiveness of electrolyzed oxidizing water on the reduction of *Salmonella* contamination on shrimp

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This study was undertaken to evaluate the effectiveness of electrolyzing oxidizing (EO) and chemically modified water with properties similar to the EO water for inactivation of *Salmonella enteritidis* (ATCC 13076) and *Salmonella typhimurium* (ATCC 14028) on shrimp (*Parapenaeus longirostris*). Shrimps were experimentally inoculated with *S. enteritidis* (6 log CFU/g) and *S. typhimurium* (7 logCFU/g) and subjected to dipping treatment (1 and 5 min) with tap water (control), EO water (50 and 80 ppm active chlorine), chlorine water (50 and 80 ppm active chlorine), acidic water (pH: 2.5). Significant differences were observed between the all treatments on the reduction of *S. enteritidis* and *S. typhimurium* contamination on shrimp.

S3.16. Use of essential oil of oregano (*Origanum compactum*) for the biopreservation of fish (*Sparus aurata*)

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The antimicrobial effect of Oregano essential oil (EO) (*Origanum compactum*) from Maroc and its main chemical components (Carvacrol 38.1%, Thymol 19.59% and γ-Terpinene 16.71%) were examined *in vitro*, using two target microorganisms, *Listeria innocua* and *Lactobacillus sakei*. Further, the antimicrobial effect of them was also discussed in front of the native microflora of fresh gilthead sea bream fillets *(Sparus aurata)*. In both studies, the result was expressed compared with the nisin effect.
The sensibility of the microorganisms was determined by the agar diffusion method. The *Listeria innocua* (10^5 cfu), *Lactococcus sakei* (10^5 cfu) and the native microflora of fresh fish (obtained from the dilution of the overnight extract) were inoculated in BHI and MRS agar. The essential oil and its main chemical components were added in the agar cells and the inhibition halos obtained were calculated.
The obtained results show a higher effect of the oregano essential oil on the *L. inocua* and *L. sakei* growth. The carvacrol was the component with higher antimicrobial effect while the thymol was the lower. The same behaviour was observed in the study on the native microflora of fresh gilthead sea bream fillets.

S3.17. Effect of major phenolic compounds found in cold-smoked salmon on *Listeria monocytogenes* growth

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Introduction: The human pathogenic bacterium *Listeria monocytogenes* is sometimes present in cold-smoked salmon freshly processed and it is important to prevent growth during the storage and guarantee that *Lm* is under the tolerated level of 100 *Lm*/g till the end of the shelf-life. Effect of salt, pH, temperature, CO_2 and some organic acids on *Lm* growth has been studied, whereas the antimicrobial effect of smoke is misunderstood. The aim of this study was therefore to determine the effect of 10 major phenolic compounds present in natural smoke on *Lm* growth.
Method: The sensitivity of 28 *Lm* isolated from seafood to one of the compound was first tested to check the variability within the collection. The maximum growth rate (μ_{max}) as a function of concentration of the ten phenolic compounds was then determined for one selected strain. Growth was measured by absorbance (OD_{600nm}) with an automatised spectrophotometer Bioscreen C and μ_{max} was determined with the serially dilution detection time technique. Then, μ_{max} as a function of phenolic compound concentration (C) was modelled using a cardinal model $\mu_{max}=\mu_{opt} * [1-(C/MIC)^p]$. μ_{opt} corresponds to μ_{max} with no phenolic compound, MIC to the minimum inhibitory concentration and p is a shape parameter.
Results: Isoeugenol, 4 propyl guaïacol and eugenol were the compounds with the highest inhibitory effects (MIC appro. 400 ppm) whereas guaïacol, syringol and phenol had the lowest one (MIC appro. 8000 ppm), 4-methyl and ethyl guaïacol and o or p cresol having an intermediate one. In cold-smoked salmon, the phenolic compounds that are in a majority are those with the lowest inhibitory effect. A model taking into account the ten phenolic compounds together will be developed.
Discussion: In smoked salmon, total phenolic compounds are below 20 ppm. The predicted antimicrobial effect is lower than observed *in situ*, probably due to interaction between smoke and the food matrix or presence of other antimicrobial compounds still unidentified.

F3.16. Microbiological spoilage of cooked and peeled brown shrimp (*Crangon crangon*)

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Brown shrimp (*Crangon crangon*) is a typical Belgian fishery product. In Belgium, brown shrimps are fished during night, cooked and brought to land within 12 hours. Without conservation, cooked brown shrimps spoil at a very rapid rate. This spoilage is mainly due to a rapid increase of microbial growth. In this study the microbial population of cooked and peeled brown shrimps was investigated during shelf life at different temperatures.

First, the shrimps were caught, sorted, washed and cooked on board according to normal fishery procedures but without adding preservatives. At these different stages of processing, shrimps were collected and put on ice for transport. Microbiological analyses started one day after catch. In the laboratory, some cooked shrimps were peeled manually as sterile as possible. From all different stages of processing, shrimps were stored on ice (0 ±0.5 °C) and at 7.5 ± 0.5 °C for several days. Microbiological analysis was performed at regular time intervals during storage. The study of the microbiota was done on three different general media (Plate Count Agar, Marine Agar and Long and Hammer's medium) and four group-specific (Iron Agar, MRS, VRBGA and Pseudomonas CFC) media.

The results showed that the boiling process leads to a two ^{10}log decrease in microbial count. In addition, a steep decrease in bacterial count was noticed after peeling. Results of total counts during storage showed that cooked and peeled shrimps at the highest temperature were microbial spoiled (>10^8 cfu/g) after 7 days, while iced shrimps had a shelf life of 12 days.

Morphologically different colonies of the initial microbiota and the microbiota growing during shelf life and spoilage were collected and clustered based on $(GTG)_5$ rep-PCR techniques. Representatives were selected and will be identified based on 16S rDNA gene sequence.

F3.17. Predicting growth of *Listeria monocytogenes* and lactic acid bacteria in lightly preserved seafood under dynamic temperature storage conditions

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Introduction: A combined model for growth of *Listeria monocytogenes* and lactic acid bacteria (LAB) including the effect of 11 environmental parameters has previously been developed at DTU Aqua. In addition, this Lm-LAB model includes the effect of interaction between all the environmental parameters as well as microbial interaction between *L. monocytogenes* and LAB. The Lm-LAB model has been successfully validated for seafood stored at isothermal conditions; however, to be useful in "real-life" situations it would be most important to evaluate its ability to predict growth under dynamic temperature conditions. The objective of the present study was to evaluate the performance of the Lm-LAB model under dynamic temperature conditions.
Methods: Data for model validation was generated in eight challenge tests with brined and drained shrimp in modified atmosphere packaging (MAP) and MAP cold-smoked salmon. Products were inoculated with *L. monocytogenes* or a mixture of *L. monocytogenes* and *Lactobacillus sakei* isolates, and subsequently stored at periodically changing temperatures. Product characteristics and storage conditions of the different products were determined carefully in order to predict growth of *L. monocytogenes* and LAB.
Results and Discussion: The Lm-LAB model was validated successfully at dynamic temperature conditions. The effect of periodically changing temperatures on growth of *L. monocytogenes* and *Lb. sakei* in lightly preserved seafood was predicted accurately by the Lm-LAB model (Fig. 1.). Complex growth models including the effect of many environmental parameters are important to accurately predict microbial responses in food and thereby obtain useful information on safety and shelf-life. Several complex predictive microbiology models are available in the scientific literature but evaluation of their performance under dynamic temperature conditions has rarely been reported. The present study is therefore important and the results obtained are most promising for the practical use of predictive microbiology models in evaluation and management of seafood safety and quality.

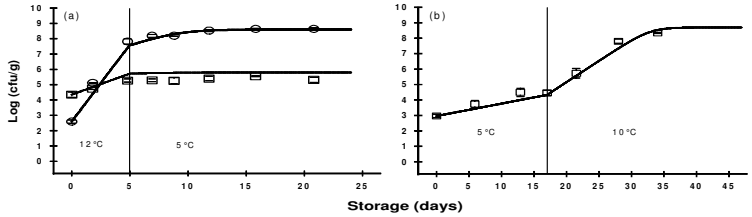

Fig. 1. Comparison of observed (□,○) and predicted growth (solid lines) of *L. monocytogenes* (□) and *Lb. sakei* (○) in (a) brined and drained MAP shrimp and (b) MAP cold-smoked salmon stored at periodically changing temperatures.

F3.18. New techniques to achieve more cost efficient selective breeding for improved consumer acceptance of aquaculture products

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Quality salmon has a desirable red colour, appropriate fat content, firm texture and normal appearance. The shape of the fish and a normal skeletal development is also of importance for the general impression of the fish as well as for the filleting yield.
It is well documented that both fillet fat and fillet colour show significant genetic variation. These characteristics have therefore been included in breeding programs for salmon. So far breeding programs have been based on family selection where a number of individuals from each family are slaughtered and then analysed for quality. Methods for analyzing colour and fat in live fish would be very useful as it enables selection of salmon within families which then can be utilized as parents. In this way one would achieve breeding success in a shorter time.
Our results have shown that fat and colour can be measured in live salmon by using VIS/NIR technology. We are now continuously investigating 150 families of salmon, which were transferred to the sea in spring 2007, where we are following the development in fat and colour regularly until slaughter. By performing multiple recordings on the same individual during the life cycle we can get a better understanding of causes and effects of quality variation. The results will also show if it is possible to select fish with desired quality characteristics at an early stage during the sea phase.
Furthermore we have investigated the variation in texture between families, liquid holding capacity, accumulation of melanin, as well as the interactions between the analysed characteristics. Preliminary results show that it might be possible to select for fish with low amounts of melanin in the abdominal fillet part, which would improve utilisation of salmon fillets for high quality products and increase fillet yield.

F3.19. Nanotechnology and seafood production - A review

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Nanotechnology can be defined as working systems with materials which are under 100 nm scale (still needs approval by ISO). In last ten years, it opened ways to new frontiers and horizons in many industrial branches. Food industry is focused on antibacterial, packaging and digestive specifications and contributions of nanomaterials. Many governmental and private institutions have established new departments to investigate benefits and risks of nanomaterials. Nanofood market is being expanded in last few years and big food companies are trying to get share with new safer, functional and tastier products that produced by nanotech which is still limited in seafood production. Nanomachines can not be acceptable as realistic for now, but materials made by nanoparticles for different purposes may be used. Some companies still hesitate to develop and apply this technique due to many disputations and conflicts on these nanoparticles. In this review, possible applicabilities of these materials and techniques to seafood production were discussed.

S3.18. Rapid identification of spoilage and pathogenic bacteria in seafood by MALDI-TOF and genomic analysis

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Introduction: Seafood safety is a major concern for the seafood industry, administration and consumers. In this sense, the rapid detection of undesirable microorganisms is of relevance since this may provide us with useful tools to undertake a timely response to any critical situation. Accordingly, this study is aimed at developing rapid molecular methods, based on genomic and proteomic tools, to identify seafood-borne pathogenic and spoilage bacteria.
Methods: A bacterial collection of pathogenic and spoilage bacteria was compiled. A wide number of strains were isolated at our laboratory from more than ten commercially-relevant fish species. The collection was completed with relevant reference strains obtained from international type culture collections. A 800 bp ribosomal fragment was amplied in all strains with the universal bacterial primer pair p8FPL/p806R, this being followed by DNA sequencing. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was also considered to obtain highly specific mass spectral fingerprints.
Results: A database with ribosomal sequences of the most relevant seafood-borne pathogenic and spoilage bacteria was generated in this study. In addition, genus-specific as well as species-specific peptide biomarkers were identified to allow the rapid identification of bacterial species. The phylogenetic classifications of the different strains and species based on the genomic and proteomic approach were compared these leading to similar conclusions. However, in the case of some genera such as *Bacillus*, the proteomic approach allowed the identification at species and subspecies level, this being more difficult by means of genomic analysis of the 800 bp ribosomal region.
Conclusions: This work provides evidence of the usefulness of MALDI-TOF analysis for the rapid identification of seafood-borne spoilage and pathogenic bacteria.

S3.19. Low-salt restructured Mediterranean horse mackerel (*Trachurus mediterraneus*) products using microbial transglutaminase as cold-set binder

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Low-salt restructured fish products without cooking were obtained using Mediterranean horse mackerel muscle. The additives used were NaCl at three levels (0-control, 10 and 20 g/kg) and microbial transglutaminase (MTG) also at three levels (0-control, 5 and 10 g/kg). Changes in physicochemical (pH, Water Holding Capacity, a_w) and mechanical (texture profile analysis and puncture test) properties were evaluated. Hardness was in the range from 2.053 to 3.885 N, cohesiveness varied from 0.193 to 0.393 and springiness varied from 2.743 to 5.480 mm. MTG needed the addition of NaCl to improve the mechanical properties of these restructured products. Increasing the amount of both additives improved the mechanical properties of the restructured products. In products obtained without NaCl, the use of MTG has no significant effect on WHC and a_w. There were also no significant differences between WHC and a_w when using different amounts of MTG at any level of salt. The microbiological results (Total Viable Bacteria, *Shewanella putrefaciens*, *Pseudomonas* spp. and *Enterobacteriaceae*) suggested a short shelf-life (less than 4 days) of the refrigerated (2±2 °C) restructured products. The cooked restructured products were easily accepted by the panellists. The best sensory results were obtained when the amount of both additives was increased.

F3.20. A miniaturized gas-chromatographic system for the evaluation of fish freshness

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The objective of this work is the development a new microsystem for the evaluation of fish freshness. The main purposes were the possibility to perform fast, reliable and non-destructive analyses of fish samples for the quantification of volatile amines. Using a simplified gas-chromatographic approach based on metal oxide (MOX) sensors used as detectors, a stand-alone GC prototype was fabricated, which can be used for headspace analyses of specific fish species without requiring carrier gas cylinders for operation.

Due to several microbiological and chemical processes the properties of fishes change during their storage. This fact results in the emission of different volatile compounds that could be used for the quality assessment. Trimethylamine, dimethylamine and ammonia were chosen for the development of the new device.

The proposed miniaturized gas chromatographic system is based on two silicon micromachined main components, namely a 50 cm long packed GC separation column with an integrated heating element and a micro-hotplate based thin film MOX sensor array, together with some commercial fluidic components (a mini-valve and a mini-pump) and custom made control electronics. The devices are interconnected by a temperature controlled stainless steel pneumatic circuit, in order to avoid as much as possible condensation and adsorption phenomena of the sample. The carrier gas is generated on-board by means of an active carbon filter which provides a constant flow of filtered air through the separation column.

Sampling protocols suitable for the new developed device have been optimized and the system has shown good sensitivity and reproducibility in laboratory conditions. Preliminary real sample characterizations have shown a good agreement between the increase of TMA concentrations measured with the miniaturized GC and the results obtained from the sensory evaluation (QIM) and from traditional chemical methodologies for the determination of volatile amines.

F3.21. Automatic cod fillet inspection by imaging spectroscopy

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Automatic inspection of cod fillets by imaging spectroscopy has been thoroughly investigated over the last few years. The results have shown that defects such as nematodes, blood spots and black lining can be detected with the same overall accuracy as by manual inspection. The previous work has been done under lab conditions and focused on proof of concept. The system is now being moved from the lab to fully industrial operation with the requirements of one fillet per second.

One of the key elements when analyzing imaging spectroscopy data is to reduce the variation of spectra within the same class, while at the same time keep the difference between classes. We present a novel method for preprocessing imaging spectroscopy data of fish fillets such that local variations representing nematodes, blood spots etc. are preserved while variations due to illumination and thickness are removed. We show how this preprocessing method improves the detection of nematodes and blood spots in cod fillets, and preliminary results from an industrial test is presented.

F3.22. Fish freshness assessment as part of an automatic fillet inspection

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A fully automatic solution for fillet inspection is under development. This is an instrumental quality assessment tool designed for detection of “defects”, such as nematodes, blood stains, black lining and skin remnants. This instrumentation is based on imaging spectroscopy, and therefore it is possible to include freshness assessment as an extra feature for the instrumentation.
Earlier work has showed that spectral data from the loin part of a cod fillet can be used for freshness assessment. No validated theories for this behavior are found, but it is assumed that changes in fish muscle structure and color influence the absorption and scattering of light. In this way storage time influence the recorded fish muscle spectra and makes it possible to assess the freshness.

From the imaging spectroscopy data both spatial and spectral information is available, and it is possible to extract the spectral data that is needed for freshness assessment. Making a mean spectrum, consisting of spectra from the loin part of the fillet, it is possible to get results comparable to what has been reported previously. By including a second mean spectrum calculated from spectra collected from the centerline it is possible to improve the result. This is due to the fact that in the centerline a lot of blood vessels are cut open, and the oxidation state of the blood provides extra information.

The spectral data are analyzed by multivariate techniques, where storage time in ice and/or the Quality Index Method (QIM) can be used as references. Results obtained so far shows that freshness assessment is possible from imaging spectroscopy data, and will be a valuable extra feature for the automatic fillet inspection instrumentation.

F3.23. Simple extraction and rapid HPLC method for tocopherol analysis in seafood

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A rapid high-performance liquid chromatographic (HPLC) method with simple extraction procedure for the determination of tocopherols in seafood was developed. A continuous gradient elution system was used for analysis with a mixture of acetonitrile and methanol. HPLC gradient profile was 50% acetonitrile and 50% methanol and flow rate was 1.0 ml/min throughout the whole separation. The total separation time was 15 min to ensure full separation. The injection volume was 2 µl and detection was monitored with a fluorescence detector at 295 and 330 nm wavelengths for excitation and emission, respectively. Tocopherols were identified by comparison of retention times and area values with standard of alpha, gamma, delta and beta tocopherols. Total tocopherol content was calculated as the sum of alpha, gamma, delta and beta tocopherol and expressed as mg/kg for fish samples.

The application of this method to detect tocopherols in 20 fresh fish was carried out. Determination of the tocopherol in some of the fish are whiting (*Merlangius merlangus*), red mullet (*Mullus barbatus barbatus*), Atlantic salmon (*Salmo salar*), Atlantic horse mackerel (*Trachurus trachurus*), common sole (*Solea solea*), common seabream (*Pagrus pagrus*), gilthead sea bream (*Sparus aurata*), Northern pike (*Esox lucius*).

Total tocopherol contents were ranged from 9.78 to 72.88 mg/kg in fish muscle. The concentration of the tocopherol in Atlantic salmon, red mullet, gilthead sea bream was 72.88, 29.58 and 27.20 mg/kg, respectively, which are high amount of tocopherol content as an antioxidant source.

F3.24. FishPopTrace: a new European project on the structure of fish populations and traceability of fish and fish products

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Owing to the central role that fish population plays in sustainable utilisation and conservation of exploited stocks, FishPopTrace aims to develop robust traceability systems that incorporate major spatial and temporal differentiation in four commercially exploited fishes from European waters. This project has begun in March 2008 and is funded for 3 years from the EU's 7th Framework Programme (KBBE-212399**,** Gary Carvalho from University of Wales Bangor is the coordinator).
FishPopTrace will address several inter-related objectives:

- to integrate recent and on-going data from European fish species traceability projects. The outputs will comprise a new database and associated web links with access to recently generated data on fish species and population identity, together with an archive of associated tissue samples from external and consortium outputs.
- To examine single nucleotide polymorphisms (SNPs) and otolith microchemistry and morphometrics in widely distributed populations of cod, hake, sole and herring, as tools for discriminating biologically differentiated populations and as a basis for traceability. Outputs will comprise population-level signatures associated with fish origins in early life and representative spawning groups.
- To undertake validation of traceability tools in relation to end-user technology. Outputs will produce Standard Operating Procedures (SOPs) to allow transfer of technologies to other laboratories throughout EU member states.
- To develop a population monitoring system based on otolith and genetic data that will assess population stability in a temporal and spatial framework. For each species, alternative parameters will be identified as indicators of population stability, and parameters will be validated using a combination of archived data and tissue samples.
- To test the utility of additional novel traceability systems (fatty acid profiles, proteomics, gene expression, microarray platform for SNP genotyping).

To facilitate technology transfer in relation to enforcement and conservation policies of the CFP (Common Fisheries Policy).

F3.25. Identification of the farm origin of salmon by using fatty acid and high resolution ^{13}C nuclear magnetic resonance profiles

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In 2003, the Norwegian Ministry of Fisheries set up a national committee to examine the tagging of farmed fish and the Director of Fisheries set up the Tagging Committee with representatives of the aquaculture industry, the research community and the authorities, with the mandate to present a concrete range of tagging/tracing systems for farmed salmon. Several techniques were selected for testing, including the ones presented here. The aim of this work was to examine the suitability of lipid analyses by gas chromatography and by nuclear magnetic resonance as tracers to identify 1) the farm where the fish had been reared and 2) in the case of fish captured outside pens in the fjord whether it was possible to first identify the wild and the farmed and in the case of farmed, to classify them as originating from a given farm.

15 Atlantic salmon collected from each of 4 different farms (a total of 60 fish) around Hardangerfjord (Norway) in July 2006, and 17 freeliving salmon, caught in the fjord during the period October 2005-October 2006, were analyzed by GC and 600 MHz HR ^{13}C NMR. Four of the freeliving fish were easily identified as wild by their FA profile, n3/n6 ratio and by PCA and Bayesian Belief Networks were used to classify the rest of samples according to their farm of origin. All the farmed fish were correctly classified except one fish from farm 1 that was classified together with fish from farm 3. Thus, this analysis was able to classify correctly 98.7% of the fish.

Of the 13 free-living fish identified as escapes, 4 were identified as originating from farm 2, 1 from farm 3 and 3 from farm 4. According to the Bayesian analysis, the rest were probably originating from other farms not included in this analysis.

S3.20. Fatty acids profile as a tool for traceability of bluefin tuna (*Thunnus thynnus*) canned products

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Authentication of fish and seafood products can results difficult for industry, trade and consumers, because of similarity in appearance of many species and of the loss of external characteristics, such as shape, size, appearance, during processing. Geographical origin and method of production, are the principles issues of seafood traceability and quality.
Fatty acid (FA) tissue profiles, in particular, were widely utilized to describe fish quality, being very sensitive to species, environment, physiology and process conditions, it can also be used as marker of traceability.
Among edibles fish species, tuna fish are of great commercial value worldwide, both fresh and canned. In western Sicily (Trapani, Italy), in particular, high quality Bluefin tuna (BFT) (*Thunnus thynnus*), is caught and marketed fresh or frozen, mostly, in Japan. A small quantity of BFT caught by local fleet is utilized to prepare high quality canned product. Thus, it could be important to distinguish analytically this niche product from those originated from others tuna species such as yellowfin (YFT) (*Thunnus albacares*).
In the present study, we analyzed gross composition and FA profile of BFT caught in the "tonnara" of Favignana (Trapani, Italy), both on fresh or canned by a local industry. Results were compared to data obtained from commercially available canned YFT and canned tuna (CT) of not identified species.
Obtained results indicate that lipid composition and FA profile are different from fresh and canned BFT ($P<0.05$), due to the origin of the processed cuts and to the transformation process. FA profile, indicating a higher PUFA n-3/PUFA n-6 in BFT and YFT, respect to CT ($P<0.05$), seems to be useful to characterize and, then, distinguish species, origin and quality of tuna product.

S3.21. Molecular identification and phylogenetic analysis of commercial shrimp species based on the cytochrome b mitochondrial gene

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Introduction: Although the cytochrome b (*cytb*) mitochondrial gene has been used successfully as a molecular marker for species identification in a broad variety of animal *taxa*, this molecular marker has not yet been considered for the identification of prawns and shrimp. The main goal of the present study was to gain deeper insight into the mitochondrial sequences of the *cytb* gene of commercial penaeid shrimps as a preliminary step to developing a novel PCR-RFLP method for their differentiation.
Method: PCR amplification with *crustF/crustR* primers, targeted to the amplification of a *ca.* 181 bp region of the cytochrome b (*cytb*) mitochondrial gene in penaeid shrimps, was performed. DNA sequencing allowed the selection of endonucleases *Cvi*JI, *Dde*I and *Nla*IV. The method was applied to fresh and frozen specimens and to complex processed foods where this type of shellfish is included as an added-value food ingredient.
Results: This study reports the first *cytb* mitochondrial sequences described to date for the species *Farfantepenaeus notialis*, *Parapenaeus longirostris* and *Pleoticus muelleri*, and nearly triplicates current knowledge of reference nucleotide sequences in this mitochondrial region for this group of species. The endonucleases considered in this study allowed the differentiation of the six species considered. Moreover, different populations with different origins could be distinguished at the intraspecific level. The small size of the molecular target proposed in this study facilitates amplification from fresh, frozen or pre-cooked samples, where DNA fragmentation may be relevant and fragment size critical. A detailed comparison of phylogenetic trees constructed on *cytb* and 16S rRNA genes, respectively, revealed only slight differences. Thus, in both cases all samples were grouped in six large clades, each of them corresponding to each species.
Conclusions: The *cytb* mitochondrial gene may be considered as a useful molecular marker for identification and phylogenetic purposes in penaeid shrimp species.

F3.26. Authentication of the commercial denomination *white tuna* by real time PCR

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The labelling *light tuna* is a commercial denomination created by the Spanish canning industry that has been recently recognised by the Spanish legislation (RD 1193/2000). The *light tuna* label refers to yellowfin or *Thunnus albacares*. The yellowfin tuna is a worldwide distributed species which share the habitat with other tuna species such as the bigeye tuna (*Thunnus obesus*). As a mater of fact, both species are generally caught simultaneously and then processed together by the canning industry. Nowadays, the authentication of seafood products is a major concern in order to assure the traceability system from *fish to fork*, and one of the most important problems for canning industry is actually the presence of tuna mixtures in canned products. To date, none of the analytical techniques enable to evaluate the real scale of this problem. In fact, the methodologies currently used are based on the genetic analysis of only a small tissue portion. These approaches are insufficiently accurate to detect and quantify the presence of tuna species mixtures.

We have developed a specific system to authenticate canned products labelled as *light tuna*. This system is based on the use of DNA probes that specifically identify and quantify the presence and proportion of different tuna species in mixtures. The method has been successfully validated with binary mixtures constructed in the laboratory. In fact, we were able to detect up to 10 % of bigeye tuna has been detected in *white tuna* canned products adulterated in the laboratory. An evaluation of the Spanish market has also been launched in order to analyse the real scale of this problem.

Summarizing, we have developed an innovative detection system to authenticate the *light tuna* label in order to assure the traceability system in the caning industry which eventually will result in a benefit for the consumer.

F3.27. Genetic identification and traceability in Pacific salmon: implications for management and marketing

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Traceability appears to be a relatively simple concept; however, the actual process of creating an informational link between the origin of materials and their processing and distribution can be complicated, especially for seafood products due to the variety of species and myriad of distribution chains. Achieving traceability throughout the food supply chain requires the building of strong relationships in both directions along the food chain and a level of vertical integration surpassing what is currently found within the industry. Although challenging, vertically integrating can result in more cooperative relationships, greater efficiency, and longer term market success by increasing consumer knowledge and satisfying their need for safe and good quality products. In this presentation we will use examples in the Pacific salmon industry to describe how traceability, whether mandatory or voluntary, can have an impact on food systems, improving food quality and safety while providing new market opportunities and creating brand recognition. A multi-disciplinary research team at Oregon State University, working in partnership with the salmon industry, have begun a program using “real time” genetic analysis to identify the basin of origin of individually harvested salmon and allow salmon to be marketed according to their home stream. More than 72 vessels participated in the project and 3,097 fish were sampled. Of these fish, 2,567 were assigned to a genetic baseline by microsatellite-DNA analyses, while 2,097 fish were assigned with > 90% probability of river basin identification. This type of “branding” could increase the demand and price to fishermen and create new niche markets of wild-caught salmon as well as provide useful information that would allow for improved management of ocean salmon.

F3.28. Proteome analysis of Atlantic and Pacific cod, two gadiform subspecies used in products commercially labelled ”cod”

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Introduction: Proteome analysis was used to distinguish the two subspecies *Gadus morhua* and *Gadus macrocephalus* which can both appear in the market as fresh fish, salted fish and salted fish products commercially labelled “cod”. These subspecies have different commercial values, *Gadus morhua* being of considerably higher value. European regulations concerning the labelling of seafood products have been introduced in order to overcome fraudulent practices. However, as these species are normally presented as fillets or salted products identification of the species at product level becomes complicated.
In this project we have employed post-genomic analysis for objective identification of the two subspecies in order to obtain bio-markers that could be used for developing commercial identification methods.
Methods: Ten *G. morhua* and ten *G. macrocephalus*, weight class 3-5 kg, were sampled. The fish was caught by long line, headed, gutted and frozen on board the vessel.
Proteome analysis (2-DE and identification by MS) was performed on muscle extracts from both groups. Ludesi Redfin software was used to interpret and analyse the gel-images.
Results: At least eight proteins were differentially expressed in the two subspecies. Six of these proteins were present only in *G. morhua* and two of the proteins were present only in *G. macrocephalus*. The proteins were identified as muscle structural protein or muscle enzyme isoforms.
Discussion: The consequences of the different protein pattern between *G. morhua* and *G. macrocephalus* will be discussed.

S3.22. Comparison of PCR assays for detection of fish

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Introduction: Fish and shellfish are food commodities of great benefit for human health. However, they have a certain allergic potential, like many other foods. Once established, seafood allergy is usually a life-long problem. According to the Directives 2003/89/EC and 2006/142/EC fish, crustaceans and molluscs and products thereof must be indicated in the list of ingredients.
As it is important for protection of consumers to control the observing of these directives, methods for detection of seafood are under development. Besides immunological tests, also PCR-based techniques are used to detect the presence of fish and shellfish.
Fish fillet is rich in parvalbumins, heat-stable, acidic, calcium-binding proteins of low molecular mass (~12 kD). Parvalbumin is the major allergenic protein of many fish species.

Methods: 1. We have analysed parvalbumins on three levels:

- Performance of PCR by amplification of short sequences of parvalbumin encoding genes (level of DNA)
- Performance of RT-PCR (PCR of reversed transcribed RNA) of parvalbumin mRNA (level of mRNA)
- Performance of isoelectric focusing of sarcoplasmic proteins (level of protein)

2. Three commercially available PCR kits based on amplification of mitochondrial or nuclear genes were tested for reactivity against a large number fish species and products.

Results and Discussion: Fish species could be identified by PCR of parvalbumine genes, RT-PCR of parvalbumin mRNA and IEF of sarcoplasmic proteins. The PCR systems reacted with a large number of species, but were not “universal” as they failed to detect some species.

The commercial kits using primers for mitochondrial genes gave a better performance than the kit working with a nuclear gene.

F.3.29. Detection and enumeration of histamine-producing bacteria in scombroid fish

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Many methods have been developed to detect histamine-producing bacteria in foods. Most of these detection systems are culture-based methods utilizing specific media that are selective and/or differential for histamine-producing bacteria. Some culture-based methods require a preliminary isolation step so bacterial isolates can be assessed for histamine formation. Others are time consuming and often end with false positive results.

More recently a number of rapid molecular-based methods have been developed for the detection of specific enzymes responsible for histamine and other biogenic amine formation in foods. The formation of biogenic amines in food requires three events; the availability of free amino acids in food, the presence of decarboxylase-positive microorganisms, and the conditions allowing for bacterial growth and decarboxylase activity. Prevention strategies to reduce these risks associated with histamine fish poisoning (HFP) are based on the control of bacterial growth conditions at point of harvest and throughout distribution. The need to identify hurdles for control of toxigenic bacteria growth besides time and temperature during various food poduction processes is self-evident.

The ability to detect and enumerate the causative agents of HFP will greatly aid in the establishment of production methods that include low histamine-technology for preventing the foodborne chemical intoxication caused by the consumption of spoiled, or bacterially contaminated, fish. The aim of this study was to develop a molecular-based assay for detection and enumeration of histamine-producing bacteria in scombroid fish. Our approach used DNA-specific probes for detection of the histidine decarboxylase gene and colony lift hybridization for enumeration of histamine-producing bacteria.

Session 4

Health aspect of seafood consumption

Bianca Maria Poli, Giuliana Parisi (edited by), *Seafood from Catch and Aquaculture for a sustainable Supply*. *Book of Abstracts*,
ISBN 78-88-8453-746-1 (online), ISBN 978-88-8453-745-4 (print), © 2008 Firenze University Press

F4.01. Evidence for a potential role of peptides derived from fish and crustacean protein hydrolysates in the regulation of satiety - Stimulation of CCK secretion in enteroendocrine cells

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This work was included in an aim to industrially produce marine ingredients that are beneficial for human health, and can, in a preventive manner, provide workable solutions to major public health issues. Fish by-products Protein Hydrolysates (FPH) are of significant interest due to their potential application as a source of bioactive peptides in nutraceutical domain. Here, we investigated the potential action of FPH from fish (*Micromesistius poutassou*) and crustacean (*Penaeus aztecus*) by-products on satiety via their capacity to stimulate intestinal cholecystokinin (CCK) release on enteroendocrine cells (STC-1). Stimulating peptides were partially purified, their action on the CCK secretion tested, and finally the mechanism of action of the CCK secreted molecules was defined using AR42J cell model. CCK is an endocrine peptide family that is found in both the brain and the gastrointestinal tract. It regulates a number of physiologic gastrointestinal functions and is involved in the control of satiety and in pathologies like obesity. Mouse enteroendocrine intestinal STC-1 cell line harbors high similarity with human CCK-secreting intestinal I cells, and was described to be a suitable model for in vitro CCK secretion study. Rat pancreatic acinar AR42J cell line expressed the two receptors CCK1 and CCK2, and was used to characterize the metabolic pathway followed by CCK molecules secreted, in response to FPH.
We demonstrated for the first time that peptide molecules from fish and crustacean by-products hydrolysates were able to highly stimulate CCK secretion in intestinal endocrine STC-1 cells. This stimulating effect was mainly due to peptides of molecular weight lower than 3000 Da. We realized the purification, and defined the in-vitro mode of action of these peptides. For the industrial utilization of this FPH as potential appetite-suppressive products, further studies should be performed in both the rat (in vivo) and human (clinical), to confirm the effects of FPH on the regulation of food intake.

F4.02. Influence of dietary intake of differently fed sea bream on lipid and hemorheological parameters

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Introduction: Fish intake has long been indicated as a protective dietary factor for cardiovascular diseases, due to the beneficial effects of its content of omega-3 polyunsaturated fatty acids (EPA and DHA). However, the mechanisms underlying this protection have not been fully elucidated. Aim of this study was to evaluate the influence of short-term dietary intake of fish on biomarkers related to the atherosclerotic process.
Materials and Methods: In a single-blinded intervention study, for a period of 10 weeks, 20 healthy subjects (12 males; 8 females) with a mean age of 48.6 ± 12.9 years (range: 23-67) were randomly allocated to two groups consuming approximately 650 g of fillets per week from portion size sea bream fed 100% fish meal (FM) or 50% of fish meal substituted by a plant source mixture meal (50PM). The first trial was then followed by an equivalent "crossover" trial. Fish fillets lipid and fatty acid contents were analysed. Lipid (total cholesterol, HDL-cholesterol, LDL-cholesterol and tryglicerides), and haemorheological parameters [whole blood viscosity (WBV), plasma viscosity (PV), erythrocyte filtration rate (EF)] of the patients, were determined in blood samples obtained at the beginning (T0) and at the end of each experimental period (T1).
Results: The serum fatty acid profiles of patients after the intervention period resulted to be significantly improved, in terms of total cholesterol, LDL, and triglycerides levels with significant differences ($P<0.01$ and $P<0.05$) for circulating risk markers of subjects eating the two differently fed fishes, mostly for total and LDL-cholesterol. Additionally, significant improvement of haemorheological parameters, at both highest and lowest shear rates was reported during FM intervention period versus those obtained during the 50PM diet.
Conclusions: Dietary short-term intake of fish seems to impose beneficial biochemical changes in healthy subjects, as showed by lipid and haemorheological parameters. In particular the intake of FM fishes determined a more favourable biochemical profile with respect to 50PM diet.

F4.03. Fish oils from Alaskan seafood processing: an unexploited resource of omega-3 rich nutraceuticals

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Alaska fish oils produced during seafood processing have the potential to increase the availability of this food ingredient for nutraceutical use. In Alaska, fish oils are produced from the byproduct stream of sustainable food fisheries and current production, while difficult to document, is probably between 30,000 and 45,000 mt per annum. Interestingly, it is estimated that the annual production of oil in Alaska could reach 70,000 mt under appropriate conditions.

The fatty acid profiles (mg/ g oil), lipid classes' distribution (% total lipids), levels of organic contaminants (ppm), and concentration of fat soluble vitamins A, D and E (IU/100g) were examined in commercial Alaska crude oils produced from walleye pollock (*Theragra chalcogramma*), pink salmon (*Oncorhynchus gorbucha*) and sockeye salmon (*Oncorhynchus nerka*), Pacific Ocean perch (*Sebastes alutus*) and sablefish (*Anapoploma fimbria*). Additionally, analysis of commercial menhaden (*Brevoortia tyrannus*) and canola (*Brassica napus)* oils were conducted for comparison. All Alaska fish oils investigated had high levels of ω-3 and ω-9 fatty acids, particularly 22:6ω3 (DHA), 20:5ω3 (EPA) and 18:1ω-9 (oleic acid), and very low levels of 18:1ω-7 (palmitoleic acid) and 18:2ω6 (linoleic acid). The major lipid class detected in Alaska oils was triacylglycerides at about 95%. Diacylglycerides, monoacylglycerides and free fatty acids were determined at less then 1% each. Phospholipids ranged from a maximum of 2% in pollock oil to a minimum of 0.3% in sablefish oil. Analysis of 29 commonly occuring pesticides (PCB's) reveled that all PCB's concentrations were very low with the exception of 4,4'-DDD and 4,4'-DDE in sablefish oil quantified at 0.5 and 0.25 ppm, respectively. Levels of fat soluble vitamins were highly variable according to fish species.

As global demand for omega-3 rich edible oils increases, purification of fish oil derived from Alaskan seafood processing for nutraceutical use may be of interest to food ingredient manufacturers.

F4.04. Namibia hake: a healthy fish with a high content of ω-3PUFA which contributes to prevent cardiovascular diseases

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Introduction: The consumption of fish is highly recommended specially due to the potential benefits on human health associated to the content of ω-3 PUFA. Lately, a number of studies are being directed to establish the beneficial effects of a fish diet in the promotion of health and prevention of diseases. Among commercial fish species, Namibia Hake which comprises *Merluccius capensis* and *Merluccius paradoxus*, is one of the most considerable fish species all over the world with a turnover of 150 million to US$ 250 million. It's widely distributed as frozen fillets and is also the basis of a large number of seafood products. The aim of this work is to characterise the lipid fraction of Namibia hake, particularly its content of ω-3 PUFA determining the effect of processing and cooking and to relate this aspect with the benefits of its consumption in the promotion of health.
Material and Methods: Two different batches of *Merluccius capensis* and *Merluccius paradoxus* caught in Namibia waters during 2006 and 2007, were supplied by Inesma. They were processed under industrial conditions and then analysed for lipid composition. For dietary intervention, 52 participants were randomised to one of three different fish content diets. Anthropometric measurements, determination of blood lipids and the content of vitamins and minerals were performed.
Results and Discussion: The lipid content of hake muscle ranged between 1.5-2.3 %. The concentration of ω-3 PUFA was high, having an average value higher than 500 mg /100 g muscle and showing a proportion of DHA, EPA and DPA of 70%, 20% and 6%, respectively. Processing hake did not decrease the proportion of PUFA on muscle. Hake intake improved several health related measures, providing an increase of weight loss, decreasing circulating triacylgycerols and cholesterol, and incrementing the levels of vitamin E.

As conclusion of the study, regular Namibia hake consumption provides a good source of ω-3 PUFA and can be considered a well way to increase the prevention of cardiovascular diseases.

This work has been performed into the Integrated Research Project SEAFOODplus, contract No FOOD-CT-2004-506359 and the research project AGL2006- 602-C03-01.

S4.01. Antioxidant activity of potato peel extracts in bulk fish oil and oil in water emulsions

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In the present study the antioxidant effect of potato peel, a waste product from the potato processing industry was investigated. Potato peel has been reported to possess high amount of phenolic compounds and can be utilized as a source of natural antioxidants. The objectives of the present work were twofold: (a) to extract the phenolic fraction from the potato peels, and to examine its antioxidant capacity and (b) to evaluate the effect of these potato peel extracts on the storage stability of fish oil in bulk and oil in water emulsions. The water and ethanolic extracts of the two common varieties of Danish potatoes, *viz* Sava and Bintje were screened for their antioxidant activity by employing four *in vitro* established systems such as antioxidant activity in liposomal model systems, 1,1-diphenyyl-2-picrylhydrazyl (DPPH) radical scavenging activity, reducing power and metal chelating activity. The multiple antioxidant activity of the potato peel extracts was evident from the preliminary screening as it showed strong reducing power, radical scavenging ability and ferrous ion chelating activity. It was found that Sava had better effect than Bintje. As water and ethanolic extract had different effects in different antioxidant model systems we chose both water and ethanolic extract of the Sava varieties at a concentration of 800, 1600 or 2400ppm for further oxidation studies in fish oil and oil in water emulsions. The analysis of Peroxide value (PV), volatiles, α-tocopherol, changes in the content of the phenolic compounds during storage and sensory analysis were used as criteria to assess the antioxidant activity and the results from these analysis will be presented.

F4.05. Seafood and health – What is the full story?

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Since the 1970´s, there has been an overwhelming focus on the long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) EPA and DHA as the only carriers of health effects from seafood. However, consumption of seafood also ensures a long series of other important nutrients and micronutrients. During the past decade, an increasing body of studies have accumulated indicating that these “non-omega-3” constituents may also contribute to the cardioprotective and neuroprotective effects documented e.g. from a low to moderate fish consumption. In the Nordic Innovation Centre-granted project MARIFUNC, an attempt has been made to review available in vitro and in vivo studies where bioactivity of these “non-omega-3” constituents have been studied. The review includes an introductory part about health effects from whole seafood, whereafter the focus is on vitamin D, proteins, peptides, amino acids and selenium, all which can be found in the edible parts of seafood. However, a number of compounds that can be achieved by utilizing by-products of fish/shellfish are also discussed; chitin, chitosan, glucosamine and chondroitin sulfate.
In the MARIFUNC-review, a broad approach has been used in the sence that a wide span of health effects have been considered, ranging from those linked to cardiovascular diseases (CVD) and other metabolic diseases to those related to inflammatory diseases, brain functions (well-being) and bone health. Since a main difference between knowledge on LC n-3 PUFA and other seafood-derived compounds lies in the kinds of studies that so far have been used to investigate their effects, information about study-type has been highly important for the current review.
In this presentation, the main findings from the MARIFUNC-based review will be given.

S4.02. Influence of smoking parameters on PAH content in rainbow trout

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During smoking of fish, polycyclic aromatic hydrocarbons (PAH) can be formed, some of which are potent carcinogens. In 2005 a maximum level for benzo[a]pyrene in smoked fish of 5 µg/kg fresh muscle was set by the European Commission. For the average Danish consumer less than 10 % of the human exposure to PAH comes from smoked fish.
A preliminary survey on smoking techniques used in Denmark for production of smoked trout was performed, and revealed that 20% of smoked fish in Denmark is trout. 36% of the smoked products are produced by local fish mongers, which causes smoking techniques to vary substantially.
Therefore, the influence of a variety of smoking process parameters on the concentration and distribution of PAH in smoked trout as a model fish was studied. Parameters include e.g. direct vs. indirect smoking, duration of smoking, temperature in the smoke generation chamber or penetration into the fillet.
The project will provide general guidelines for the Danish smokehouses for best available smoking technology and a better risk assessment of the products and hereby an improvement of the food safety for the consumer.

S4.03. Arsenic speciation in seafood from the North Adriatic sea

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The recommendations on seafood consumption should take into account also the toxicological hazards of seafood in order to perform a correct risk/benefit analysis. A recent survey showed that the composite fish group contributed 94% to the total population dietary exposure for arsenic. Since the toxicity of arsenic varies with the chemical form, speciation is mandatory to assess the safety of seafood. An IC-ICP/MS method for the determination of arsenobetaine, arsenocholine, dimethylarsinic acid, arsenic III and arsenic V in seafood was then developed. The extraction procedure was based on liquid-solid extraction. The method was used to analyse 4 samples of mussel (*Mytilus edulis*), 4 samples of mullet (*Mullus barbatus*) and 4 samples of mantis shrimps (*Squilla mantis*) pre and post grill cooking in order to investigate arsenic speciation in raw products and the variations induced by thermal treatment. Total arsenic was also determined by ICP-AES after acid digestion. The mean concentration of total arsenic in mantis shrimps, mullet and mussel was 23.5, 12.4 and 3.07 mg/kg, respectively, in raw products and 34.7, 15.5 and 3.13 mg/kg in cooked products. The mean concentration of arsenobetaine in raw products was 18.8, 11.9, and 1.22 mg/kg in mantis shrimps, mullet and mussel, respectively, while in cooked products was 30.8, 15.2 and 0.92 mg/kg. The mean concentration of arsenocholine in raw products was 2.21 mg/kg in mantis shrimps and 0.26 mg/kg in mullet. The mean concentration of arsenocholine in cooked products was 2.42 mg/kg in mantis shrimps and 0.31 mg/kg in mullet. Arsenocholine was never detected in mussels. Dimethylarsinic acid was detected only in mussels (0.26 mg/kg in raw, 0.28 mg/kg in cooked). Arsenic III and V were not detected. The results show that arsenic is present in seafood mainly as arsenobetaine and that thermal treatment does not affect arsenic speciation.

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